EFFECTS OF INOCULATION AND LIMING ON NODULATION AND NITROGEN FIXATION IN SOYBEAN (*Glycine max*) ON SELECTED ACIDIC SOILS OF KENYA

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DECLARATION AND RECOMMENDATION

Declaration

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

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Recommendation

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DEDICATION

This thesis is dedicated to my parents, family, and my sons Hajj and Abdulhafidh Abdikarim.

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ABSTRACT

Soybean (*Glycine max*) is a legume of tropical to sub-tropical origin, used as a source of food, feed and in oil production. Soybean yields in Kenya are low due to detrimental effects of soil acidity on soybean and rhizobia inoculants. The objectives of this study were; first, to determine the optimal soil pH range for effective nodulation and biological nitrogen fixation (BNF) of soybean varieties TGx1740-2F (SB19) and Nyala inoculated with Biofix and Legumefix. Secondly, to evaluate effects of soil fertility on nodulation and biological nitrogen fixation (BNF) of soybean inoculated with Biofix and Legumefix. Thirdly, to determine the effectiveness of agricultural lime and Mijingu phosphate rock (MPR) in raising soil pH and fourth, to evaluate the impact of liming on nodulation and BNF of inoculated soybean. For the 1st and 2nd objectives, soils of pH range 4.5-6.3 obtained from Central Rift Valley, Central Kenya and Lower Eastern regions were used for greenhouse experiment at IITA, Nairobi, with soybean variety Nyala and SB19 under inoculation with two commercial products; Biofix and Legumefix and a non-inoculated control. At mid podding, numbers of pods, nodules fresh weight, nodule effectiveness, shoot and root dry weight, nodule occupancy, nitrogen (N) and phosphorus (P) uptake and N fixation were determined. For objective three, two acidic soils were used in the laboratory, with three lime levels (agricultural lime, MPR and without-lime treatment), two lime requirement methods (Shoe-Maker, McLean and Pratt (SMP) and exchangeable acidity) and the treatments incubated with lime for 12 weeks in the laboratory. Soil pH was determined after every 2 weeks and on the 12th week of incubation, soil available P was determined. For objective four, two acidic soils were limed with agricultural lime and soybean varieties SB19 and Nyala inoculated with Biofix and Legumefix and data collection was same as in the 1st objective. The experiments were in a completely randomized design (CRD). In the first greenhouse experiment, soils with pH range 5.5-5.9 had the highest measured growth parameters. Inoculation of soybean in soil S7 had highest amount of nitrogen uptake (1.36g N plant^{-1}) and nitrogen fixation (43.03%). In the soil-lime incubation study, liming with agricultural lime resulted in increased soil pH6.0 while liming MPR resulted increase of soil pH to 5.8. Lime rate estimation by SMP methods had significance influence in raising soil pH $p<0.05$ compared to exchangeable acidity. Co-application of lime and inoculation increased nodule fresh weight (5.8g plant^{-1} in Kuresoi soil) and nitrogen fixation (58.3% in Murang’a soil). Co-application of lime and inoculation has potential to increasing soybean nodulation and BNF and hence yield increase.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ARDRA	Amplified Ribosomal Deoxyribonucleic Acid Restriction Analysis
ASAL	Arid and Semi-Arid Lands
BNF	Biological Nitrogen Fixation
CCE	Calcium Carbonate Equivalent
CFU	Colony Forming Unit
DGGE	Denaturing Gradient Gel Electrophoresis
IGS	Intergenic Spacer region
IITA	International Institute of Tropical Agriculture
KEPHIS	Kenya Plant Health Inspectorate Services
MPN	Most Probable Number
MPR	Mijingu Phosphate Rock
MT	Metric Tons
NDFA	Nitrogen derived from atmosphere
PCR	Polymerase chain reaction
rDNA	Ribosomal Deoxyribonucleic Acid
RFLP	Restriction Fragment Length Polymorphism
rRNA	Ribosomal Ribonucleic Acid
SSA	sub-saharan Africa
TGGE	Temperature Gradient Gel Electrophoresis
TGx	Tropical Glycine Crosses
YEMA	Yeast Extract Mannitol Agar

CHAPTER ONE
INTRODUCTION

1.1 Background information

Soybean (*Glycine max*) is a legume of tropical to subtropical origin and an important source of food and income (Maingi *et al*., 2006). Soybean production in sub-Sahara Africa (SSA) remains low compared to South America and USA. Biological nitrogen fixation (BNF) is the major source of nitrogen in soybean (Staton, 2011). Nitrogen fixation by soybean is affected by factors including the population, presence and effectiveness of rhizobia present in soil, soil physiochemical characteristics, amount of nitrogen in soil, plant- soil interaction and soybean genotype (Mathenge, 2017). Rhizobia are not commonly present in soils and those present are not always highly effective, thus it is often necessary to inoculate legumes to ensure effective nodulation and nitrogen fixation (Thilakarathna *et al*., 2018). Soil acidity affects rhizobia effectiveness and in turn, nodulation and nitrogen fixation are impaired. Acidic soils have a high concentration of hydrogen (H^+), aluminum (Al^{3+}), iron (Fe^{3+}) and manganese (Mn^{2+}) and low amounts of calcium (Ca), magnesium (Mg), molybdenum (Mo), and available phosphorus (P). Soybean production regions in Kenya include Western, Nyanza, Rift valley as well as Central and Eastern Provinces (Infonet Biovision, 2018). These regions receive adequate rainfall, with well-drained soils; however, they are highly affected by soil acidity (Kisinyo *et al.,* 2014). Soil acidity is a major constraint to food production in tropical and subtropical regions. Soil acidity limits soybean nodulation and yields thus poor soybean production levels (Muleta *et al.,* 2017). High concentration of Al, H, Fe and Mn constrains legume root-nodule formation, functioning, and subsequently grain production and *Rhizobium*-plant association (Yakubu *et al*.,2010).

Soil pH has shown a strong correlation with changes in microbial communities particularly bacterial communities (Lauber *et al*., 2009) while soybean is considered a crop sensitive to pH and perform well in soils of pH between 6.00-6.50 (Infonet Biovision, 2018). Soil liming to correct acidity is necessary to make nutrients available to the crop and provision of favorable conditions for microbial functioning. Lime estimation is crucial in ameliorating soil acidity; various methods have been tested to estimate liming rates including exchangeable acidity, Shoe-maker, McLean and Pratt method (SMP), soil-lime incubation method and Mehlich 3 method (Tunney *et al.,* 2010). Soil liming should be cost efficient thus liming method should not underestimate or overestimate lime application. The limitation to lime use in Kenya includes cost, availability in the markets, intensive hand application method by small-scale farmers and lack of knowledge on the benefits of liming (Kisinyo *et al.*, 2014).

1.2 Statement of the Problem

Soil is the most valuable and widespread natural resource that supports agricultural based livelihoods. Soil fertility in smallholder farmers is declining due to lack of replenishment of lost nutrients. Acidic soils cover about 13% (7.5 million Ha) of Kenyan arable land (Kisinyo *et al.*, 2014). Soil acidity limits crop production regardless of the application of fertilizer. Soybean production in Kenya ranges between 5000-10000 tons (T) year^{-1} which is lower than the industrial demand of more than 120,000 T year^{-1}. Soybean growing areas in Kenya produce an average yield of 0.8 T Ha^{-1}which is lower than the potential of 3.0-3.5 T Ha^{-1} (Infonet Biovision, 2018). Major soybean production regions face soil acidity limiting productivity. Soil acidity affects not only the development of rhizobia and nodule formation but also the growth and uptake of nitrogen by plants. Nutrients availability including nitrogen, phosphorus, potassium, sulfur, calcium, magnesium, and molybdenum is highly dependent on soil pH and their uptake by soybean is impaired at low soil pH levels. The use of inoculants can be effective only when applied at the optimal soil pH. Soybean inoculation is necessary in soils with low rhizobia population. In Kenya, the use of inoculants is not common among farmers, leading to poor nodulation, nitrogen fixation, and hence low yield. Soybean inoculation in some regions in Kenya has not been effective in increasing yields and this is attributed to soil acidity as one of the limiting factors (Kihanda & Gachingiri, 2013). Major areas of high agricultural potential including Rift valley and highlands of Rift Valley face soil acidity problem (pH< 5.5) limiting crop production (Kisinyo *et al.,* 2014). Soybean as well as rhizobia inoculants are all sensitive to low soil pH (<5.5). Soil liming is one of the methods of ameliorating soil acidity increasing nutrients availability to plants. Studies done in soil acidity-affected regions on the use of lime to improve soybean yields including the Central parts of Kenya, Eastern and Western regions have proven to be effective. However, a suitable lime requirement method for Kenyan acidic soils that will ensure accurate application is not established. This study will provide insight for soil liming to an optimal pH and provide small-scale farmers with a cost-effective liming material and in turn, increasing their yields. The determination of optimal soil pH for use of rhizobia inoculants in soybean is crucial in ensuring effective nodulation and BNF.

1.3 Objectives

1.3.1 Broad objective

To contribute to increasing soybean yields in Kenya through soybean inoculation and liming of acidic soils.

1.3.2 Specific objectives

i. To determine optimal soil pH range for effective nodulation and BNF of soybean varieties TGx1740-2F (SB19) and Nyala inoculated with Biofix and Legumefix.
ii. To determine nodulation and BNF in soils of different fertility using soybean varieties SB19 and Nyala inoculated with Biofix and Legumefix.
iii. To evaluate the effectiveness of agricultural lime and Mijingu phosphate rock (MPR) in raising soil pH.
iv. To assess the effects of liming on BNF and nodulation soybean varieties inoculated with Biofix and Legumefix.

1.4 Hypotheses

This study tested the null hypotheses that:

i. Soil pH does not affect nodulation and BNF of inoculated SB19 and Nyala soybean varieties.
ii. Soil fertility has no effect on nodulation and BNF of SB19 and Nyala under inoculation.
iii. Agricultural lime and MPR are not effective in raising soil pH.
iv. Soil liming does not have an impact on nodulation and BNF on soybean under inoculation.

1.5 Justification of the Study

Soybean is an important food legume in sub-Saharan Africa due to its high nutritive value. In Kenya, mainly small-scale farmers produce soybean and the production levels are low. Potential areas of soybean production in Kenya have acidity problem thus contributing to low yield, this leads to high soybean importation to meet the demand. Nutrients deficiency caused by acidic soils affects not only the crop but also the rhizobia inoculants. This leads to poor nodulation, low nitrogen fixation and in turn reduces yields. Rhizobia inoculants are sensitive to acidic and their effectiveness is impaired at low pH levels. Soil liming is one of the amelioration ways of reducing soil acidity effects on crop through reduction of Al, H, Mn and Fe toxicity and increasing the availability of nutrients to crops. However, lime application needs to be to the optimal range in which it will favor crop and inoculant used. There is little research and information on optimal range for soybean and rhizobia inoculants. This study will provide directions in the use of rhizobia inoculants for soybean production under variable pH conditions. It will therefore provide a guide for the most optimal soil pH range for soybean inoculation to improve yields. The findings and recommendations of this research work will also be beneficial to the national government and development partners in making decision regarding the applications of soil amendments to improve and increase yields in soybean.

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CHAPTER TWO
LITERATURE REVIEW

2.1 Soybean production in Kenya

Soybean (*Glycine max*) is a legume of tropical to subtropical origin and is a multipurpose crop ranked as number two in oil production in Kenya (Mahasi *et al*., 2011). Soybean grows in areas where maize and common beans are mainly grown. It grows to a height of 60-120cm and matures in 3 to 6 months depending on the variety, climate, and location. Depending on the variety, the crop can grow up to 2200m above sea level (Mathu *et al*., 2009) and under rainfall ranging from 300 to 1200mm.

Major soybean producing regions in Kenya include Western, Rift Valley, Eastern, Nyanza and Central (Table 2.1) in over about 2500 ha (Chianu *et al.,* 2008). The Western region is the leading soybean production region accounting for about 50% of total national production. Small-scale farmers are major producers of soybean in Kenya, and the production levels are lower than the demand leading to importation.

Table 2.1: Major soybean production regions in Kenya

Province	**Districts/ County**
Western	Busia, Bungoma, Teso, Butere/Mumias, Kakamega, Mount Elgon, Lugari, Vihiga
Rift valley	Nakuru, Nandi, Trans Nzoia, Koibatek, Narok, Trans, Mara, Laikipia, Bomet
Eastern	Meru, Embu, Mbeere, Machakos
Nyanza	Rachuonyo, Homabay, Gucha, Kisii, Nyamira, Siaya
Central	Kirinyaga, Murang'a, Maragwa, Nyeri

Source: Chianu *et al*. (2008)

Soybean varieties introduced in Kenya by 2009 include Blackhawk, EAI 3600, Nyala, Gazelle (Mahasi *et al*., 2011) released and registered by Kenya Plant Health Inspectorate Services (KEPHIS), having a yield potential of up to 2.0 T Ha^{-1}. Nyala is an early maturing variety with a yield potential of 0.7 T Ha^{-1} (Tropical Legume II, 2013).

The International Institute of Tropical Agriculture (IITA) introduced promiscuous soybean varieties, Tropical Glycine crosses (TGx) series and in 2010 TGx 1740-2F (SB 19), TGx 1895-33F (SB 8) were introduced in Kenya and have been tested and found to have a high ability to fix nitrogen (Mahasi *et al*., 2011). The variety SB19 is a medium maturity, and has been found to best for mono-cropping with yields potential of about 2.7 T Ha^{-1} (although yields of 4 T Ha^{-1} have been recorded), while SB 8 best for intercropping with high grain yield of 2.5 T Ha^{-1} and biomass accumulation (Tropical Legume II, 2013).

Recently, IITA introduced two new TGx varieties i.e. TGx 1988-5F which is an early maturing variety (90-100 days after sowing) with promiscuous nodulation and high resistance to disease (IITA, 2015), and TGx 1989-19F which is medium maturing (101-110 days after sowing). The two varieties were released in 2014 and trial tests have shown that they have potential yields of about 2.5 T Ha^{-1}.

2.2 Inoculants use in Kenya

Nitrogen is a major limiting plant nutrient in sub-Saharan Africa (SSA), resulting in low yields in crop production. Inorganic N fertilizers such as Di-Ammonium Phosphate (DAP) and Urea frequently used to supply the needed nitrogen to the farming system in SSA. The presence of *Bradyrhizobium* in the roots of soybean enables the crop to fix nitrogen in the soil thus making nitrogen available to soybean. The soil factors affecting inoculation include excessive soil moisture, drought, soil acidity, P deficiency, excess mineral N and deficiency of micronutrients such as molybdenum, cobalt and boron (Muleta *et al.,* 2017). When *Bradyrhizobium* population is low or no soybean has ever grown in the field, this will necessitate the use of inoculants containing nitrogen-fixing bacteria. In a study conducted in Kenya (Lesueur *et al*., 2012) it found, that the application of *Rhizobium* inoculants significantly increased the soybean yields in all tested areas (about 75% of the farms).

The limitation in the use of commercial inoculants in Kenya includes lack of access by the small-scale farmers, limited production in Kenya and insufficient distribution network (Mutuma *et al.,* 2014). Biofix is a peat-based inoculant, produced by MEA Ltd and commercialized in Kenya. It contains industrial standard *Bradyrhizobium diazoefficiences* strain USDA 110. Biofix is widely adopted but has shown low efficacy thus posing a limitation to its use (Mungai & Karubiu, 2011). Low inoculants efficacy is associated with high population of indigenous rhizobia, which outcompete the introduced rhizobia. Unfavorable soil conditions will also influence the efficacy of the inoculant; this includes soil pH of below 5.0.

Legumefix is a rhizobia inoculant manufactured by Legume Technologies in the United Kingdom. it contains *Bradyrhizobium japonicum* stain 532C. Research on Legumefix in Kenya has found it to boost the natural population of beneficial nitrogen-fixing bacteria, hence effective in increasing nodulation and BNF (Mburu *et al.*, 2011). Legumefix also increases nodule fresh weight and yields. Commercial inoculants increase nodulation, dry matter yield, and grain yield hence necessary for increasing soybean yields (Ulzen *et al.,* 2016)

2.3 Methods for Determination of biological nitrogen fixation

The determination of biological nitrogen fixation (BNF) can be done by measuring parameters including dry matter yield, nodule index based on nodule color, nodule size, nodule number, and weight (Prevost & Antoun, 2008), nodule fresh weight, shoot and root dry weights. A well-nodulated soybean will have 8-10 nodules of about 2-4 mm in size. The color should also be red-pink due to the presence of leghemoglobin (Staton, 2012).

The N- difference method is another method for N fixation and is widely used when total N analysis is available. The N fixed is estimated by the difference between the total amount of N fixed by soybean and non-fixing plant both grown under same conditions (Prevost & Antoun, 2008; FAO, 1993). The main assumption with this method is that both plants assimilate the same amount of soil nitrogen; it is, however, limited in that plants differ in their root morphologies (Prevost & Antoun, 2008).

Acetylene reducing assay method also determines BNF and the method is on the principle that rhizobia cells produce nitrogenase, which is an enzyme that reduces nitrogen to ammonia and other compounds like ethylene. The roots, nodules, and even plant parts incubated with 10% acetylene and the ethylene gas produced measured by gas chromatography. It is a sensitive method and the total nitrogen not measured due to nitrogenase activity (Roger and Ladha, 1990).

Another method for BNF determination is the ^{15}N dilution method where the fixing legume plant is labeled with a ^{15}N inorganic or organic fertilizer. The principle is that the fixing legume will assimilate both the ^{15}N labeled as well as the atmospheric nitrogen (Prevost and Antoun, 2008; FAO, 1993). The ^{15}N natural abundance method involves no addition of labeled N fertilizer in the soil. During nitrogen uptake in the soil, ^{14}N is lost to atmosphere thus causing an increase in ^{15}N:

^{14}N ratio in soil than in the atmosphere (Prevost and Antoun, 2008). Both methods involve the use of a reference crop that does not fix nitrogen. Although they are accurate methods for BNF determination, they are however expensive. The N difference method is a cheap method of determining BNF, thus combined with nodule index to give a good estimation of BNF.

2.4 Methods for Determination *Bradyrhizobium* population

Soil microbial population is determined using various methods including biochemical, physiological and molecular methods. Various techniques have developed to quantify the population of *Bradyrhizobia* in the soil. This includes plate counts which is a simple and rapid method, it involves incubation of soil with a nutrient medium yeast extract mannitol agar (YEMA) at several serial dilution and determination of the colony forming units (CFU) to evaluate the population of the indigenous *Bradyrhizobia* population (Germida & Freitas, 2008). Gram staining indicates if the rhizobia are gram negative or gram positive (Somasegaran & Hoben, 1985).

Another technique of enumeration is the most probable number (MPN) method, which relies on the presence or absence of microorganisms in the samples of several series of dilution (Prevost & Antoun, 2008). The MPN method is applicable on soil capable of nodule formation to determine the population of indigenous rhizobia responsible for nodulation (Germida & Freitas, 2008; FAO, 1993). However, plate technique and MPN are disadvantaged in the choice of appropriate media and adsorption of microbes to pipette walls.

Direct methods of studying population of *Bradyrhizobia* include the use of molecular methods. Molecular methods used includes nucleic acid reassociation and hybridization, DNA microarrays, polymerase chain reaction (PCR) based approaches including restriction fragment length polymorphism (RFLP), denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE) and amplified ribosomal DNA restriction analysis (ARDRA) (Prevost and Antoun, 2008). The PCR-RFLP method is widely used for determination of rhizobia diversity and by sequencing of the 16S rRNA gene for identification of rhizobia species and the 16S-23S rDNA intergenic spacer (IGS) which allows the differentiation of strains within the same species (Sikora & Sulejman, 2003). Molecular methods are advantageous over traditional methods in that they allow identification of the cultivatable and non–cultivatable microorganism (Prevost & Antoun, 2008).

2.5 Soil suitability for soybean production

Kenya has about 4.5 million ha as arable land out of about 60 million ha of the land in the country. The Arid and Semi-Arid Lands (ASAL) cover about 80% of the land and about 13% of the land has acidity problem (Kanyanjua *et al.*, 2002). The arable lands in the country face acidification and aluminum toxicity while the ASAL have salinity and sodicity problems (Matolo *et al*., 2000). The medium to high rainfall potential regions are most affected by soil acidity with the East of Rift valley and Western region of the country being most affected. These conditions make agricultural productivity a challenge in these regions. Soybean is produced in a broad range of well-drained soil with the medium-textured soils (loam) being ideal for soybean than heavy clay soil or sandy/gravy soils (Upfold & Olechowski, 2000).

Soil pH in the regions with high agricultural potential ranges from extreme acidity to slightly alkaline with Western regions having a range 4.20- 7.42, Rift valley 5.00-7.80, Kisumu 4.1-8.14, Eastern 4.3-7.74 and central 4.1-8.1 (NAAIAP & KARI, 2014). Soils with pH below 6.00 limit agricultural production and especially soybean, which is sensitive to soil pH.

2.6 Impact of soil pH on soybean nodulation and BNF

Soil acidity constrains symbiotic nitrogen fixation in both tropical and temperate soils by limiting rhizobial survival and reducing nodulation. Phosphorus is highly dependent on soil pH, and in acidic soils, P usually fixed by Al, Mn or Fe, thus unavailable to plants hence limiting nodulation. Aluminium is soluble at low soil pH, and the more the soil is acidic the more soluble aluminum becomes hence causing aluminum toxicity in the soils. The presence of available aluminum in acid soils will inhibit nodulation directly and indirectly by stunting root growth and tends to compound the effects of low-level calcium by inhibiting its uptake. Soils of pH below 6.0 have low molybdenum (Mo) availability (Erker & Brick, 2014), as Mo solubility and availability is pH dependent. Molybdenum is an important micronutrient in nitrogen fixation since it is an essential component of one of the two proteins, which together form nitrogenase (Eaglesham & Ayanaba, 2005). Soil acidity affects the survival of soil microorganism, with fast-growing rhizobia being more sensitive to soil acidity than slow-growing Rhizobia including *Bradyrhizobium*.

The management of soil pH is important when high yields and profitable soybeans are expected. Soybeans mostly perform well at a soil pH of between 6.0 and 7.0 (Staton, 2012). At the optimal pH, there is maximum nutrient availability like nitrogen, potassium, phosphorus, magnesium, and calcium. Factors such as unfavorable soil pH, mineral toxicity, and nutrient deficiency and plant diseases limit inoculants strains due to lack of expression their full capacity for nitrogen fixation regardless of strains' competitiveness (Hassen *et al*., 2014).

A study conducted in Kenya (Lesueur *et al*., 2012) on soybean, concluded that soil factors such as pH, P, C, N can affect the inoculants efficiency whether the strain is occupying the nodules or not. The study also reported that soil pH significantly affects nodulation and yield, although this effect varies from regions to region. A study also conducted to assess the effects of varying soil pH on soybean production in several soil types across the state of Madison (Wisconsin) (Peters *et al*., 2004), reported that soil liming to pH of between 5.5 and 6.6 resulted in optimum yields and at low soil pH (< 5.0) there was significant reduction in soybean yields. Soybean will show limited nodulation when soils are strongly acidic (5.2-5.4) (Hassen *et al*., 2014). Legumes usually fail to nodulate under acidic soil conditions leading to impaired symbiotic efficiency and reduced yields. The determination of optimum soil pH range for inoculation is important and when found to be outside the range, inoculation should not be done unless soil pH amendment has been considered.

2.7 Soil pH amendment

Liming is an important practice to achieve optimum yields on crops grown in acidic soils. Soil liming will result in the increase of soil pH, base saturation, Ca, and Mg content and a reduction in Al concentration. It also enhances microbial activities in the rhizosphere, improving root growth thus increase in nitrogen fixation. The efficiency and quality of liming material depend on the neutralizing value of the material expressed as calcium carbonate equivalent (CCE), particle size distribution, and initial soil pH, clay content of the soil and buffering capacity of the soil. Methods, frequency, depth, and time of application are important factors on lime efficiency. Various methods can used to apply lime including spot application, band application, and broadcasting. Broadcasting is the most recommended method of lime application due to its effectiveness.

Lime requirement is the amount of a basic material needed to raise soil pH to the desired level. Accurate and rapid methods to determine the amount of liming material needed is an essential step in soil liming. Lime requirement is affected by factors such nature of acidity, neutralizing sequence (initial and desired pH), soil properties including parent material and texture and organic matter content (Thomas, 1996). Methods for lime requirement determination include soil-lime incubation, soil-base titration, soil buffer equilibrations, and exchangeable Al (Edmeades *et al*., 2012). For accuracy in lime requirement methods, different lime requirement test will give the recommendation based on a specific geographic region. The buffer equilibrations methods include Shoemaker-McLean-Pratt (SMP) single buffer method, Adams and Evans single buffer methods, and Mehlich single buffer method. The SMP buffer method is quick, cost efficient and has a good reproducibility, however, the chemicals it uses including p-nitrophenol and chromate ions are hazardous thus posing a health risk to the user. Sikora buffer developed in the USA to eliminate the use of p-nitrophenol and chromate in SMP. Brazil has been able to come with a new buffer suitable for tropic and subtropical soil Maria Santa (TSM) buffer that mimics the SMP uses some of SMP chemicals and Sikora chemicals (Maria *et al*., 2010).

2.8 Lime use in Kenya

Agricultural lime has been used in several studies in Kenya, and proven to be an effective liming material. A study in Uasin Gishu showed that the use of agricultural lime with MPR increases soybean and maize yields (Nekesa *et al*., 2008). Mijingu phosphate rock (MPR) has been widely used as a source of P and liming material. The addition of rock phosphate in soils increases P availability, Ca and Mg thus leading to a reduction in soil acidity (Anetor & Akinkunmi, 2007). Use of Mijingu phosphate rock (MPR) as a liming material resulted in high soybean yield in western Kenya (Nekesa *et al*., 2008). Application of MPR at a rate of 60 kg P ha^{-1} resulted in the increase in soil available P and maize grain yields in Western Kenya.

There is no documented study of the use of wood ash as a liming material in Kenya. However, use of wood ash as an alternative liming material in Canada, has proven to be an effective liming material as its mode of action attributed to being same as that for agricultural lime (Lickacz, 2002). Calcium carbonate equivalent (CCE) of most wood ash ranges between 10-90% (Mbah & Deborah, 2010). Wood ash improves the availability of nutrients C, P, K, Zn, Mn, Ca, and B. Use of wood ash on pea and barley lead to increase in yield (Arshap *et al*., 2012). Increase in yield of barley and mixed forage was observed in using wood ash as a liming material than use of agricultural lime (Lickacz, 2002).Wood ash when applied at same rate as agricultural lime improves on soil chemical and physical properties thus increasing crop production (Mbah & Deborah, 2010).

MEA Ltd has produced a new lime material called physiolith, mainly contains 36% CaO and 2.5% MgO and has a recommended rate of application of 100kg/acre. No documented research on the effectiveness of this product has been published in its use has a lime material.

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CHAPTER THREE

EFFECTS OF SOIL ACIDITY ON NODULATION AND BIOLOGICAL NITROGEN FIXATION OF SOYBEAN (GLYCINE MAX)

3.1 Abstract

Kenya arable lands face acidity problems limiting soybean production. Soil pH is one of the limiting factors to effective nodulation and biological nitrogen fixation (BNF) causing low soybean yields. A greenhouse study was conducted at the International Institute of tropical agriculture (IITA) in Nairobi, to evaluate the effect of soil acidity on soybean nodulation and BNF when inoculated with Biofix and Legumefix using soybean varieties Nyala and TGx1740-2F (SB19). Ten soils with a pH range of 4.3-6.3 (labeled S1-S10) obtained from Central Kenya (Murang'a), Central Rift Valley (Kuresoi) and Lower Eastern regions (Kitui) were used for this study. The soils were analyzed for physical and chemical characteristics and amended using nutrient solutions, and packed in 2 kg capacity pots maintained at field capacity. Soils S5-S8 (pH range 5.5-5.9) had the highest measured growth parameters. Inoculation of extreme acidic soil (pH <5.5) had no significant influence on nodulation and BNF. The interaction of soil × variety × inoculation was significant for the number of pods ($p<0.05$), root biomass ($p<0.05$), nodules effectiveness ($p<0.0001$), nitrogen (N) uptake ($p<0.05$) and nitrogen derived from the atmosphere ($p<0.05$). Soybean variety SB19 inoculated with Biofix in soil S5 (pH 5.6) had the highest number of pods (89 pods plant^{-1}) and nodules effectiveness (85.8%). Highest nodules fresh weight was in soil S6 in soybean inoculated with Legumefix (11.37 g plant^{-1}). Soil S7 (pH 5.8) planted with SB19 and inoculated with Biofix had the highest N uptake (1.368 g plant^{-1}) and Ndfa (43.09%). The results suggest that at soil pH 5.6-6.0, inoculation of soybean increased BNF and nodulation, hence a high number of pods that may translate to high grain yield. Soybean varieties differ in terms of nodulation, biomass, and nitrogen fixation. Inoculants effectiveness highly depends on the soybean variety inoculated and soil fertility level. In addition, there is need to lime acidic soils to soil pH 5.5-6.0 to improve soybean nodulation and BNF.

Keywords: Biological nitrogen fixation, effective nodulation, nodule effectiveness, soil pH

3.2 Introduction

Soil pH plays a major role in influencing nutrients availability for plant growth. Soil fertility has been declining over years due to lack of sufficient replenishment of lost nutrients. Soils nutrients including nitrogen (N) and phosphorus (P) are most liming to crop production. Nitrogen demands are meet by use of mineral nitrogen fertilizers, which pose a high cost in crop production and causes environmental pollution. An alternative source of N for legumes is through biological nitrogen fixation (BNF). Inoculation of legumes such as soybean results in increase of BNF and yield (Zarei *et al.,* 2012). The use of effective high quality rhizobium inoculants in Kenya has resulted in increased soybean yields (Lesueur *et al.,* 2012). Soil pH is one of the liming factors to symbiotic efficiency between host legume and rhizobium (Cooper & Schere, 2012).

Soybean grows well at pH 5.6-6.8 where there is maximum nutrients availability. Acidic soils are usually deficient in P, Mg, Ca, Mo and K with a high concentration of Fe, Al, H, Cu and Mn ions (Osundwa *et al.,* 2013). In Kenya, acidic soils occur in high rainfall areas, including highlands of east Rift valley and it occurs in about 13% of Kenya land area (Kanyanjua *et al.,* 2002). Soybean production in Kenya is low and this could be due to the sensitivity of the crop to soil acidity (or low pH). Soil pH below 5.2 and above 6.8 does not favor soybean growth hence poor yields at these pH ranges. High levels of aluminum and low levels of phosphorus in acidic soils affect the growth of symbiotic nitrogen-fixing bacteria (Nisa *et al.,* 2012). Most acidic soils will require re-inoculation after harvesting due to poor survival of rhizobia in the soil. Soils of pH <5.5 causes nodulation failure in terms of formation and functioning leading to impaired symbiotic efficiency and reduced yield (Ferguson *et al.,* 2013).

Acidic soils face reduced organic matter breakdown, nutrient cycling by microorganisms, reduced uptake of nutrients by plant roots and inhibition of root growth (Fageria *et al*., 2013). Soybean require a high amount of nutrients, with P and K being most crucial for optimal production (Sikka *et al*., 2012). Acidic soils have high concentration of Al and Fe ions in solution and these causes P sortion making it unavailable for plant use (Osundwa *et al.,* 2013). Soil fertility has significant influence on rhizobial efficiency and soils with different fertility status respond differently to inoculation (Korir, 2016). Rhizobium in culture media has shown a critical range of pH 4.0-6.0 (Goncalves *et al*., 2000) and *Bradyrhizobium japonicum* is tolerant to low pH compared to other fast-growing *Rhizobium* (Nisa *et al*., 2012). This study investigated the impact of soil pH on soybean nodulation and BNF on soils that differ in fertility.

3.3 Material and methods

3.3.1 Soil collection sites

The soils for the greenhouse study were obtained from Nakuru County (Kuresoi and Mauche), Murang'a (Kangema), and Kitui (Kyangwithya East). Kuresoi is located at about 0.2993°S longitude and 35.5302°E latitude while Mauche occurs at Mauche 0.3316°S longitude and 35.9449°E latitude, at an elevation of 1480-1550m (Figure 3.1). The mean annual temperature is 18.1°C and an average annual rainfall of 1200-1900mm (MoALF, 2016). The soils from Kuresoi are humic Andosols while soils from Mauche are mollic Andosols. These soils are well drained, deep to very deep, dark brown, friable and smeary, with acid humic topsoil. Kuresoi soils are sandy clay to clay and Mauche soils are silty clay-to-clay (Jaetzold *et al.*, 2006).

Kangema (Murang'a) lies at 0.7957°S longitude and 37.1327°E latitude, at an elevation of 2150m above sea level (Figure 3.1). The area has a mean annual temprature of 17.6°C and average annual rainfall 1980mm (Climate-Data.org, 2016). These region occurs in upper midland zones i.e coffe tea zones and the soils are humic Nitosols, well drained, extemely deep, dusky red to dark reddish brown, friable clay, with acid humic topsoil (Jaetzold *et al.*, 2006).

Kyangwithya (Kitui) lies at 1.3751°S longitude and 37.952°E latitude and occurs at an elevation of 1700m above sea level in the lower midlands i.e. marginal cotton zone (Figure 3.1). The area has a mean annual temperature of 21.4°C and an annual average rainfall of 1068 mm (Climate-Data.org, 2016). The soils in these regions are humic Cambisols, well-drained, dark reddish brown-to-brown, friable, rocky stony, sandy clay to clay with acidic humic topsoil (Jaetzold *et al.*, 2006).

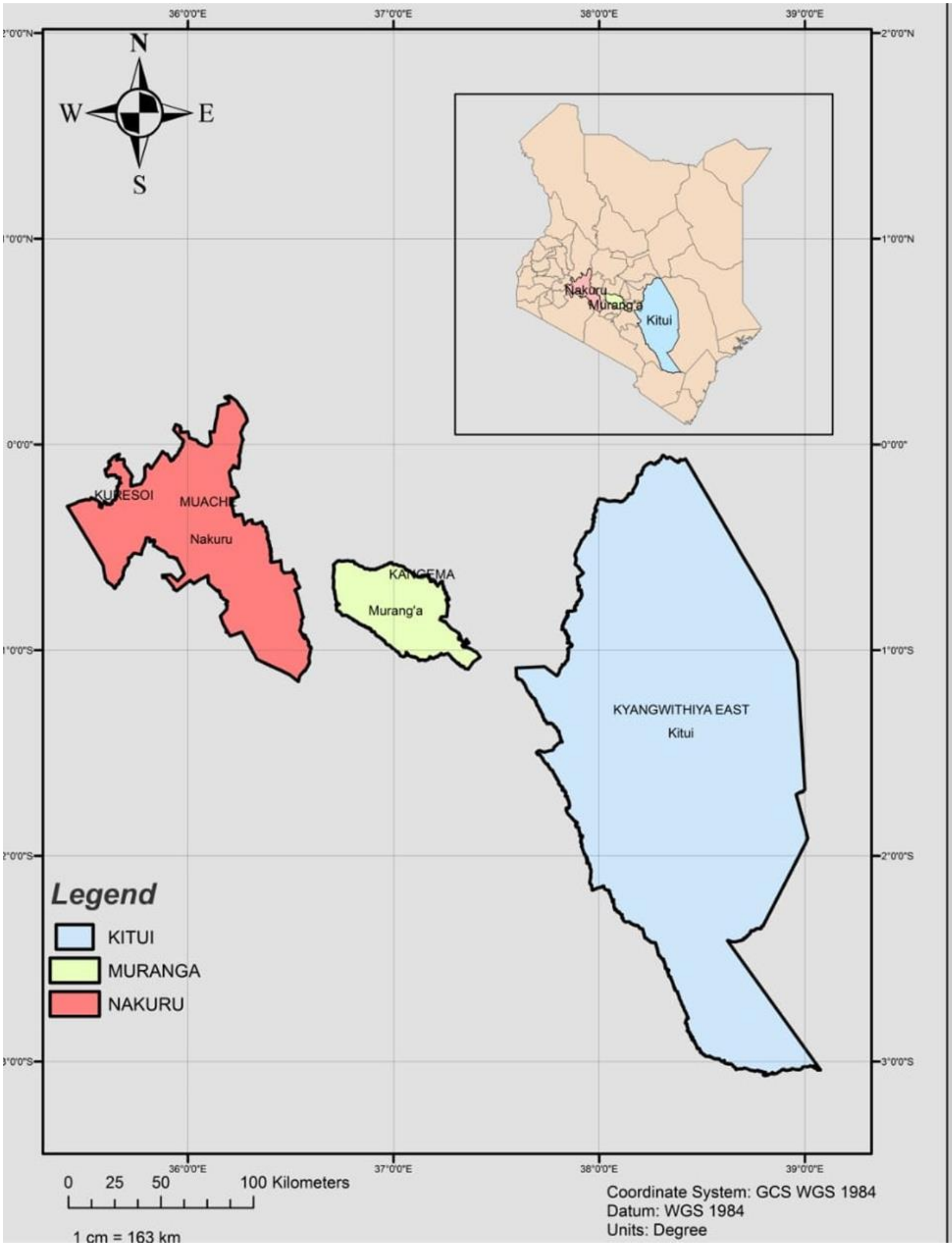

Figure 3.1: Map of Kenya showing soil-sampling sites. Source: (ArcGIS [GIS software], 2010)

3.3.2 Soil sampling

Soil collection was at a depth of 20cm and ten subsamples randomly collected per site using hoe for digging out soils. The soil samples from each site were homogenized and the composite samples obtained put in 50kg capacity sack and transferred to the greenhouse at IITA Nairobi. The samples were then air-dried for 48 hours and sieved through a 2mm sieve and a subsample (50g) used in physical and chemical analyses of the soils.

3.3.3 Experimental treatments

The experiment was set in a greenhouse with the soils obtained from Murang'a, Kuresoi and Kitui. Treatments were; inoculation with Legumefix-soybean, Biofix-soybean, and a negative control (no inoculation) with two soybean varieties TGx1740-2F (SB19) and Nyala and the experiment laid in a completely randomized design (CRD) in factorial arrangement. Soybean variety SB19 is promiscuous and medium maturing with high biomass yield while Nyala is early maturing, non-promiscuous and does well as an intercrop (ICRISAT, 2013). The two varieties differ in their N fixation and pod formation (Thuita *et al.,* 2011), hence yield differences. The commercial inoculants used differ in strain content; Legumefix manufactured from UK (Legume Technology LTD-UK) contains strain 532C and Biofix manufactured by MEA LTD Kenya, contains strain USDA110. Biofix is a widely used inoculant due to its availability. Recent studies indicate the effectiveness of Legumefix inoculants in Kenyan soil (Thuita *et al.,* 2011).

3.3.4 Determination of soil physical and chemical properties

The soil samples collected were analysed for physical and chemical characteristics following procedures described in Okalebo *et al* (2002). Soil pH- water was determined using glass electrode pH meter in a 1: 2.5 soil/water ratio while Exchangeable acidity was analyzed using buffered neutral salts (KCl). The soil particle size analysis was determined using the hydrometer method and the amount of soil total nitrogen was determined using Kjeldahl method. Soil organic carbon was determined using the Walkley-black method (sulfuric acid– dichromate digestion followed by back titration with ferrous ammonium sulfate). Available P, exchangeable bases (Ca, Mg and K) and micronutrient were extracted using Mehlich 3 method and available P was then determined using ammonium vanadate method and amount determined using a spectrophotometer. The amount of extracted exchangeable bases was determined using atomic absorption (emission for K) spectrophotometry after extraction with ammonium acetate (pH 7.0). Micronutrients Cu, Mn and Zn were measured using atomic absorption.

3.3.5 Soybean Inoculation and Planting

Plastic containers of 2kg capacity filled with the soil of different pH to a bulk density of $1kgm^{-3}$ were used for planting soybean. Basal nutrients application containing 300 mg P, 0.06 mg Cu, 0.2 mg Zn, 0.04 mg B and 0.008 mg Mo pot^{-1} (Somasegaran & Hoben, 1994) was applied 2 days prior to planting. The soils were maintained at 80% field capacity and reference crop pots. The nutrient solution did not include any source of Ca, Mg, or N. Seeds were surface sterilized with sodium hypochlorite for 1 minute and rinsed 5 times with sterilized distilled water. Planting of the negative control (uninoculated) was first to avoid any potential contamination. Inoculation was at a rate of 1g of inoculant/100g seeds following instruction on each pack of the inoculants and three seeds planted per pot, and later thinned to one plant per pot on 10^{th} day after planting. Sorghum was planted as a reference crop for BNF estimation.

Table 3.1: Treatments outline for determination of optimal soil pH for effective nodulation and BNF in soils of different fertility

Factors	Levels	Description
Soil	10	Soils of different pH (4.3, 4.6, 4.7, 4.8, 5.6, 5.7, 5.8, 5.9, 6.2 and 6.3)
Inoculation	3	Inoculation with Biofix or Legumefix and uninoculated (control)
Varieties	2	TGx1740-2F (SB 19) and Nyala

Number of pots (10×3×2×3) =180 pots.

Sorghum for BNF estimation (10 soils × 3replicates) = 30 pots

Total number of pots 180+30=210 pots

3.4 Data collection

3.4.1 Number of pods and nodulation

Plant harvesting was done on 10^{th} week after planting and the number of pods counted from each plant and recorded. The shoot was cut from the pots and soil was removed from roots and the nodules separated from roots by washing in running water (Somasegaran & Hoben, 1985), obtaining the fresh nodules and their weight recorded for each pot. After nodule weight determination, the nodules from each plant were, subgrouped into two, one group was used determination for nodule effectiveness, and the other group stored in glycerol and used for nodule occupancy analysis. Nodule effectiveness was determined by cutting half of the counted nodules into two and observed for color. A pink-red color indicated nitrogen fixation, whitish or greenish nodule indicating nodule was not effective at fixing nitrogen (FAO, 1993). The percentage effectiveness of the nodules was determined as:

$$\% \text{ nodules effectiveness} = \frac{\text{number of pink nodules}}{\text{Total number of nodules}} \times 100$$

3.4.2 Biomass yield, nutrient uptake and biological nitrogen fixation

The shoots and roots were dried in the greenhouse before transferring to the oven for further drying at 70˚C for 24 hours and the dry weights were determined. Nitrogen fixation was determined using the N difference method. This was by procedure described by Unkovich *et al.*, (2008). The dry shoot of soybean and sorghum shoots were grinded and used to determine tissue N concentration using Kjeldahl method (Rutherford *et al*., 2008). The total N determined together with dry matter yield to give the amount of N_2 fixed (Mary *et al*., 1995).

$$\text{Total N uptake in plants} = \frac{\text{Dry matter weights} \times \%\text{N in plants}}{100}$$

$$\text{N fixed (Ndfa)} = \text{Total N uptake in legume} - \text{Total N uptake in reference crop}$$

$$\%\text{Ndfa} = \frac{\text{Total N uptake in legume} - \text{Total N uptake in reference crop} \times 100}{\text{Total N uptake in legume}}$$

3.4.3 Phosphorus uptake

The dried and grounded soybean shoots were digested with HNO_3 and 30% H_2O_2, then analyzed using inductively-coupled plasma atomic emission spectroscopy (ICP-AES) as described in Okalebo *et al.,* (2002).

3.4.4 Assessment of nodule occupancy

The assessment of nodule occupancy was by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to determine the composition and actual characteristic of microorganisms involved in nodulation. Two sites were chosen Mauche (S6) and Kitui (S9) to be representative based on their nodulation effectiveness (soil S6 had 85.5% and S9 had 85.2%). Eight nodules per plant were analyzed. Deoxyribonucleic acid (DNA) extraction from nodules was done by use of standard phenol-chloroform-isoamyl in presence of sodium acetate (Krosova-Wade *et al*., 2003). A 930–1100bp intergenic region between the 16S and 23S rDNA was amplified by PCR using rhizobia-specific primers derived from the 3′ end of the 16S rDNA (FGPS 1490-72; 5′-TGCGGCTGGATCCCCTC CTT-3′)(Navarro *et al.,* 1992) and from the 5′ end of the 23S rDNA (FGPL 132-38; 5′-CCGGGTTTCCCCATTCGG-3′)(Ponsonnet & Nesme, 1994).

The PCR amplification was carried out in a 25µl reaction volume containing 2µl of total DNA extract, 10pmol of each primer, and one freeze-dried bead (puReTaq Ready-To-Go PCR beads, GE Healthcare UK Ltd) containing 2.5U of Taq DNA polymerase, 200µM in 10mM Tris-HCl (pH 9 at room temperature) of each dNTP, 50mM KCl, and 1.5mM MgCl2. The PCR amplification was performed in a Bio-Rad iCycler™ thermal cycler adjusted to the following program: initial denaturation for 5min at 94°C, 35 cycles of denaturation (30s at 94°C), annealing (30s at 58°C) and extension (30s at 72°C) and a final extension (7min at 72°C). The PCR products were visualized by electrophoresis of 3µl of the amplified DNA on 2% horizontal agarose gel in TBE buffer (1.1% TrisHCl, 0.1% Na2EDTA·2H2O, and 0.55% boric acid), pre-stained with 0.033mg ml^{-1} of Ethidium Bromide. The gel was photographed under UV illumination with Gel Doc (BIO-RAD) Software (USA). Aliquots (10µl) of PCR products were digested with the restriction endonucleases MspI and HaeIII (5U) in a total volume of 15µl for 2h at 37°C. The restriction fragments were separated by horizontal electrophoresis in 1X TBE buffer with 3% agarose gel pre-stained with 0.033mg ml^{-1} of Ethidium Bromide. The gels were run at 100V for 3h and photographed under UV illumination with Gel Doc (BIO-RAD, USA) software. Strains with identical restriction fragment profiles (in individual fragment size and number) were classified into the same intergenic spacer (IGS) group.

3.5 Data analysis

All the data of the number of pods, nodulation, shoot and roots dry weight, nutrients uptake and BNF were subjected to Analysis of Variance (ANOVA) to evaluate the effect and interaction between soils, soybean varieties and inoculation on the measured parameters using the procedure glm of the SAS System (SAS Institute Inc, 2006). The effects of the different treatments were compared by computing their standard errors of the differences (SED); the significance of difference was evaluated at $P<0.05$. The model for the experiment was:

$$Y_{ijkl} = \mu + S_i + T_j + ST_{ij} + V_k + SV_{ik} + TV_{jk} + STV_{ijk} + \varepsilon_{ijkl}$$

Where;

Y_{ijkl} is observation due to the effect of i^{th} soil, with j^{th} inoculation and k^{th} variety

μ is the overall mean

S_i is the effect due to i^{th} soil

T_j is effect due to j^{th} inoculation

ST_{ij} is the interaction due to i^{th} soil and j^{th} inoculation

$V_k$is the effects due to k^{th} variety

SV_{ik} is the interaction due to i^{th} soil and k^{th} variety

TV_{jk} is interaction due to j^{th} inoculation and k^{th} variety

STV_{ijk} is the interaction due to i^{th} soil, j^{th} inoculation, and k^{th} variety

ε_{ijkl} is the random error term.

3.6 Results

3.6.1 Physical and Chemical properties of the study soil

The soils differed in fertility level (Table 3.2); soils from Murang'a (S1 with pH 4.3 and S2 with pH 4.8) and Kuresoi (S3 with pH 4.6 and S4 with pH 4.7) were strongly acidic. Mauche soils (S5-S8) were moderately acidic while Kitui soils (S9-S10) were slightly acidic. Available P ranged from medium to high (Okalebo *et al*., 2002) expect for soil S2 and S3 which had 6 mg kg^{-1} of soil available P. Basic cations in soils S3 and S4 were very low contributing to their low CEC. Although Soil S9 and S10 had moderate acidity, they however had low basic cations and total N content. Soil S2 had low total N (0.07%) and organic carbon (0.42%).

Table 3.2: Top soil (0-20cm) physical and chemical characteristics

Soil code	pH	Total N	Organic C	Available P	K	Ca	Mg	Fe	Cu	Zn	Clay	Sand	Silt	Soil texture
	H_2O	------ % -----		--------------------------------mg kg^{-1}						--	-----------%		------------	
S1	4.3	0.07	0.42	6	0.77	1.09	0	134.4	0	0	24.4	50.4	25.2	SL
S2	4.8	0.27	1.76	60	1.09	1.17	0	228.7	0	20.6	11	65.6	23.4	SL
S3	4.6	0.23	2.97	6	1.5	2.41	0	371.3	0	11.6	14.3	51	34.7	L
S4	4.7	0.2	2.3	20	1.5	3.66	0	281.7	0	11.3	10.4	54.2	35.4	L
S5	5.8	0.2	2.97	36	2	1.46	17.1	357.6	0	40.2	23.4	12.2	47.6	L
S6	5.6	0.27	3.21	50	1.51	14.59	1.83	282.8	0	38.5	14.2	39.6	46.2	L
S7	5.7	0.24	3.15	26	1.5	13.37	2.56	302.5	0	44.4	8	46	46	L
S8	5.9	0.26	3.27	66	1.51	14.48	2.47	301.4	0	9.03	16	41.8	42.2	L
S9	6.2	0.09	0.85	40	0	10.42	1.83	27.53	1.79	3.17	12.2	47.6	40.2	SCL
S10	6.3	0.13	0.81	30	1.34	7.79	2.08	39.41	10.59	5.11	24.4	44.4	31.2	SCL
Mean	5.39	0.20	2.17	34.00	1.27	7.04	2.79	232.73	1.24	18.39				
SD	0.72	0.07	1.12	20.66	0.55	5.77	5.15	124.03	3.33	16.64				
CV%	13.38	37.76	51.77	60.75	43.08	81.84	84.64	53.29	69.22	90.47				

Where S1 and S2 are soils from Murang'a, S3 and S4 are soils from Kuresoi, S5, S6, S7 and S8 are from Mauche, S9 and S10 are soils from Kitui. SCL is sand clay loam, L-loam and SL is sandy loam. SD is standard deviation of the means and CV is coefficient of variation.

3.6.2 Number of pods

Soybean number of pods was significantly affected by soil, inoculation and variety at P<0.01 (Appendix 2). There were significant interactions among soils, varieties and inoculation. In terms of soils, S5, S6, S7 and S8 soils (Mauche soils) had the highest pods while S1 and S2 soils (Murang'a soils) had the lowest number of pods. Soil S6 with pH 5.8 had the highest number of pods (47.61 pods plant^{-1}) while S1 soil with pH 4.3 had an average of one pod plant^{-1}. Inoculation of soybean varieties with Biofix resulted in high average number of pod (33.6) compared to Legumefix (32); however, control had 4.35 pods plant^{-1}, which was the least. Soybean variety SB19 produced high number of pods (27.44 pod plant^{-1}) compared to Nyala (19.25 pods plant^{-1}). In the soil by variety by inoculation interactions, soybean variety SB19 inoculated with Biofix had the highest number of pods (89 pods plant^{-1} in S6) followed by SB19 inoculated with Legumefix (87 pods plant^{-1}) this was higher compared to Nyala inoculation (figure 3.2). Nyala inoculation with Legumefix in S6 had the highest number of pods (58.7 pods plant^{-1}). Control plants had the least number of pods in both varieties compared to inoculated plants.

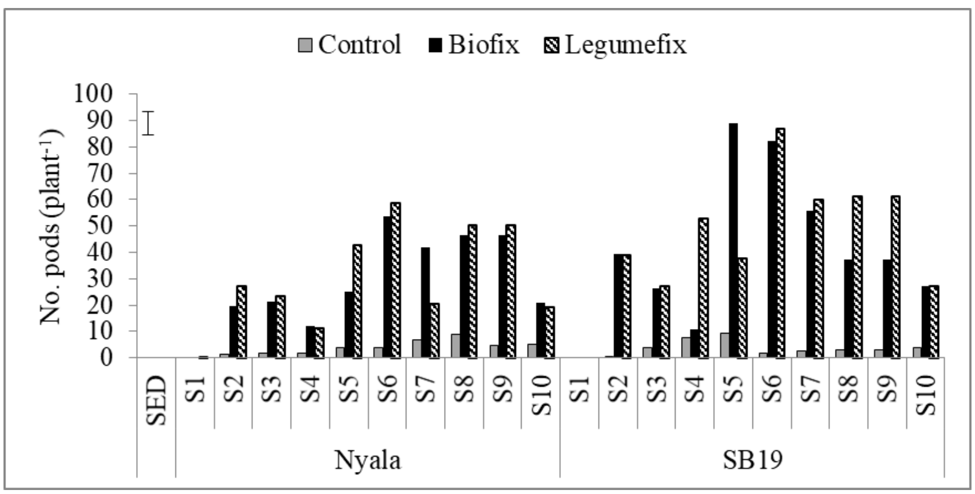

Figure 3.2: Number of pods as influenced by soil, varieties and inoculation interaction. The error bar represents standard error of the differences (SED) for the soil × variety × inoculation interaction.

3.6.3 Nodule fresh weight

Soils, variety and inoculation had significant effect on nodule fresh weight at p<0.05 (Appendix 2). Soybean in soils from Mauche (S5, S6, S7 and S8) had the highest nodule fresh weight than in other soils. Soybean in soil S7 (pH 5.9) recorded highest nodules fresh weight (7.19g plant^{-1}) and in soil S1 from Murang'a had the lowest nodule fresh weight (0.02g plant^{-1}). Soybean variety

Nyala had high nodule fresh weight (3.62g plant^{-1}) compared to SB19 (2.98 g plant^{-1}) (figure 3.3a). Inoculation of soybean with Legumefix (regardless of variety and soil) produced highest nodules fresh weight (4.87g plant^{-1}) compared to Biofix (4.84g plant^{-1}) and control had the least nodule fresh weight (0.2g plant^{-1}). The three-way interaction of soil by variety by inoculation and two-way interactions of variety × inoculation and variety × soil were not significant for nodules fresh weights. Soils and inoculation interaction was significant $p<0.05$ (Appendix 2). Inoculation resulted in significant increase of nodules fresh weight while control plants did not produce nodules except for S9 and S10 (figure 3.3b); however, these were still low compared to inoculated plants. Inoculation of soybean with Legumefix on soil S6 resulted in high nodule fresh weight (11.37g plant^{-1}) while Biofix had highest nodule fresh weight on soil S6 (10.22 g plant^{-1}). Soil S1 had minimal nodules even after inoculation and only Legumefix led to nodulation (0.06 g plant^{-1}) and Biofix having no effect on nodulation.

3.6.4 Nodules effectiveness

Nodules effectiveness was significantly influenced by soil and inoculation ($p<0.05$). Soil S7 from Mauche had the highest percentage nodule effectiveness (53.58%) while S1 did not have nodules. Soybean inoculation with Legumefix resulted in high nodule effectiveness (97.66%) compared to inoculation with Biofix (56.22%), control had least effective nodules (5.97%). The three-way interaction of soil, variety and inoculation was significant $p<0.05$ (Appendix 2). Soybean variety SB19 in soil S5 inoculated with Biofix had the highest nodule effectiveness (85.8%) while Nyala inoculated with Legumefix had the highest effective nodulation (80.9%) on soil S5 (figure 3.4).

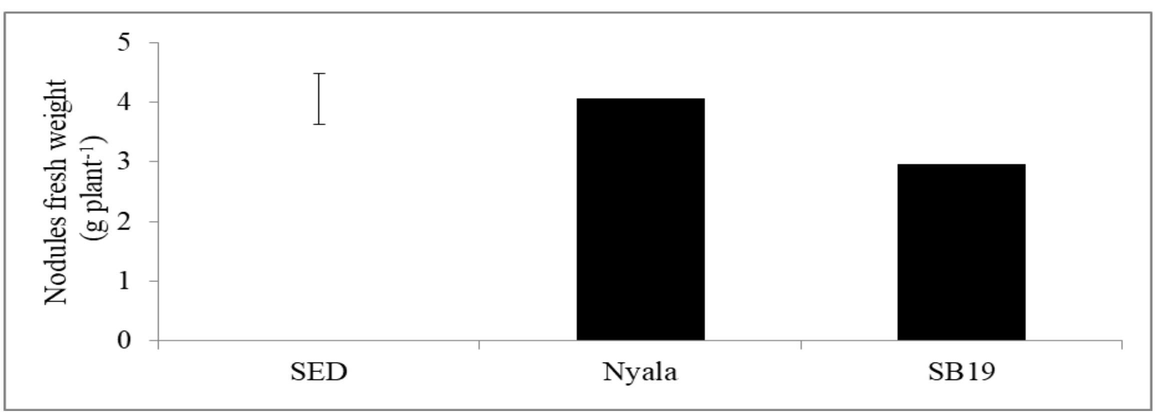

Figure 3.3a: Nodule fresh weight as affected by soybean varieties. The error bar represents standard error of the differences (SED) for variety effect.

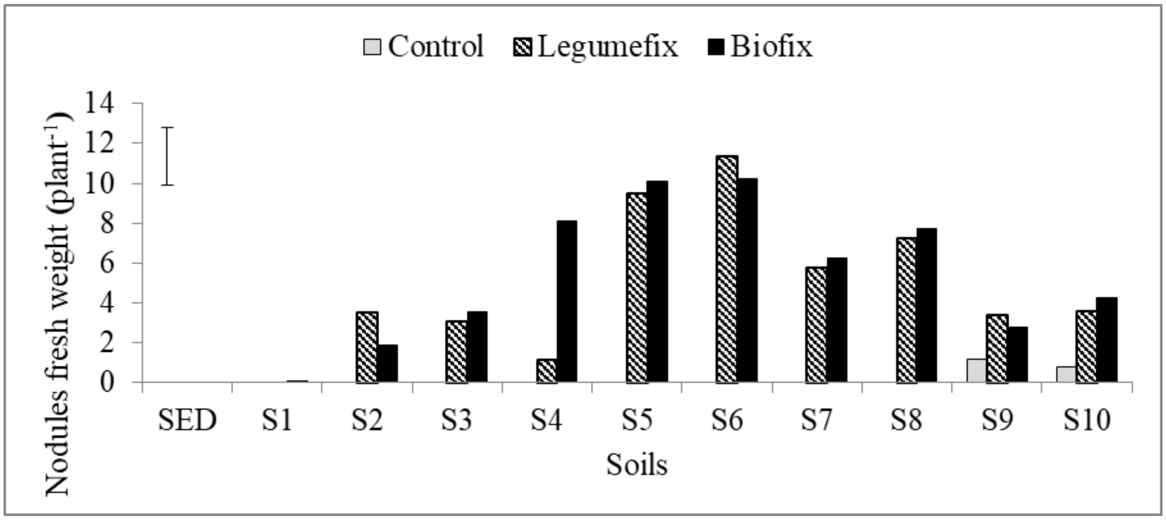

Figure 3.3b: Nodule fresh weight as affected by soil and inoculation interaction. The error bar represents standard error of the differences (SED) for the soil × inoculation interaction.

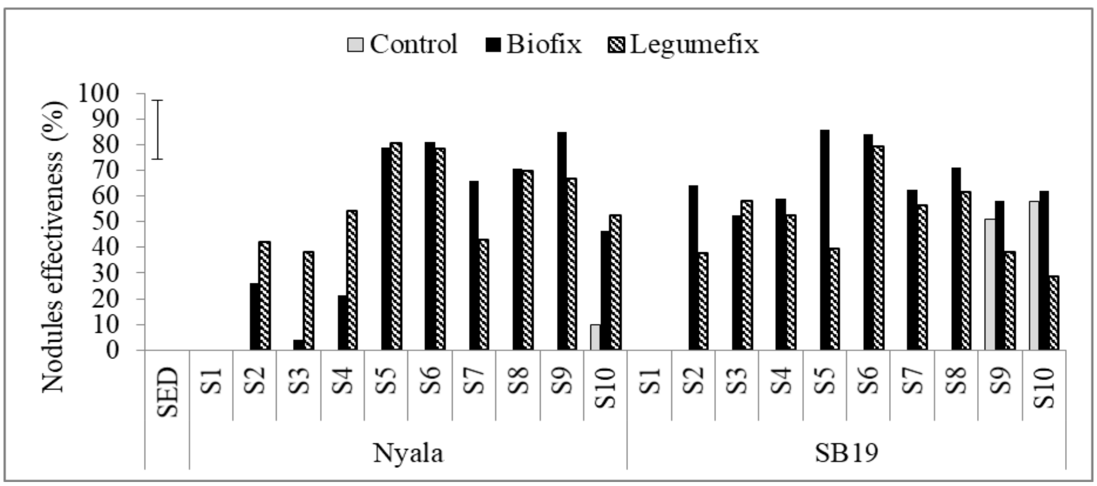

Figure 3.4: Nodules effectiveness as affected by soil, variety and inoculation. The error bar represents standard error of the differences (SED) for the soil × variety × inoculation interactions.

3.6.5 Shoot dry weight

Soil and inoculation had significant influence on shoot dry weight $p<0.05$ (Appendix 2). Soybean in soil S7 had the highest shoot dry weight (40.49 g $plant^{-1}$) while in S1 soil had the least shoot dry weight 0.63 g $plant^{-1}$ (Figure 3.5a). Soybean in soils S5-S8 had the highest shoot dry weight compared to the other soils. Biofix and Legumefix inoculants had significant effects on shoot dry weight (21.27 and 21.55 g $plant^{-1}$ respectively) while control plants had the least shoot dry weight 8.12 g $plant^{-1}$ (Figure 3.5b).

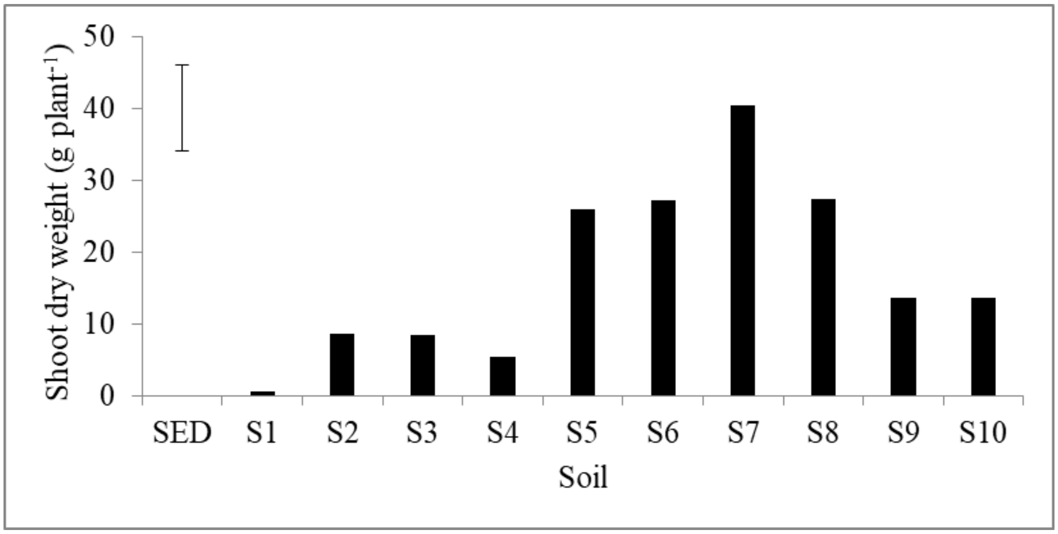

Figure 3.5a: Shoot dry weight as influenced by soil. The error bar represents standard error of the differences (SED) for the soil effect.

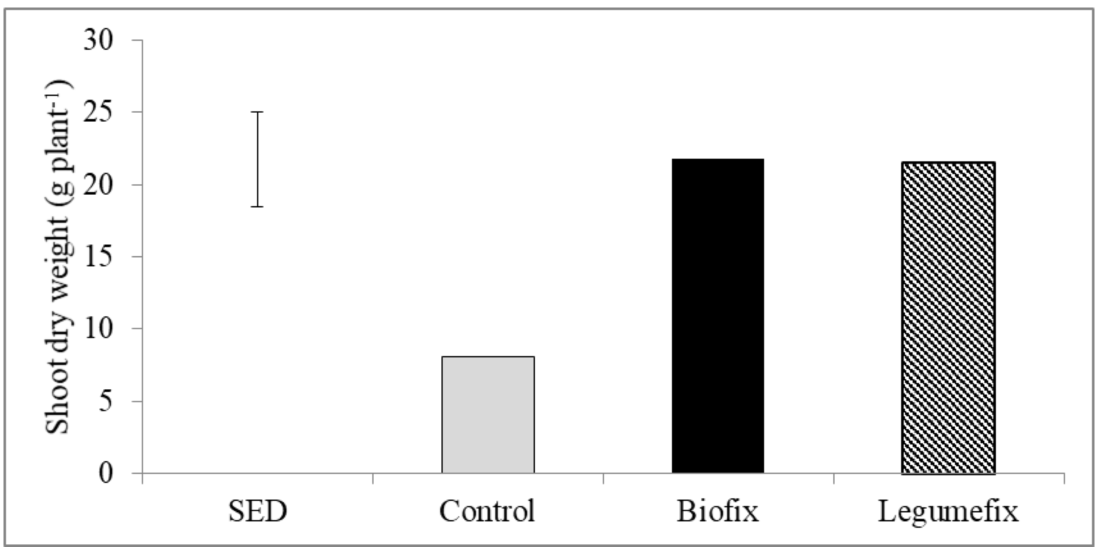

Figure 3.5b: Shoot dry weight as influenced by inoculation. The error bar represents standard error of the differences (SED) for inoculation effect.

3.6.6 Root dry weight

Soil, variety and inoculation had significant effect on root dry weight. Soil S6 had the highest root dry weight (6.57g plant^{-1}) while S1 had the least root dry weight (0.14g plant^{-1}). Soybean in soils S10 from Kitui had the highest root dry weight (5.2g plant^{-1}) had high root dry weight compared to other soils. Soybean variety SB19 had highest root dry weight (3.65g plant^{-1}) compared to Nyala (2.84g plant^{-1}) root dry weight. Soybean inoculation with Legumefix resulted in high root dry weight (4.33g plant^{-1}) while plants inoculated with Biofix had 4.33g plant^{-1} which was above control (0.92g plant^{-1}). Roots dry weight was significantly affected by soil, variety and inoculation interaction ($p<0.05$) (Appendix 2). Inoculation had significant effect on root dry weight with control plants having the lowest root dry weight compared to inoculated plants (Figure 3.6). Soil S8 with SB19 inoculated with Legumefix had the highest root biomass (14.22g plant^{-1}) while inoculation of the same with Biofix had 10.26g plant^{-1} root dry weight. Nyala inoculated with Biofix had 8.77g plant^{-1} root dry weight, which was high, compared with Nyala inoculated with Legumefix (8.18 g plant^{-1}) root dry weight.

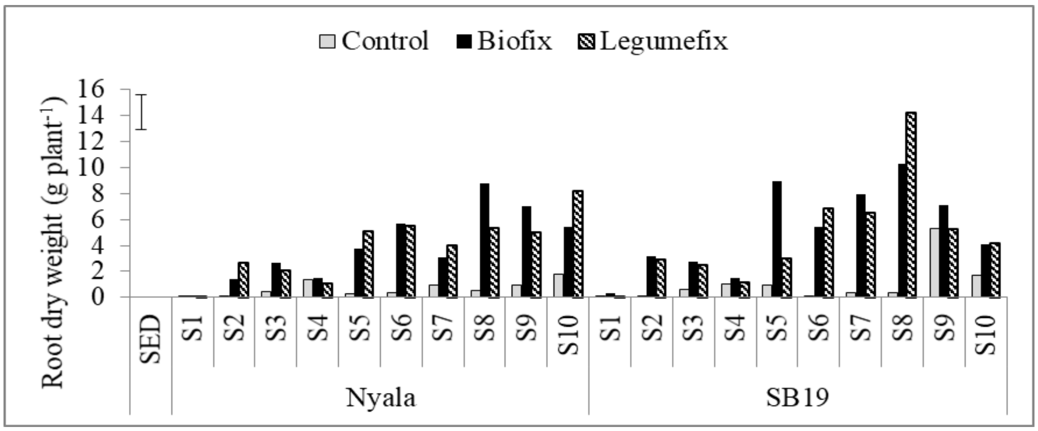

Figure 3.6: Root dry weight as affected by soil, inoculation and soybean varieties. The error bar represents standard error of the differences (SED) for the soil × variety × inoculation interactions.

3.6.7 Biological Nitrogen fixation and N uptake

Soil and inoculation had significant influence on N uptake and Ndfa (Appendix 2). For N uptake, soybean in S5 soil had the highest level of N uptake (0.76g N plant^{-1}) while soybean in S1 soil had the least N uptake (0.01g N plant^{-1}). Soybean inoculation with Legumefix had the highest level of N uptake (0.56g plant^{-1}) while inoculation with Biofix resulted in 0.53g N plant^{-1} and control had the least N uptake (0.05g plant^{-1}). The interaction of soil, variety and inoculation was significant for N uptake $p<0.05$. Soybean variety SB19 inoculated with Biofix in soil S7 had the highest level of N uptake (1.36 g N plant^{-1}). Control plants had low to inestimable amount of N uptake in all soils (regardless of variety and inoculation) and varieties (regardless of soils and inoculation) and soil S1 had low amounts of N uptake even after inoculation (figure 3.7).

For amount of nitrogen derived from atmosphere (Ndfa), soil S6 had the highest amount of Ndfa (73.20%) while S1 soil had the least (0.53%). Inoculation also improved nitrogen fixation; soybean inoculated with Legumefix had a high amount of nitrogen fixation (52.64%) and inoculation with Biofix resulted in 50.35% Ndfa and was higher than control (0.97%). The soil, variety, and inoculation interaction had significant effect on nitrogen fixation at $p \leq 0.01$, (Appendix 2). Soils S1- S4 had the least level of N fixation on both varieties under inoculation while soils S5-S8 had the highest N fixation amounts on both Nyala and SB19 under inoculation (Figure 3.8). Nitrogen fixation on control plants for both Nyala and SB19 was very low in all the soils.

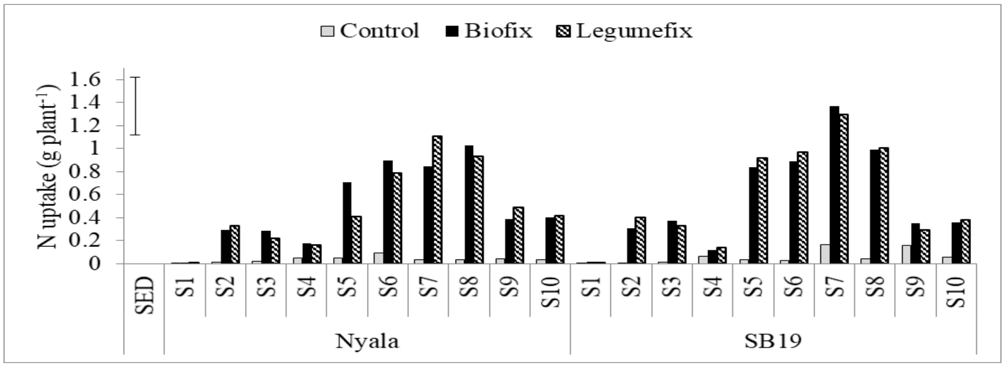

Figure 3.7: Nitrogen uptake as affected by soil, inoculation and soybean varieties. The error bar represents standard error of the differences (SED) for the soil × variety × inoculation interactions.

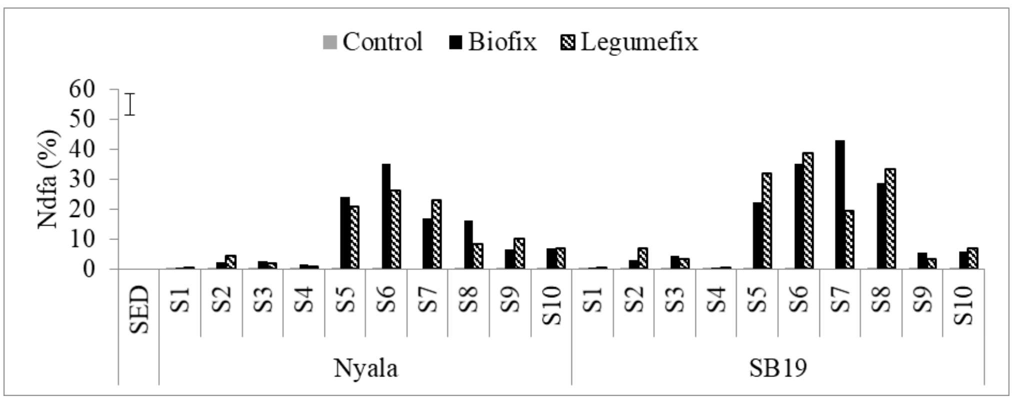

Figure 3.8: Nitrogen fixation influenced by soil, inoculation and soybean varieties. The error bar represents standard error of the differences (SED) for the soil × variety × inoculation interactions.

3.6.8 Phosphorus uptake

Soil, inoculation and their interaction had significant influence on P uptake $p<0.05$ (Appendix 2). Soybean in soil S8 had the highest uptake (299 mg P $plant^{-1}$) while in soil S2 had the least uptake (55mg P $plant^{-1}$). Inoculation with Legumefix resulted in high uptake (262 mg P $plant^{-1}$) compared to Biofix (240 mg P $plant^{-1}$) and control (148 mg P $plant^{-1}$). All inoculated plants had a high amount of N fixed above the control (Figure 3.9).

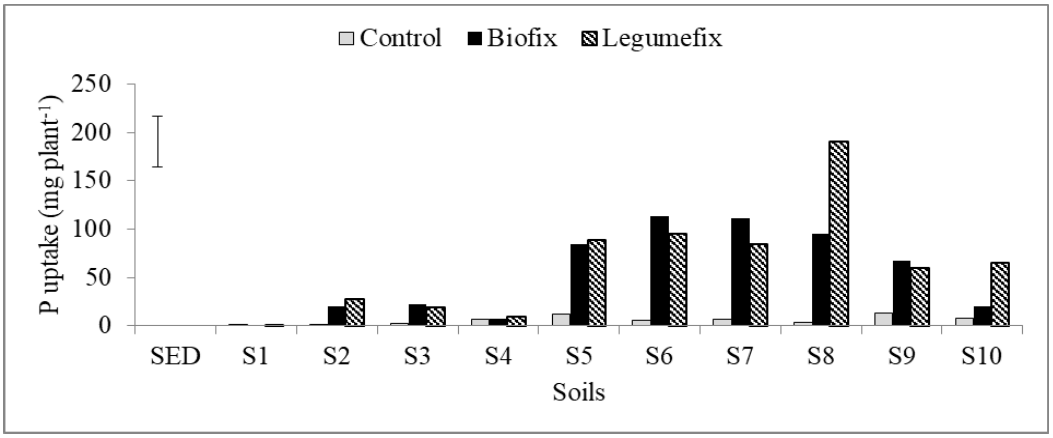

Figure 3.9: Phosphorus uptake as affected by soil and inoculation. The error bar represents standard error of the differences (SED) for the soil × inoculation interaction.

3.6.9 Nodule occupancy

Soybean in soil S9 (kitui) had nodules in the control plants for soybean variety SB19. The nodules from S6 (Mauche) showed two profiles; profile I (Biofix inoculant), and profile II (Legumefix inoculant) while nodules from soil S9 showed three profiles; I, II and III (control) (Table 3.3). The percentage of intergenic spacer regions (IGS) for soil S9 planted with SB19 and soil S6 planted with Nyala inoculated with Biofix did not reach 66%, which is the critical rate for the inoculation effectiveness. On average, nodule occupancy rate was high for Legumefix (73.96%) compared to Biofix (72.71%).

Table 3.3: Profile summary of the nodules in soil S6 and soil S9 soils in percentage

		Nyala			SB19		
	Inoculants	I	II	III	I	II	III
Soil S9	Control	0	0	0	0	0	37.5
	Biofix	83.33	0	0	70.83	0	0
	Legumefix	0	62.5	0	0	87.50	0
Soil S6	Biofix	68.33	0	0	68.33	0	0
	Legumefix	0	75	0	0	70.83	0

Total number of nodules analyzed was eight per treatment.

3.7 Discussion

Acidity greatly affected pod formation with soils of pH >5.5 having a high number of pods compared to those with pH <5.5. A study by Bekere (2013) showed low number of pods in soil pH 4.42 compared to limed soil and that inoculation of an acidic soil did not increase number of pods in soybean. Acidic soils are low in available P necessary for pod formation (Xiurong *et al.,* 2010). Fageria *et al.* (2013) reported low number of pods in control plants (pH 5.3) compared to soybean in limed soils which had a higher pH. Bekere (2013) observed increase in number of pods after adjustment of soil pH to >5.5 in inoculated plants than control. Inoculated plants had high number of pods compared to control plants, this is attributed to nitrogen fixation hence plants growth contributing to pod formation. Tamiru *et al.* (2012) also observed inoculation of soybean with different strains resulted in high number of pods. Soils with near neutral pH had the best perform when inoculated with Legumefix and Biofix and from the soil analysis, these soils had above optimal available P and high CEC.

The difference in nodules fresh weight is due to the availability of nutrients, soils with low pH levels had low essential nutrients compared to soils with near neutral pH. Nutrients availability have a direct relation with inoculants effectiveness. Lack of nodules in acidic soil has been observed in Leucaena inoculated with *rhizobium* (Goncalves *et al*., 2000) this was attributed to aluminium toxicity. Nodules fresh weights is affected by H^+ concentration in soils, this is due to the toxic effects of these ions on the roots of the plants. Bekere (2013) observed low nodule dry weight in soybean planted in soils of pH 4.42 compared to limed soil. Inoculation of soybean with Biofix at soil pH 5.2 had shown good nodulation compared to controls (Thi *et al.,* 2012). Tamiru *et al.* (2012) also observed low nodulation in control plants compared to inoculation of soybean with different strains. The FAO (1984) reported that the application of P and K had been seen to increase number of nodules and nodules fresh weight. Low Nodule fresh weight in acidic soils can be associated with rhizobia growth, at low pH the expression of rhizobia nodulation genes and production of nod factor is inhibited (Meng-Han *et al*., 2012). This causes disruption of the signal exchange between rhizobia and host plant and leads to root hair deformation and curling reducing nodulation. Nodule effectiveness was influenced by soil pH, soils with pH <5.5 had low nodules effectiveness compared to soil pH >5.5. This can be attributed to P availability and K, soils with extreme acidity (pH<5.5) had low level of available P and K which play vital role in contribution to leghaemoglobin (Tamiru *et al.,* 2012). Inoculation also had significant influence on nodules effectiveness. The control plants did not nodulate (except for Kitui soil (S9) indicating absence of native rhizobia population specific to soybean. Inoculation was effective based on nodule effectiveness results hence nitrogen fixation.

Soils with pH <5.5 had low shoot dry weight compared to soils with pH > 5.5. this is attrubuted to low levels of essential nutrients at pH<5.5 resulting in stunted growth (Keino *et al.*, 2015). Low shoot biomass in control plants had been observed in many research work while the inoculated plants response to the inoculants can be attributed to the effectiveness of the inoculant to influence plant growth (Goncalves *et al*., 2000). Soil pH had a direct effect on shoot biomass, this is due to its influence on nutrients availability to plants. Magnesium plays a vital role in plant growth when it's limiting like in acidic soils, it interferes with photosynthesis and P reactions (Keino *et al*., 2015). Soil P plays an important role in soybean production, when fixed in soils plant growth is negatively affected. Al toxicity will cause sorption of P, reduced uptake of the basic cations. Root hairs are also sensitive to H^+, this is prone in acidic soils (Goncalves *et al*., 2000) thus attributed to lower root biomass regardless of inoculation. Calcium deficiency in acidic soils affects plants cell growth and root development, impaired roots limits nutrients uptake hence poor plant growth (Keino *et al*., 2015).

Nitrogen fixation and uptake is influenced by soil pH; soils with pH <5.5 had low level of N fixation and uptake compared with soil pH >5.5; this is attributed to poor roots development and poor nodulation in acidic soils. Nitrogenase activity increases with increasing K in the soil (Keino *et al*., 2015) hence high rate of BNF in soils. Nodules effectiveness and BNF are directly related to number of nodules per plant, hence affected directly as a result of soil pH and nutrient content. Calcium in soil is used for adhesion by rhizobia hence its deficiency in acidic soils affects rhizobia attachment, infection and infection thread formation (Meng-Han *et al*., 2012) hence nitrogen fixation is negatively affected. The *Rhizobium* in the inoculants has the ability to solubilize the precipitated P hence making it available for uptake (Fatima 2007) hence high shoot P in inoculated plants over the control. The high shoot P in the soils of pH 5.5-6.3 can be attributed to P availability in the soil hence ease uptake by the plants. Acidic soils have low soil available P due to sorption on the soil surface. Fageria *et al.* (2013) observed low shoot P content and they concluded that soil P uptake influences the number of pods.

3.8 Conclusion

Soybean response to inoculation was high in soil of pH range 5.5-6.2, at this range there was highest % effective nodules, number of pods, nodules fresh weight, shoot and root biomass among other measured parameters. The soybean varieties did not differ in the measurement of growth parameters both varieties did better at pH 5.5-6.2. Poor results of nodulation, BNF among other measured parameters were recorded in acidic soils (pH<5.2) from Murang'a and Kuresoi. To improve on nodulation and BNF of these soils, there is need for liming.

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CHAPTER FOUR

EVALUATION OF LIME REQUIREMENT METHODS AND THE EFFECTS OF AGRICULTURAL LIME AND MIJINGU PHOSPHATE ROCK ON SELECTED ACIDIC SOILS

4.1 Abstract

Soil acidity is a major problem in Kenyan arable lands and liming using accurate lime estimation method and effective materials are necessary when ameliorating acidic soils. A laboratory incubation study using soils from Kuresoi (pH 4.9) and Murang'a (pH 5.0) was set up using agricultural lime and Mijingu phosphate rock (MPR) as liming materials. Exchangeable acidity and Shoemaker-Mclean-Pratt (SMP) used as lime requirement methods (LRM) and the experiment laid in completely randomized design (CRD). Soil pH was measured every two weeks for 12 weeks using destructive sampling and pH measured in 1:2.5 soil/ water ratio. Available P was determined after the twelfth week of incubation using Olsen P and Mehlich available P methods. The data were subjected to analysis of variance and t-test done between the two LRM. Soil liming with agricultural lime increased soil pH (5.6) compared to liming with MPR (5.4). The SMP method recommended high amount of lime (agricultural lime 32 T Ha^{-1} and MPR 95 T Ha^{-1}) compared to exchangeable acidity method (agricultural lime 6.2 T Ha^{-1} and MPR 4.55 T Ha^{-1}). Liming with exchangeable acidity rates did not increase soil pH to > 5.5. Olsen available P in soils amended with MPR was higher (45 mg kg^{-1}) compared to those amended with agricultural lime (37 mg kg^{-1}). The results of this study suggest that 2 weeks of soil-lime incubation is sufficient in raising soil pH. Agricultural lime had the high (78.9%) effective calcium carbonate equivalent compared to MPR (65.2%) indicating that lime quality plays a major role in its effectiveness.

Keywords: Exchangeable acidity, Shoemaker-Mclean-Pratt, Agricultural lime, Mijingu phosphate rock, Lime requirement method, soil pH

4.2 Introduction

Kenya has about 13% arable land affected by soil acidity, this is nearly 7.5 million ha, and majority of this land occur in high rainfall area with medium to high potential agricultural productivity, including the highlands east of Rift valley. Sources of soil acidity include leaching of basic cations, release of the proton during major nutrient cycles (C, N and S) in the soil-plant systems, poor farm management practices including use of acidifying fertilizers. The cause of acidic soils in Kenya include leaching of basic cations due to high rainfall leaving Al^{3+}, H^{+}, Fe^{2+} and Mn^{3+} as predominant cations (Obura, 2008), and also the non-calcareous parent material that Kenyan soils develop from are acidic in nature.

The effects of soil acidity on plants include poor yields, poor root growth, and poor nutrient uptake especially for Ca, P, Mg, Mo and K (Oguntoyinbo *et al*., 1996). Soil acidity also reduces organic matter breakdown and nutrient cyclying by microflora. Soil liming increases the availability of basic cations, reduces the concentration of toxic levels of Al and increases P availability hence increasing soil pH (Mesfin *et al.*, 2014). Different lime product are available in the market and the most common being agricultural lime. Due to the cost associated with lime, accessibility, labor in application, and low demand by farmers due to lack of knowledge about soil liming results in its limited use among farmers.

Lime rates are determined by its calcium carbonate equivalence (CCE) and fineness factor hence the effective calcium carbonate equivalence (ECCE) or the total neutralizing value. Lime requirement (LR) is the amount of lime needed to neutralize soil acidity to the desired level (Thomas, 1996). Thus for lime to be considered effective, it should be applied at the recommended rate considering its ECCE and choosing a suitable lime requirement method (LRM). An optimal incubation period of 14-28 days recommended after lime addition before planting (Ezekiel, 2007; John & Antonio , 2016 and Opala *et al*. 2012). Lime requirement is affected by factors such nature of acidity, neutralizing sequence (initial and desired pH), soil properties including parent material and texture and organic matter content (Thomas, 1996). The buffering capacity of the soil is also an important factor in soil liming; this is the ability of soil to resist change in pH after lime addition. Soils with high buffer capacity (soils with high amount of clay, oxides and organic matter) require much lime compared to soils with low buffering capacity.

Soil liming should consider the concentration of Al^{3+} ions as liming to <0.1meq Al/ 100g soil has been seen to have an impact on plants like sorghum and soybean (Reeve & Summer, 1980).

Exchangeable acidity LRM is suitable in highly weathered soils and in lime recommendation; the value of exchangeable acidity multiplied by a factor of 1.5 or 2 depending on crop sensitivity to acidity (Thomas, 1996). Application of lime increases Ca^{2+} and Mg^{2+} and reduces Al^{3+}, H^{+}, Mn^{2+} and Fe^{3+} thus increasing soil pH and available P.

The Shoemaker-McLean-Pratt (SMP) LRM relies on soil-buffer pH measurement and it was first developed in incubated soils of Ohio (Shoemaker *et al.,* 1961). The method is suited for soils with <5.8 pH, LR >4.5tons ha^{-1} and organic matter <10%. Once the soil-buffer pH has been determined the LR table for SMP is used to estimate the amount of lime to be applied and this is adjusted based on CCE >100% and fineness factor for the top 20cm of soil depth. SMP method is not widely used as a LRM. Nuwamanya (1984) found a good correlation between SMP and $CaCO_3$- incubation study. The study concluded that SMP- double buffer method is suitable for lime estimation for acidic soils in Kenyan. Kenya acidic soil have high level of exchangeable Al^{3+} thus exchangeable acidity has been used in lime requirement determination. Methods, frequency, depth, and time of application are important factors on lime efficiency. Various methods can be used to apply lime including spot application, band application, and broadcasting together with incorporation is the most recommended method of lime application due to its effectiveness.

Agricultural lime (quick lime- CaO) is one of the lime materials used in Kenya. Athi river mining company and Koru mining companies are major manufacturers. It is effective in improving yield when used alone as well as in combination with other fertilizers (Nekesa *et al.,* 2011). Mijingu phosphate rock (MPR) mined in northern Tanzania used as P source and liming effects and its recommended application rate is 60 kg Ha^{-1} (Opala *et al.,* 2012). Lime requirement methods for Kenyan acidic soils are not clear thus farmers may be miss-guided on the rate and timing of application. In addition, there is lack of information of effectiveness of lime materials in Kenyan soils and this could lead to ineffectiveness of applied lime to raise pH due to underestimation/ overestimation. This research work will determine the most effective lime material to raise soil pH using LRM exchangeable acidity and SMP and liming materials agricultural lime and MPR.

4.3 Materials and methods

4.3.1 Soil collection and analysis

Top Soil (0-20cm) were obtained from Rift Valley Nakuru County (Kuresoi) and central region in Murang’a County (Kangema) in Kenya. The soils were sieved through a 2mm sieve and their initial characteristic determined (Chapter 3). The soil physical and chemical characteristics were determined following procedures described in Okalebo *et al*. 2002. The initial soil pH was determined in 1:2.5 soil/ water using the glass electrode pH meter, soil particle size analysis was determined using hydrometer method, total nitrogen using Kjeldahl method and soil organic carbon using Walkley-black method. The available phosphorus, exchangeable cations (Ca, Mg, K, and Na) and micronutrients (Cu, Zn, Fe, and Mn) were extracted using Mehlich 3 method and amounts determined using atomic absorption spectrometer.

4.3.2 Lime analysis

Agricultural lime and Mijingu phosphate rock were used as lime materials and their calcium carbonate equivalence (CCE) was determined by back titrating a 6N HC1 solution of each lime material with NaOH to a phenolphthalein end point (Jian-Ling *et al.*, 2010). Lime materials fineness was determined by sieving through a 2mm and a 0.25mm (John, 2016). The values of fineness together with CCE were to determine the effective CCE of the lime materials (Table 4.2).

4.3.3 Methods of lime estimation

4.3.3.1 Exchangeable acidity

The soil exchangeable acidity was determined by weighing 5g of each soil in replicate in plastic bottles and adding 50ml of 1N KCl followed by shaking for 30 minutes and centrifuging at 700 rpm for 10 minutes. The samples then filtered through Whatman filter paper No.40 and the extract titrated with 0.1M standardized NaOH using phenolphalein indicator (Thomas, 1996). Lime requirement was calculated using exchangeable acidity $cmol_c Kg^{-1}$ as shown below:

Lime requirement $T\ Ha^{-1}$= Exchangeable acidity ($cmol_c\ Kg^{-1}$) × 2

4.3.3.2 Shoemaker-Mclean-Pratt (SMP) double buffer method

The SMP buffer was prepared as described in Thomas (1996). Five grams of each soil weighed into plastic bottles and 5ml of distilled water added to each sample and shaking them for 10 minutes. The soil pH was then determined using glass electrode pH meter and then 10ml of the SMP buffer was added to each bottle and shaken again for 10minutes. The pH reading for soil-buffer suspension recorded after 15 minutes. Lime requirement for the target pH was determined using the SMP table taking into consideration the CCE and lime fineness. The experiment was in CRD and replicated three.

4.4 Incubation setup

Plastic cups were filled with 100g of soil and used for incubation. Lime materials used were MPR and agricultural lime. Lime addition rates were based on lime analysis and ECCE (table 4.3). The cups were maintained at 80% field capacity and watered after every 48hrs followed by mixing. A total of 216 cups (2×3×3×2×6) were used in study and arranged in a completely randomized design (CRD). The treatment outline used is as below (Table 4.1):

Table 4.1: Treatments outline for determination of an effective liming material

Factors	Level	Description
Soils	2	Soils of pH 4.9 and 5.0
Liming materials	3	Agricultural lime, MPR and control (unlimed)
LRM	2	Exchangeable acidity and SMP
Replicates	3	Each combination repeated 3 times
Weeks of pH assessment	6	Done on 2^{nd}, 4^{th}, 6^{th}, 8^{th}, $10^{th,}$ and 12^{th} week after incubation.

Total number of samples 2× 3 × 3 ×2 × 6=216

4.5 Data collection and analysis

Destructive sampling was used to sample the cups and after every two weeks, 36 cups were removed and soil pH determined in a 1:2.5 soil: water solution using glass electrode pH meter. Available P was extracted using Mehlich-3 (Mehlich, 1984) and Olsen P (Olsen *et al.,* 1954) methods. Data was analyzed using SAS statistical software version 9.4 (SAS, 2016). Analyses of variance (ANOVA) at 95% confidence for soil incubation and available P. The t-test for the two LRM methods was also performed.

Statistical model:

$$Y_{ijklm}=\mu+A_i+B_j+AB_{ij}+C_k+AC_{ik}+BC_{jk}+ABC_{ijk}+D_l+AD_{il}+BD_{jl}+CD_{kl}+ABD_{ijl}+ACD_{ikl}+BCD_{jkl}+ABCD_{ijkl}+\varepsilon_{ijklm}$$

$\mathbf{Y_{ijklm}}$ is the observation due to ith soil pH, jth liming material, kth lime requirement method and lth week of incubation

μ is the general mean

$\mathbf{A_i}$ is the ith soil pH

$\mathbf{B_j}$ is the effect of jth liming material

$\mathbf{AB_{ij}}$ is the interaction between ith soil pH and jth the liming material

$\mathbf{C_k}$ is the effect of kth lime requirement method

$\mathbf{AC_{ik}}$ is the interaction between the ith soil initial pH and kth the lime requirement method

$\mathbf{BC_{jk}}$ is the interaction of the jth lime material with the kth lime requirement method

$\mathbf{ABC_{ijk}}$ is the interaction of the ith initial soil pH with the jth liming materials over kth lime requirement method

$\mathbf{D_l}$ is the effect due to lth week of incubation

$\mathbf{AD_{il}}$ is the interaction of the ith soil pH and lth week of incubation

$\mathbf{BD_{jl}}$ is the interaction of the jth liming material and lth week of incubation

$\mathbf{CD_{kl}}$ is the interaction of the kth lime requirement method lth week of incubation

$\mathbf{ABD_{ijl}}$ is the interaction of the ith soil pH, jth liming material and lth week of incubation

$\mathbf{ACD_{ikl}}$ is the interaction of the ith soil pH, kth lime requirement method and lth week of incubation

$\mathbf{BCD_{jkl}}$ is the interaction of the jth liming material, kth lime requirement method and lth week of incubation

$\mathbf{ABCD_{ijkl}}$ is the interaction of ith soil pH, jth liming material, kth lime requirement method and lth week of incubation

$\boldsymbol{\varepsilon_{ijklm}}$ is the random error term

4.6 Results

4.6.1 Soil and lime analyses

The two soils from Kuresoi and Muranga were acidic pH 4.9 and 5.0 respectively (Table 4.2), however Kuresoi soil had a high level of exchangeable acidity (3.19 $Cmol_c\ kg^{-1}$) compared to Murang'a soil (2.91 $Cmol_c\ kg^{-1}$) thus influencing on the level of soil available P and the liming rates for the two soils (Table 4.2). Agricultural lime had the highest CCE (Table 4.2) with >50% of its particles passing through the 0.25mm sieve thus influencing on its efficiency as a liming material and application rates.

Use of exchangeable acidity method resulted in low lime rates for both agricultural lime and MPR compared to SMP method (table 4.3). Liming rate for MPR was high (Kuresoi soil 100 T Ha^{-1} while Murang'a soil 89 T Ha^{-1}), for agricultural lime it required 34 T Ha^{-1} of lime to raise the pH to 6.0 for Kuresoi soil and 30 T Ha^{-1} of lime for Murang'a soil.

Table 4.2: Soil physical and chemical characteristics and lime analyses

Parameters	Kuresoi	Murang'a
pH (H2O 1:2.5)	4.9	5.0
pH (SMP buffer 1:2)	5	5.0
Exchangeable acidity (cmolc kg^{-1})	3.19	1.94
Ca	2.41	1.17
Mg	0	0
K	1.51	1.09
Organic carbon (%)	2.97	1.76
Total N (%)	0.23	0.27
Mehlich P (mg kg^{-1})	15	20
Texture		
Sand (%)	51	65.6
Silt (%)	34.7	23.4
Clay (%)	14.3	11
Textural class	Loam	Sandy loam

Table 4.3: Lime application rates in T Ha^{-1} determined by soil exchangeable acidity and Shoemaker-Mclean-Pratt lime requirement methods

Lime characteristic	Agricultural lime	MPR	
Moisture content (%)	0.53	8.09	
pH (H_2O 1:2.5)	8.6	9	
CCE	78.9	65.2	
Fineness% (sieve method)			
pass >2mm	0.17	48.78	
pass 0.25-2mm	40.97	36.94	
pass <0.25mm	58.86	14.28	
Lime requirement method	Lime material	Rates T Ha^{-1}	
		Kuresoi	Murang'a
Exchangeable acidity	Agricultural lime	7.5	4.9
	MPR	3.95	5.15
SMP	Agricultural lime	34	30
	MPR	101	89

4.6.2 Soil incubation

Soils, lime materials, lime requirement methods and weeks of incubation had significant influence on soil pH at $p<0.05$. Kuresoi soil had average high soil pH 5.4 compared to Murang’a soil pH 5.1 regardless of lime materials, lime requirement methods and weeks of incubation. Soil liming with agricultural lime resulted in increase in soil to pH 5.6 while soil liming with MPR resulted to change in soil pH to 5.4 and control had soil pH 4.7 on average. Lime estimation using SMP method resulted in increase in soil pH to 5.5 while lime application based on exchangeable acidity estimates resulted in low soil pH 5.0. After the second week of soil incubation, there was increase of soil pH to 5.5 and in the fourth and sixth week soil pH dropped to 5.4, there was further drop in soil pH to 5.3 in the eighth, tenth and twelfth week of incubation. The interaction of soil, lime materials, lime requirement methods and weeks of incubation was not significant (Appendix 3), however, the interaction of soil, lime materials and lime requirement methods and interaction of lime with weeks of incubation were significant $p<0.05$. Soil liming with agricultural lime estimated by SMP method resulted in increase of soil pH to 6.0 for both Murang’a and Kuresoi soil while liming with MPR resulted to increase in soil pH to 5.8 in both soil (figure 4.1). Exchangeable acidity method did not increase soil pH to >5.3 and control treatment had the least pH in both soils. For weeks of incubation and lime materials interaction, soil limed with agricultural lime had the highest soil pH >5.5 throughout the weeks of incubation (figure 4.2). Liming with MPR resulted in increase of soil pH between 5.3-5.5 in the twelve weeks of incubation with second week having the highest soil pH (5.5) and then declining. Control treatment had the least soil pH between 4.3-4.9. The ttest showed significant difference between SMP and exchangeable acidity ($p<0.05$) on the second week of soil incubation (Table 4.5) for both Murang'a and Kuresoi soil.

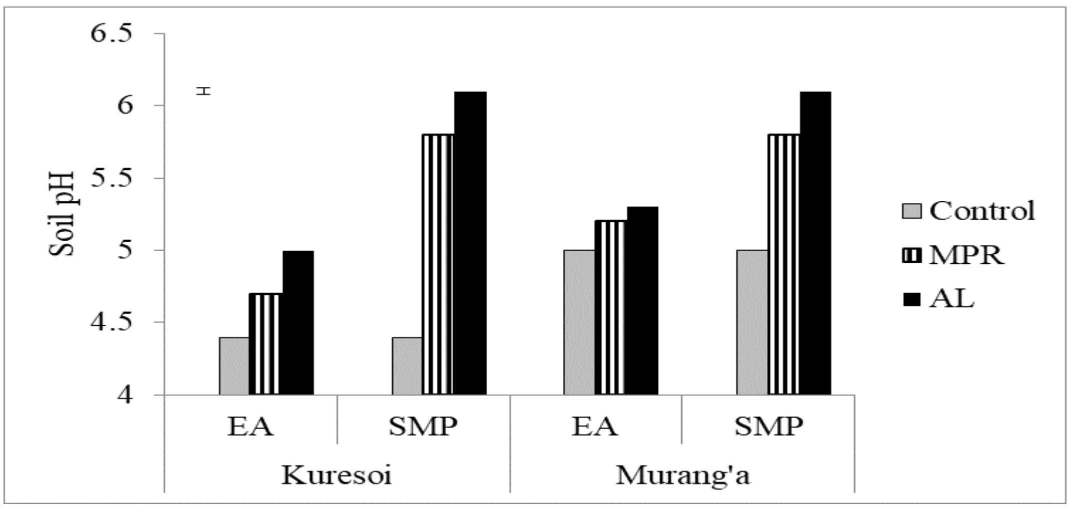

Figure 4.1: Soil pH as influenced by lime materials, lime requirement method and soils. The error bar represents standard error of the differences (SED) for the soil × lime material × weeks of incubation interactions.

Where EA is exchangeable acidity, SMP is Shoemaker-McLean-Pratt, MPR is Mijingu phosphate rock, and AL is agricultural lime.

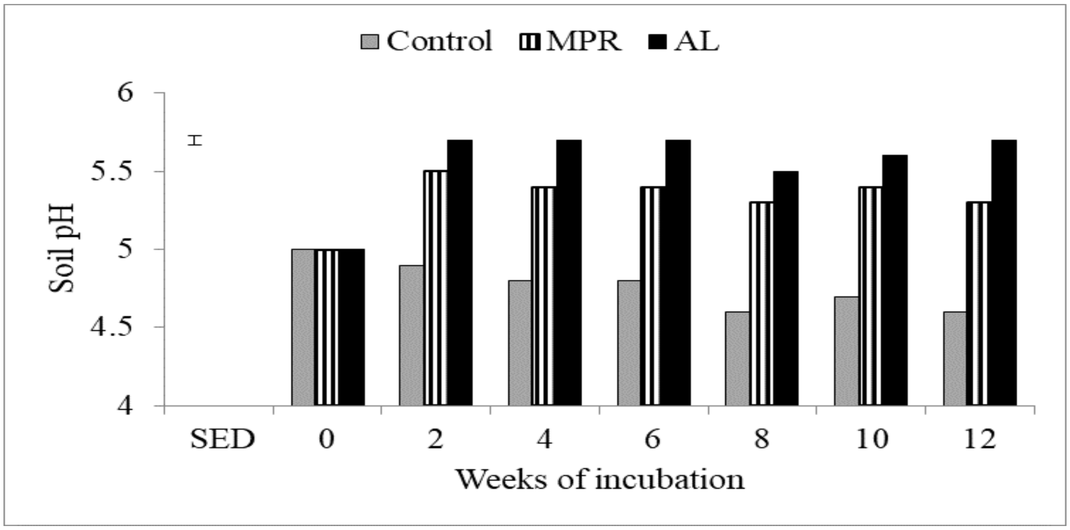

Figure 4.2: Soil pH as influenced by liming materials and weeks of incubation. The error bar represents standard error of the differences (SED) for the soil lime material and weeks of incubation interaction.

4.6.3 Soil available phosphorus

Soil available P for the two soils was not significantly different based on Mehlich 3 method. Lime requirement method and lime materials had significant influence on soil available P at $p<0.05$ (Appendix 4). The three-way interaction of soil × lime × lime requirement method was significant for Mehlich available P ($p<0.05$). Soil liming with Mijingu phosphate rock resulted in high Mehlich 3 available P 156.67 mg kg^{-1} (Figure 4.3) with liming rates determined by SMP method. There was increase in soil available P in soils limed with agricultural lime (78 mg P kg^{-1} Kuresoi soil and 36.67 mg P kg^{-1} Murang'a soil) based on SMP liming rates compared to exchangeable acidity (16.67 mg P kg^{-1} Kuresoi soil and 21.67 mg P kg^{-1} Murang'a soil).

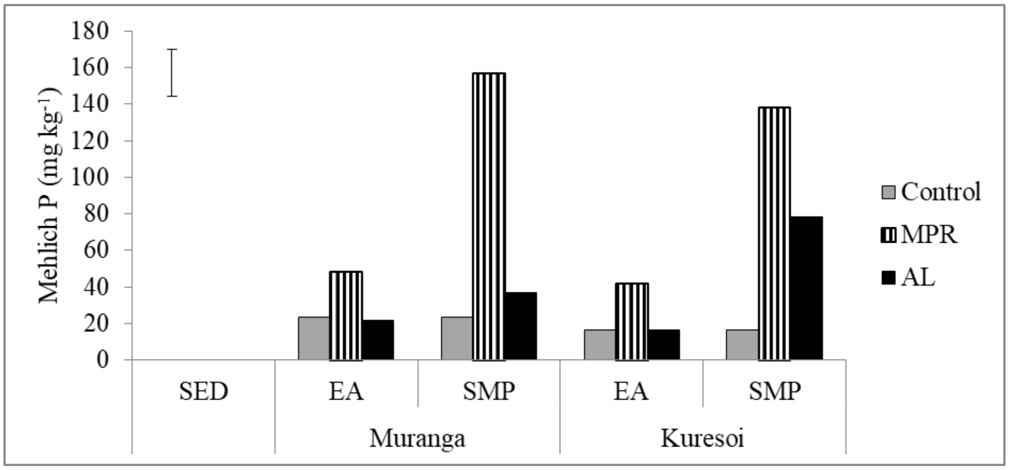

Figure 4.3: Soil available P as influenced by lime materials, lime requirement methods, and soils. The error bar represents the standard error of the difference (SED) for the lime materials, lime requirement methods, and soil interactions.

Soil and lime materials had significant influence on available P determined by Olsen P method (Appendix 4). Liming with MPR increased soil available P (45.75 mg P kg^{-1}) compared to agricultural lime (37 mg P kg^{-1}) and control (34 mg P kg^{-1}).The three-way interaction was not significant for Olsen P, however only the soil × lime requirement method was significant $p<0.05$. Soil liming with rates determined by SMP method resulted in high available P compared to exchangeable acidity with Murang'a soil having 48.89 mg P kg^{-1} and Kuresoi soil 38.33 mg P kg^{-1} (figure 4.4).

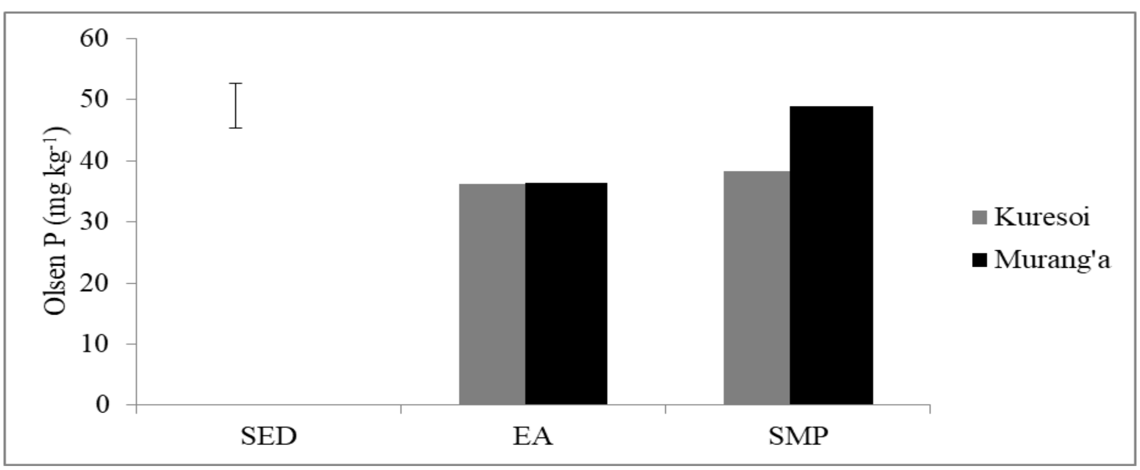

Figure 4.4: Influence of lime requirement methods on soil available P. The error bar represents standard error of the differences (SED) for the soil × lime requirement methods interactions.

4.7 Discussion

An increase in soil pH after liming indicates the ability of liming material to reduce the H+ activity (Oguntoyinbo *et al.*,2008) and precipitation of exchangeable and soluble Al in the soil solution (Opala *et al.*, 2012). The high CCE of agricultural lime increase its ability to increase soil pH compared to MPR. The low effect of MPR on soil pH compared to agricultural lime is due to the fineness of lime particles. Agricultural lime had >50% of its particles passing the <0.25mm sieve while MPR had more of coarse particles, fine particles in the lime increase lime dissolution in the soil hence more efficiency in raising soil pH (Kym *et al.,* 2010). Mijingu phosphate rock has been found to have low solubility affecting its use as a lime material alone, however, its combination with farmyard manure (Opala *et al.,* 2012) or agricultural lime has been seen to reduce acidity (Nekesa *et al.,* 2011) and increase available P as shown in this study. Soil incubation studies have shown changes in soil pH after liming within the second week (Mercy & Ezekiel, 2007). The decline in soil pH after four weeks of incubation is due to maximum lime absorption by the soil colloids.

Application of lime based on SMP method has been found to be effective in raising soil pH to target pH of 6.0 compared to exchangeable acidity. Exchangeable acidity has been on its own not to be a suitable method in lime requirement (Ssali & Nuwamanya, 1981) and researchers recommend using it along with residual acidity (Mehlich *et al.,* 1976). Mehlich (1976) recommended the use of Mehlich buffer for determining lime requirement of acid tropical soils, however, Ssali & Nuwamanya (1981) found that the method underestimates lime requirement. Incubation of soil with calcium carbonate as a reference method gave a lime requirement of 15-21 T Ha^{-1} (Ssali & Nuwamanya, 1981) and they observed that SMP was accurate in determining lime requirement for soils with high or low liming needs. The findings of the current study was in contrast with those found by Husni *et al.* (1994), concluded that SMP overestimates lime for soils with high lime requirements and underestimates for those with low lime requirements and he attributed this to the predominance of H^+ and Al^{3+} in the soils. Exchangeable acidity is suitable only when liming soil to pH 5.5 (Ssali & Nuwamanya, 1981).

Liming of acidic soil has been to increase soil available P (Raij & Quaggio, 1990) and this due to release of P from Al and Fe with the reduction in soil acidity. Calcium in the extracting solution of Olsen P prevents dissolution of labile P thus underestimating the amount of Olsen P (Raij & Quaggio, 1990; Mercy *et al.,* (2007). Mercy *et al*. (2007) also observed an increase in soil available P in limed soils over the without-lime, however, a reduction in soil available P has been observed with high rates of liming (Fageria *et al.*, 1995). Mijingu phosphate rock has high amount of P compared to agricultural lime this is due to initial levels of P in MPR. Soil lime estimation by exchangeable acidity method resulted in low level of available P for both Mehlich P and Olsen P compared to SMP method, indicating its unsuitability in amelioration of acidic soil.

The SMP method recommended high liming rates compared to exchangeable acidity this was due to the buffering capacity of the soils. Exchangeable acidity method only considered Al and H ions while SMP consider the buffering capacity of the soil. The greater the buffering capacity, the greater the quantity of base used to alter the pH (Thomas, 1996). Soil rich in organic matter with a high buffering capacity would require more lime in order to raise the pH. Previous studies have revealed that soil buffering capacity is governed mostly by acidic functional groups, dissolution or precipitation of carbonates and CEC, in soils with pH> 4.5 (Nelson & Su, 2010). Soil carbonates and nonacidic exchangeable cations can also buffer pH; carbonates consume added hydrogen carbonates and exchangeable non-acid cations exchange with hydrogen carbonates, removing it from solution.

4.8 Conclusion

Agricultural lime was most effective in raising soil pH based on the SMP LRM. There was an increase in Olsen P and Mehlich available P to above optimal level when soils were limed based on SMP rates. Although soil limed with MPR had high amounts of available P, its use as a liming material is limited to its low CCE and particle size giving it a high recommendation rates compared to agricultural lime. This study suggests more research to be conducted on field calibration of the SMP method and recommendation on the split application of liming materials to avoid the incidence of under/ over liming.

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CHAPTER FIVE

EFFECT OF LIMING AND INOCULATION ON SOYBEAN NODULATION AND BIOLOGICAL NITROGEN FIXTION

5.1 Abstract

Soil acidity is a major limiting factor to crop production and acidic soils limit soybean nodulation and BNF. Soil liming is one of amelioration ways of reducing effects of soil acidity in achieving maximum yields. This study aimed at finding the effect of soil liming on soybean nodulation and BNF, using soybean varieties TGx1740-2F (SB19) and Nyala and inoculation with Biofix and Legumefix. A greenhouse experiment was set up at IITA Nairobi using two acidic soils, Kuresoi (pH 4.9) and Murang'a (pH 5.0). The treatments included liming with agricultural lime and without-lime, two soybean varieties SB19 and Nyala and three inoculation treatments (Biofix, Legumefix, and control). The experiment was in completely randomized design (CRD) and each treatment combination replicated three times. Analysis of variance was done at 95% confidence level using SAS software. For Kuresoi soil, Soil liming and inoculation with Legumefix significantly increased nodules fresh weight (5.8 g $plant^{-1}$), nodules effectiveness (88.61%), shoot dry weight (27.82 g $plant^{-1}$), N uptake (0.27 g $plant^{-1}$). Inoculation of SB19 with Legumefix grown in limed soil increased the amount of nitrogen derived from atmosphere (Ndfa) by 58.57%. For Murang'a soil, Soil liming and inoculation with Legumefix was significant in increasing nodules fresh weight (5.3 g $plant^{-1}$) and Ndfa (62.35%). Inoculation of SB19 with Legumefix grown in limed soil increased nodules effectiveness (91.25%). Soybean variety and inoculation had significant influence on N uptake, with inoculation of Nyala with Legumefix resulting in 0.08 g $plant^{-1}$ of N uptake. The mean soil pH at the end of the experiment was 6.0 indicating consecutive planting is possible after soil liming. These results indicate that co-application of inoculants and lime has potential of increasing soybean nodulation and nitrogen fixation in acidic soils.

Key words: Biofix, Legumefix, Liming, N uptake, Ndfa,

5.2 Introduction

Soil acidity is one of the limiting factors to crop production in the tropical and subtropical soils (Joann *et al.,* 2001). Soil acidity covers 30% of world land surface, and 40% is arable land and in Kenya about 13% of arable land surface is affected by soil acidity. Soil acidity has been associated with leaching of basic cations in high rainfall areas, use of acidifying fertilizers and in larger extend soil formation from non-calcerous parent materials that are acidic in nature acidic (Kisinyo *et al.,* 2016). Crop harvesting has contributed to nutrients depletion removed through harvesting with minimal inputs to replenish lost nutrient contributing to soil acidity.

Acidic soils are associated with Al, Fe and Mn toxicity and deficiency of available P, Mo, Ca, Mg and K (Cristancho *et al.,* 2011). Crop production in tropical acid soils can be improved through liming and amelioration of soil acidity will improve plants nutrients availability, reduces solubility of Al and Fe, enhances root development, water and nutrients uptake. Kenya has a high demand for soybean but production is low and due to declining soil fertility (Keino *et al.,* 2015). Soil acidity impairs soybean root development, aerial parts and hence reduces yields (Moreira *et al.,* 2014).

Soybean yields increase with application of inoculants at near neutral soil pH and does well in a pH range 5.5-8.5. At low soil pH soybean nodulation and BNF is limited and this has been attributed to low P level at pH <5.5 due to its sorption Al and Fe. Soil acidity impairs the rhizobium-plant association limiting nitrogen fixation (Yakubu *et al.,* 2010). At low soil pH, rhizobium growth and function is impaired due to proton concentration and increase in metal ions solubility (Meng-Han *et al.,* 2012). Soil liming has been reported to improve crop production, nodulation in legumes and increased microbial activity (Shrikant *et al.,* 2015) Liming of acidic soils in Kenyan rift valley resulted in reduction of exchangeable acidity, increase in available P and high maize yield (Keino *et al.,* 2015).

Application of lime increases Ca and Mg level in soil and improves bacterial function in N fixation. This could be due to high needs of P and K by soybean for optimum yields (Sikka *et al.*, 2012). Application of P, K and inoculation of soybean with Legumefix in Western Kenya did not produce high yields and soil acidity was one of the limiting factor (Keino *et al.*, 2015). This study will investigate the impact of soil liming on soybean nodulation and BNF.

5.3 Materials and methods

Soils used in the study were collected Kuresoi (0.2993°S, 35.5302°E) and Murang'a (0.7957°S, 37.1327°E) sampling was done at depth 0-15cm and transferred to the green house at ICIPE-IITA, Nairobi. The samples were air dried for 48hours and sieved through a 2mm sieve and the initial physical and chemical characteristics were determined following standards procedure as described in Okalebo *et al.*, (2002) as in section 3.3.4. Containers of 2kg capacity were fill with soils and incubated with agricultural lime for 2 weeks at field capacity before planting. Soybean varieties SB19 and Nyala were surface sterilized with sodium hypochlorite for 1 minute and rinsed 5 times with sterilized distilled water and negative control (uninoculated) planting done first to avoid any potential contamination with three seeds sown per pot. Inoculation was at a rate of 1g of inoculant/100g seeds following instruction on each pack of the inoculants (Legumefix and Biofix) and the experiment laid in a completely randomized design (CRD). The Shoemaker-McLean-Pratt method determined the liming rates for the two soils as in section 4.3.3. In BNF determination, sorghum was the non-fixing plant and N difference method used to calculate BNF. Soil pH was determined from each pot before planting and after harvest. Nodules analysis performed as described in section 3.4.4.

Table 5.1: Treatment outline for the determination of effect of liming, inoculation and varieties on soybean growth

Factors	**Level**	**Description**
Soils	2	Soils of pH 4.9 and 5.0
Liming treatments	2	lime and without-lime
Inoculation	3	Biofix, Legumefix and Uninoculated (control)
Varieties	2	SB 19 and Nyala
Replications	3	Each combination repeated 3 times

Total pots number = 2×2×3×2×3=72 + 12 (additional pots for sorghum) = 84 pots

5.4 Data collection and analysis

Data collection was in reference to the first greenhouse experiment (section 3.4) and the analysis of variance done using SAS (SAS software version 9.4, 2016) for each soil sample considering the experiment as a three-factor experiment.

Statistical model

$$\mathbf{Y_{ijkl} = \mu + S_i + T_j + ST_{ij} + V_k + SV_{ik} + TV_{jk} + STV_{ijk} + \varepsilon_{ijkl}}$$

Where,

$\mathbf{Y_{ijkl}}$ is observation due to the effect of i^th^ liming, with j^th^ inoculation and k^th^ variety

$\boldsymbol{\mu}$ is the overall mean

$\mathbf{S_i}$ is the effect due to i^{th} soil liming

$\mathbf{T_j}$ is effect due to j^{th} inoculation

$\mathbf{ST_{ij}}$ is the interaction due to i^{th} soil liming and j^{th} inoculation

$\mathbf{V_k}$ is the effects due to k^{th} variety

$\mathbf{SV_{ik}}$ is the interaction due to i^{th} soil liming and k^{th} variety

$\mathbf{TV_{jk}}$ is interaction due to j^{th} inoculation and k^{th} variety

$\mathbf{STV_{ijk}}$ is the interaction due to i^{th} soil liming, j^{th} inoculation, and k^{th} variety

$\boldsymbol{\varepsilon}_{\mathbf{ijkl}}$ is the random error term.

5.5 Results

5.5.1 Soil physical and chemical characteristics and lime rates

The soils were acidic, with Kuresoi soil having pH 4.9 and Murang'a pH 5.0; the soils also had low level of available P Kuresoi (15mg P kg^{-1}) and Murang'a (18mg P kg^{-1}) and high exchangeable acidity. Kuresoi soil was limed with 19 g lime kg^{-1} soil, which was equivalent to 34 T Ha^{-1} while Murang'a soil was limed with 15 g lime kg^{-1} soil, which was equivalent to 30 T Ha^{-1}.

5.5.2 Number of pods

5.5.2.1 Kuresoi soil

Soil liming had significant influence on the number of pods $p<0.05$. Soybean planted in soils limed with agricultural lime had high number of pods (31.78 $plant^{-1}$) compared to those in without-lime treatments (16.89 $plant^{-1}$) (figure 5.1). Soybean inoculation with Legumefix and Biofix also had significant influence on the number of pods $p<0.05$ above the uninoculated plants, however inoculation with Legumefix produced 36.83 pods plant^{-1} which was high compared to Biofix which had 23.83 pods plant^{-1}. Soybean variety SB19 had high number of pods (28.72 plant^{-1}) compared to Nyala which had 10.4 pods plant^{-1}. Both the two and three-way interactions for the number of pods were not significant (Appendix 6). All the control plants in limed and without-lime treatments had low number of pods compared to the inoculated plants.

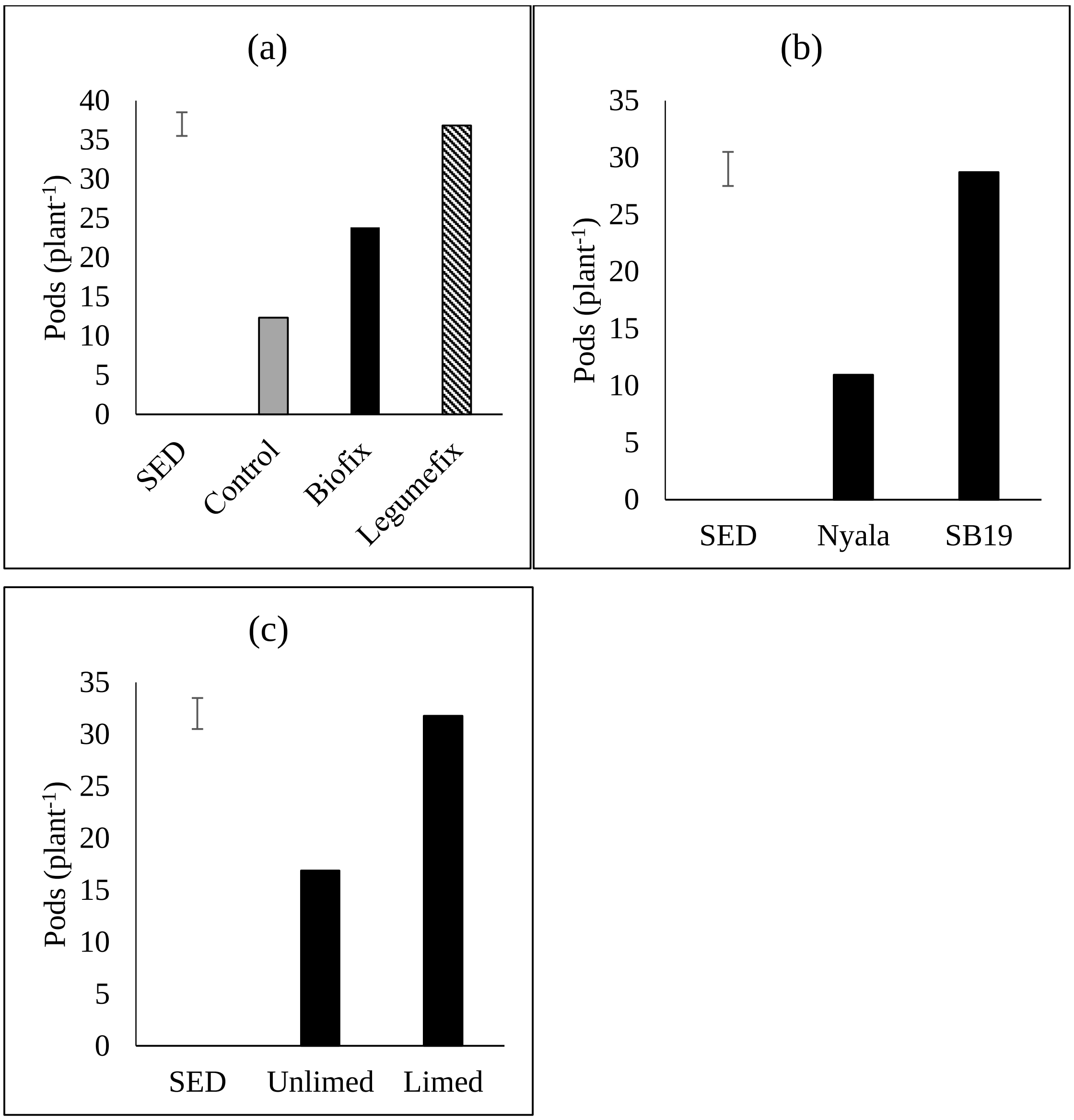


Figure 5.1: Number of pods as influenced by (a) inoculation, (b) soybean varieties and (c) liming in Kuresoi soil. The errors bars indicate the standard error of differences (SED).

5.5.2.2 Murang'a soil

Single treatment of soybean variety and soil liming did not have an effect on the number of pods in Murang'a soil; however, inoculation had significant effect on number of pods $p<0.05$. Soybean inoculation with Legumefix produced 34.8 pods $plant^{-1}$ while inoculation with Biofix produced 14.75 pods $plant^{-1}$, which was low. The three-way interaction of lime ×variety × inoculation was significant $p<0.05$ for the number of pods $plant^{-1}$. Soybean variety SB19 inoculated with Legumefix had the highest number of pods (42) in soil limed with agricultural lime. Inoculation with Legumefix performed better compared to Biofix in both limed and without-lime treatment. All the control plants in both limed and without-lime, had low number of pods $plant^{-1}$ compared to the inoculated plants.

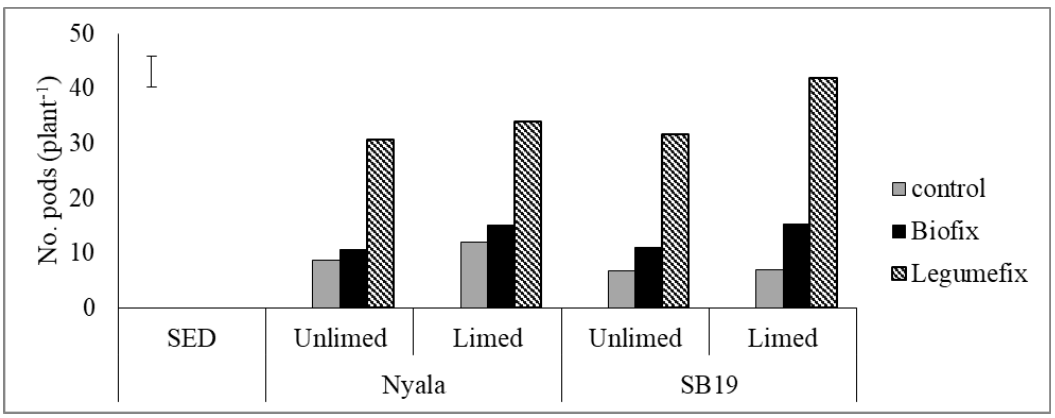


Figure 5.2: Number of pods as influenced by liming, soybean varieties and inoculation interaction in Murang'a soil. The error bar indicates the standard error of differences of the means (SED).

5.5.3 Nodule fresh weight

5.5.3.1 Kuresoi soil

The treatments had significant effects on nodule fresh weight with soybean planted on limed soil having high nodule fresh weight (2.78g $plant^{-1}$) compared to those planted on without-lime treatment (1.61g $plant^{-1}$). Soybean variety SB19 produced high nodule fresh weight (2.61g $plant^{-1}$) compared to Nyala (1.78g $plant^{-1}$). Soybean inoculation with Legumefix resulted in high nodule fresh weight (4.90g $plant^{-1}$) compared to Biofix (1.66g $plant^{-1}$). The three-way interaction was not significant for the nodules fresh weights, however, lime × inoculation and variety × inoculation were significant at $p<0.05$ and $p<0.0001$ respectively. All limed soils had high nodule fresh weight compared to without-lime treatments. Soybean inoculation with Legumefix performed better than

Biofix in both limed and without-lime treatment (figure 5.3a), however the limed soil planted with soybean inoculated with Legumefix had the highest nodule fresh weight (5.80g plant^{-1}) compared to without-lime treatment (4.00g plant^{-1}). Inoculation with Biofix resulted in high nodule fresh weight (2.52g plant^{-1}) in limed soils compared to without-lime treatments (0.83g plant^{-1}). Control plants did not produces nodules in both limed and without-lime treatments. Nyala inoculation with Legumefix had the highest nodule fresh weight (5.33g plant^{-1}) while inoculation of SB19 with Legumefix had 4.48 g plant^{-1} (figure 5.3b). Soybean variety SB19 inoculated with Biofix produced 3.35g plant^{-1} of nodules while Nyala with Biofix produced 1.23g plant$^{-1,}$ which was lower. The control plants of both Nyala and SB19 did not produced any nodules.

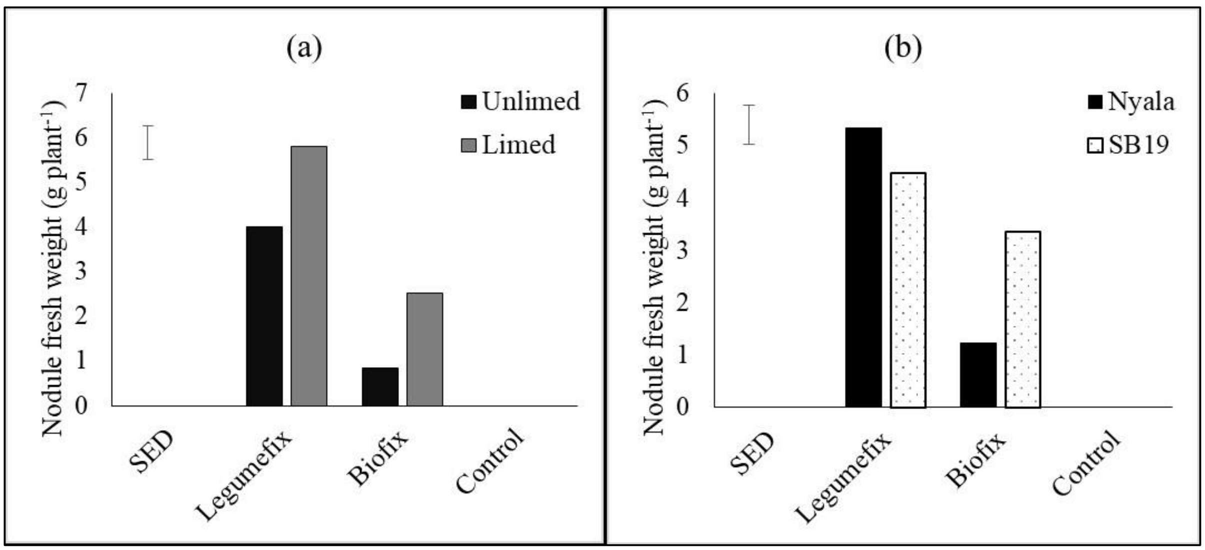


Figure 5.3: Nodule fresh weight as influenced by (a) liming and inoculation and (b) variety and inoculation on soils of initial pH 5.0 (Kuresoi soil). The error bars indicate the standard error of differences of mean.

5.5.3.2 Murang'a soil

Soil liming and soybean variety did not have significant effect on the nodule fresh weight, however inoculation had a significant effect with inoculation using Legumefix resulting in high on nodule fresh weight (4.5g plant^{-1}) while inoculation using Biofix produced 1.75g plant^{-1} of nodule fresh weight. The three-way interaction for lime, variety and inoculation was not significant. The two-way interaction of lime ×variety, lime ×inoculation and inoculation ×variety were significant at $p \leq 0.05$ (Appendix 6). Soybean inoculated with Legumefix on limed soil produced highest nodules fresh weight (5.3 g plant^{-1}) compared to inoculation with Biofix (2.97g plant^{-1}). Inoculation with Legumefix resulted in much higher nodules fresh weight compared to Biofix in both lime and without-lime treatments (figure 5.4a). Biofix inoculant was more effective in increasing nodule fresh weight in limed soils (2.97g plant^{-1}) than without-lime treatments (1.93g plant^{-1}). The control plants in both lime and without-lime treatment did not produce any nodules. For the interaction of lime with varieties, soils liming resulted in increase in nodule fresh weight (figure 5.4b) in both Nyala and SB19 with Nyala producing highest nodule fresh weight (2.47g plant^{-1}) compared to SB19 (2.1g plant^{-1}). Soybean variety SB19 did better in without-lime treatments (1.9g plant^{-1}) compared to Nyala variety (1.4g plant^{-1}). For the interaction of variety with inoculation (figure 5.4c), Nyala inoculation with Legumefix resulted in increase of nodules fresh weight (5.33 g plant^{-1}) compared to inoculation with Biofix (1.44g plant^{-1}). Inoculation of SB19 and Nyala with Legumefix had significant effect in increasing nodules fresh weight compared to inoculation with Biofix. The control plants of both soybean varieties did not produce any nodules.

5.5.4 Nodules effectiveness

5.5.4.1 Kuresoi soil

Soil liming did not have significant influence on nodule effectiveness, however, soybean varieties and inoculation had a significant effect on nodule effectiveness $p<0.05$. Inoculation with SB19 resulted in high percentage effective nodule (58.33) compared to Biofix inoculation (28.69%). The three-way interaction was not significant for nodules effectiveness, however all the two-way interactions were significant. Lime × inoculation, variety × inoculation and lime × variety were significant at $p<0.01$, $p<0.0001$ and $p<0.05$ respectively (Appendix 6). Inoculation with Legumefix on limed soil resulted in high effective nodules (88.61%) compared to Biofix in limed soil (43.26%). Both lime and without-lime with plants inoculated with Legumefix had the highest effective nodules compared to those inoculated with Biofix (figure 5.5a). Both limed and without-lime treatments planted with SB19 variety had high effective nodules compared to Nyala variety (Figure 5.5b). Effective nodules in SB19 planted in lime soil was higher (57.77%) compared to Nyala in limed soil (24.05%). In terms of varieties with inoculation, SB19 inoculation resulted in high effective nodules compared to Nyala using either Biofix or Legumefix (figure 5.5c). Inoculation of SB19 with Legumefix had most effective nodules (87.67%) compared to Nyala inoculated with Legumefix (80.92%). The control plants did not have nodules, hence no effectiveness.

5.5.4.2 Murang'a soil

Soil liming did not have significant influence on nodule effectiveness $p<0.05$; however inoculation and soybean variety had significant effect. Inoculation with Legumefix resulted in higher nodule effectiveness (80.25%) compared with Biofix (41.91%). Soybean variety SB19 produced higher effective nodules (55.17%) compared to Nyala (26.27%). The three-way interaction of lime × variety × inoculation was significant for effective nodules $p<0.05$. Inoculation with Legumefix produced high percentage effective nodules (Figure 5.6). Limed soils planted with SB19 inoculated with Legumefix did better than those inoculated with Biofix. All control for both lime and without-lime treatments had no recorded nodules.

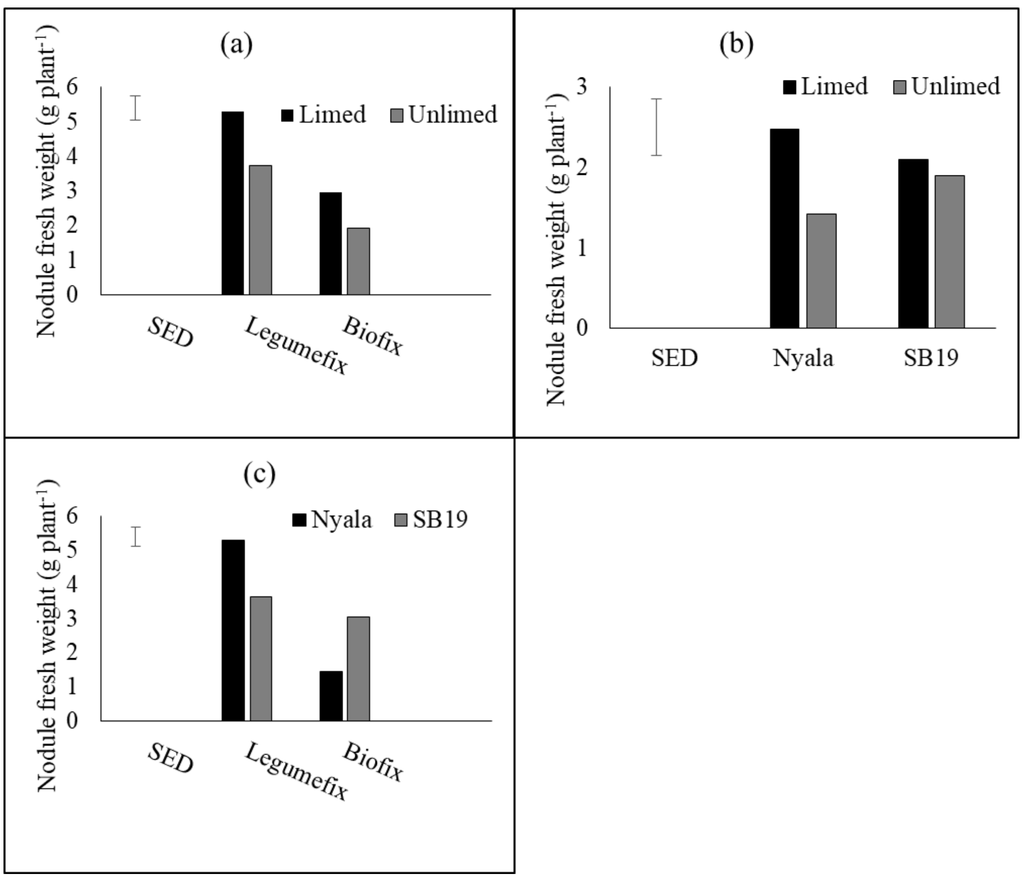


Figure 5.4: Nodule fresh weight plant as influenced by (a) interaction of liming ×inoculation, (b) interaction lime × variety and (c) interaction of variety × inoculation in Murang'a soil. The error bars indicate the standard error of differences of means (SED).

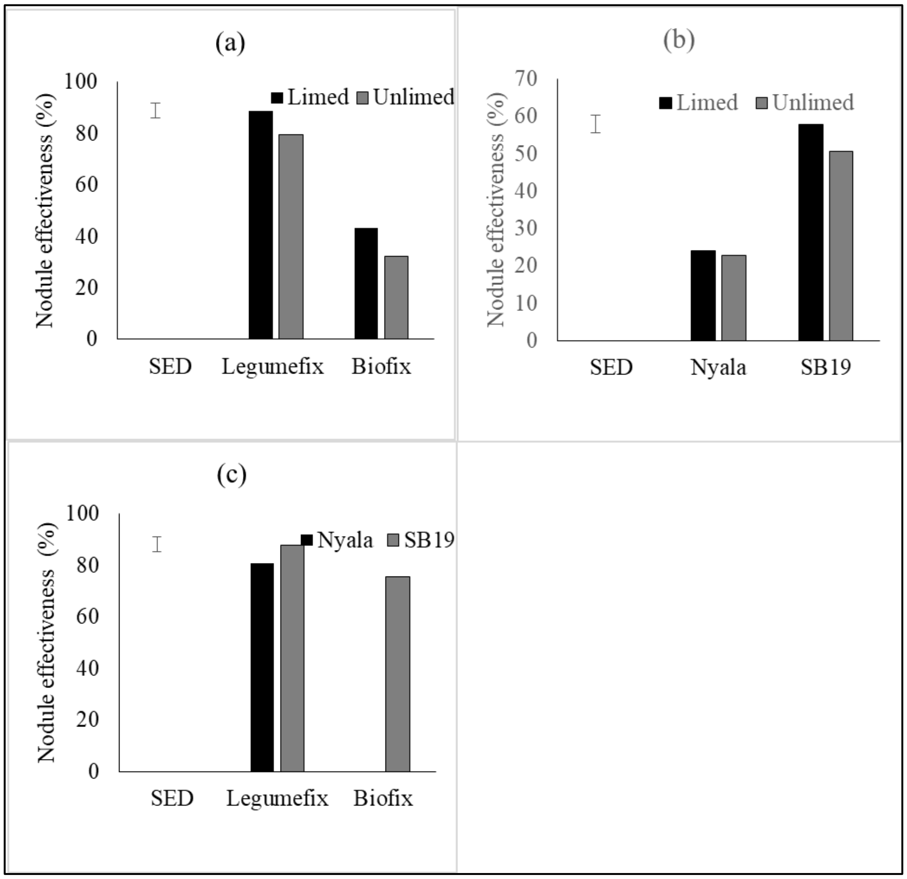


Figure 5.6: Nodule effectiveness as influenced by (a) interaction of liming × inoculation, (b) interaction of liming × variety, and (c) interaction of variety × inoculation on Kuresoi soils. The error bars indicate the standard error of differences of the means (SED).

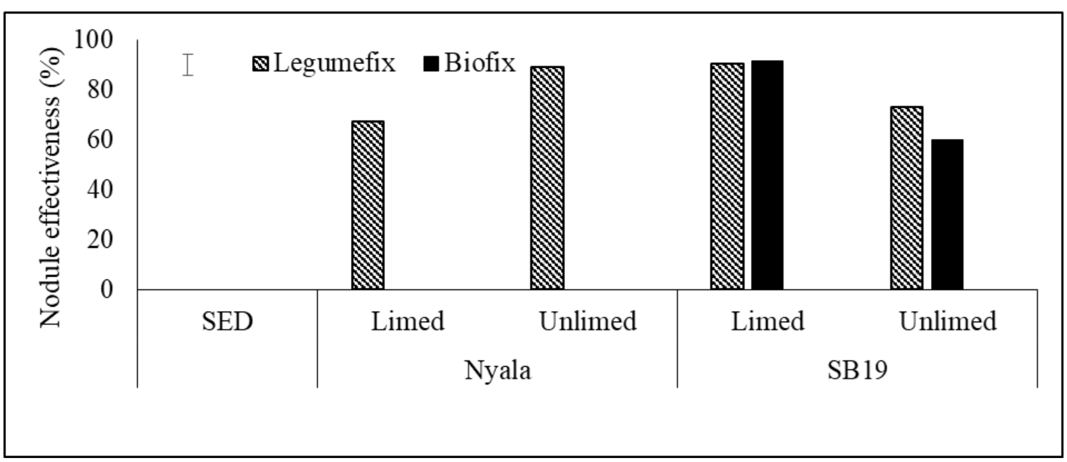


Figure 5.7: Nodule effectiveness as influenced by interaction of liming, soybean varieties and inoculation on Murang'a soil. The error bar indicates the standard error of differences of mean (SED).

5.5.5 Shoot dry weight

5.5.5.1 Kuresoi soil

Soil liming and inoculation had significant effect in increasing shoot dry weight, with limed soil having 19.29g plant^{-1} shoot which was high compared to shoot dry weight of without-lime treatment (10.5 g plant^{-1}). In terms of inoculation, use of Legumefix resulted in high shoot dry weight (20.57g plant^{-1}), while inoculation with Biofix resulting in (17.09g plant^{-1}) control plants had the least shoot dry weight (7.03g plant^{-1}). The three-way interaction was not significant for the shoot dry weight, and only lime × inoculation was significant $p<0.05$ for the two-way interactions. Planting of inoculated soybean in limed soil produced high shoot dry weight compared to planting inoculated soybean in without-lime treatment. Inoculation of soybean with Legumefix and planting in limed soils produced highest shoot dry weight (27.82g plant^{-1}) while inoculation with Biofix produced (20.65g plant^{-1}). The plants that did not receive inoculation for the both limed and without-lime treatment had the lowest shoot biomass compared to the inoculated plants (Figure 5.7).

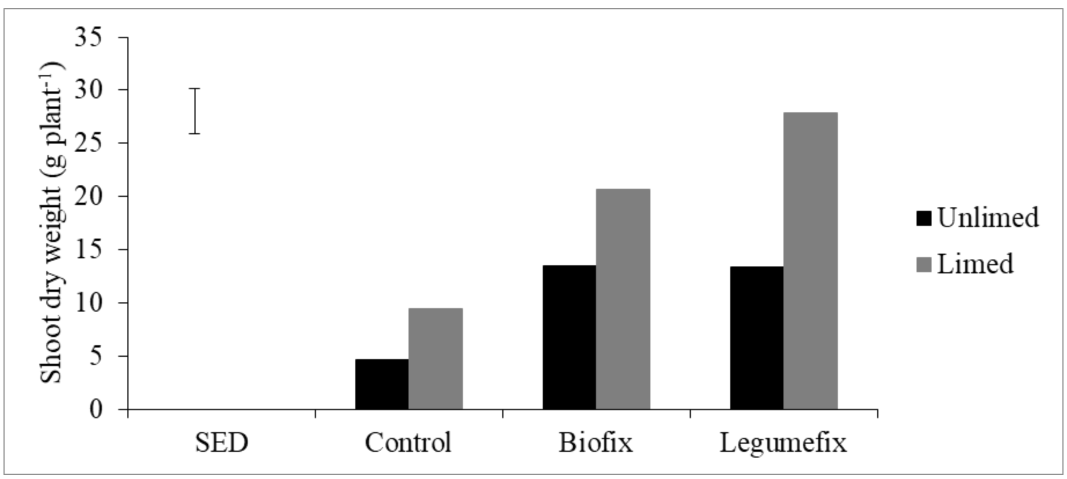


Figure 5.8: Shoot dry weight as influenced by liming, soybean varieties and inoculation on soils of initial pH 5.0 (Kuresoi soil). The error bar indicates the standard error of differences of mean (SED).

5.5.5.2 Murang'a soil

Soil liming and inoculation had significant influence on shoot dry weight. Limed soil produced 16.47g plant^{-1} while without-lime treatment had 12.84 g plant^{-1} of shoot dry weight. Inoculation with Legumefix resulted in high shoot dry weight (45.08 g plant^{-1}) while Biofix had

9.5g shoot dry weight plant^{-1} and control (1.01g plant^{-1}). The three-way and two-way interactions were not significant for the shoot dry weight.

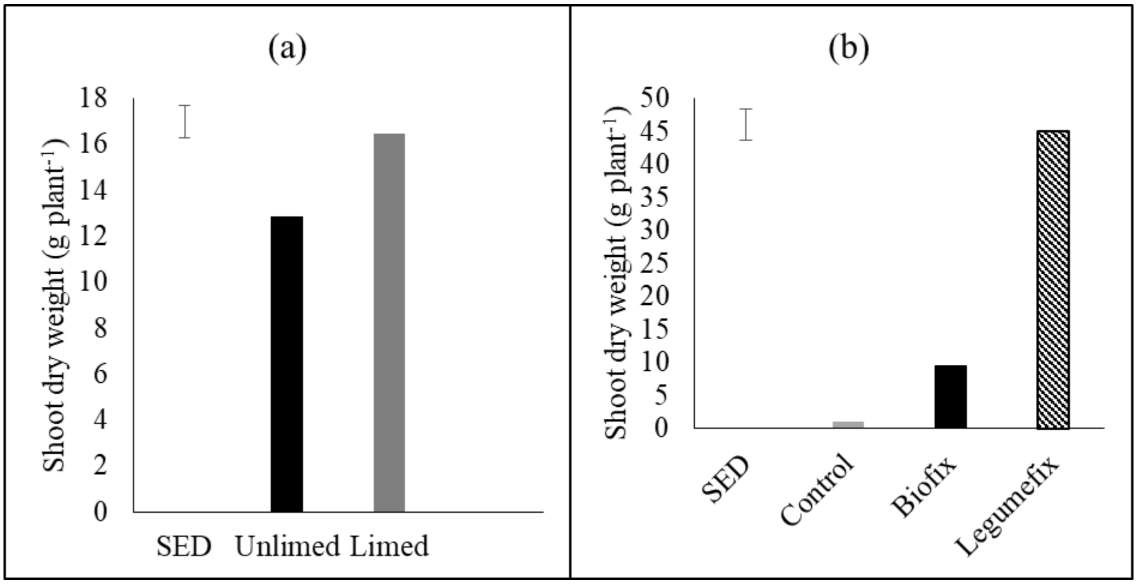


Figure 5.9: Shoot dry weight as influenced by: (a) liming and (b) inoculation on soils of initial pH4.9 (Murang'a soil). The error bar indicates the standard error of differences of mean (SED).

5.5.6 Root dry weight

5.5.6.1 Kuresoi soil

Soil liming and inoculation had significant influence on root dry weight. Limed soil had the highest root dry weight (4.3g plant^{-1}) compared to the without-lime treatment (2.9g plant^{-1}). In terms of inoculation, control plants had the lowest root dry weight (1.73g plant^{-1}) compared to those inoculated with Biofix (4.43g plant^{-1}) and Legumefix (4.72g plant^{-1}). The three-way interactions were not significant, while only the two-way interaction of lime ×inoculation were significant. Planting of soybean in limed soil and inoculation with Legumefix resulted in high root dry weight (6.01g plant^{-1}) while plants inoculated with Biofix had 4.99g plant^{-1} of root dry weight. Control plants in both lime and without-lime treatment had the least root dry weight (figure 5.10).

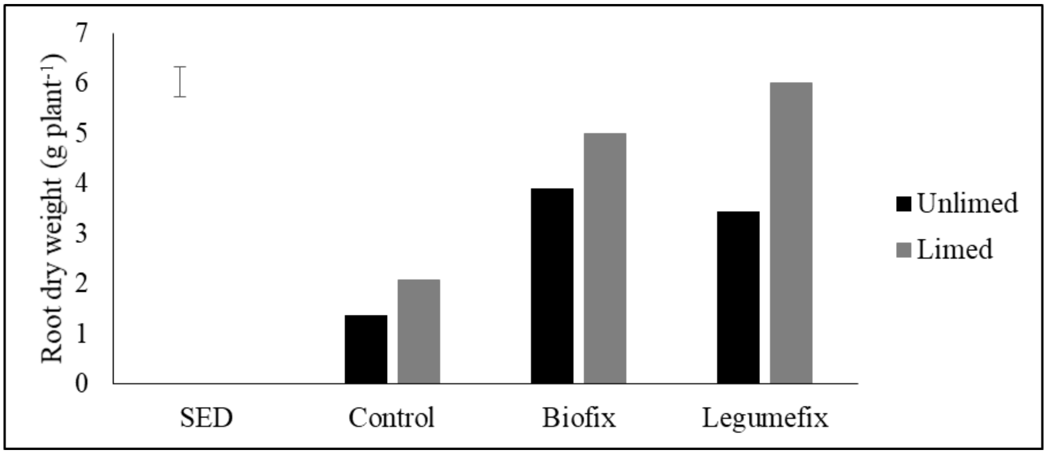


Figure 5.10: Effect of liming and inoculation on root biomass of soils pH 5.0 (Kuresoi soil). The error bar indicates the standard error of differences of mean (SED).

5.5.6.2 Murang'a soil

Soil liming and inoculation had significant influence on root dry weight. Limed soil had the highest root dry weight (4.53g plant^{-1}) compared to the without-lime treatment (4.08g plant^{-1}) (figure 5.10). In terms of inoculation, control plants had the lowest root dry weight (3.8g plant^{-1}) compared to those inoculated with Biofix (4.37g plant^{-1}) and Legumefix (4.8g plant^{-1}). Both the two-way and three way interactions were not significant for root dry weight. Soybean variety did not have any influence on root dry weight.

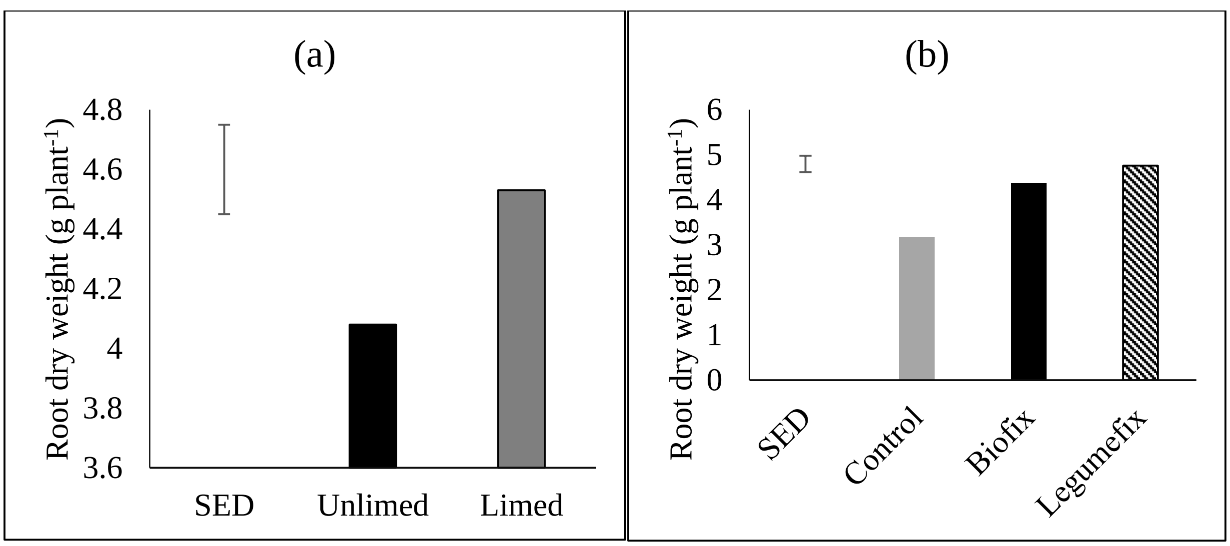


Figure 5.11: Influence of: (a) liming and (b) inoculation on the root dry weight in Murang'a soil. The error bar indicates the standard error of differences of the means.

5.5.7 Biological nitrogen fixation and phosphorus uptake

5.5.7.1 Kuresoi soil

Soil liming had significant effect in N uptake; limed recorded 0.08g $plant^{-1}$ while without-lime treatment had 0.04g $plant^{-1.}$ Inoculation with Legumefix resulted in high N uptake of 0.12g plant-1, while Biofix had 0.08g $plant^{-1}$. The three way interaction of lime, variety and inoculation was not significant, only the two-way interaction of lime × inoculation was significant at $p \leq 0.05$. Soybean inoculation in limed soil had high N uptake compared to without-lime treatment (figure 5.12a). Planting of soybean inoculated with Legumefix in limed soil resulted in high N uptake (0.27g $plant^{-1}$) while inoculation with Biofix had N uptake of 0.18g $plant^{-1}$. Control plants in both lime and without-lime treatment had the least N uptake.

Soil liming and inoculation also had significant effect on Ndfa. Planting in limed soil resulted in 79.61% Ndfa higher compared to without-lime treatment 30%. Inoculation also improved BNF, with use of Legumefix resulting in 69.25% Ndfa and for Biofix (59.73%). The

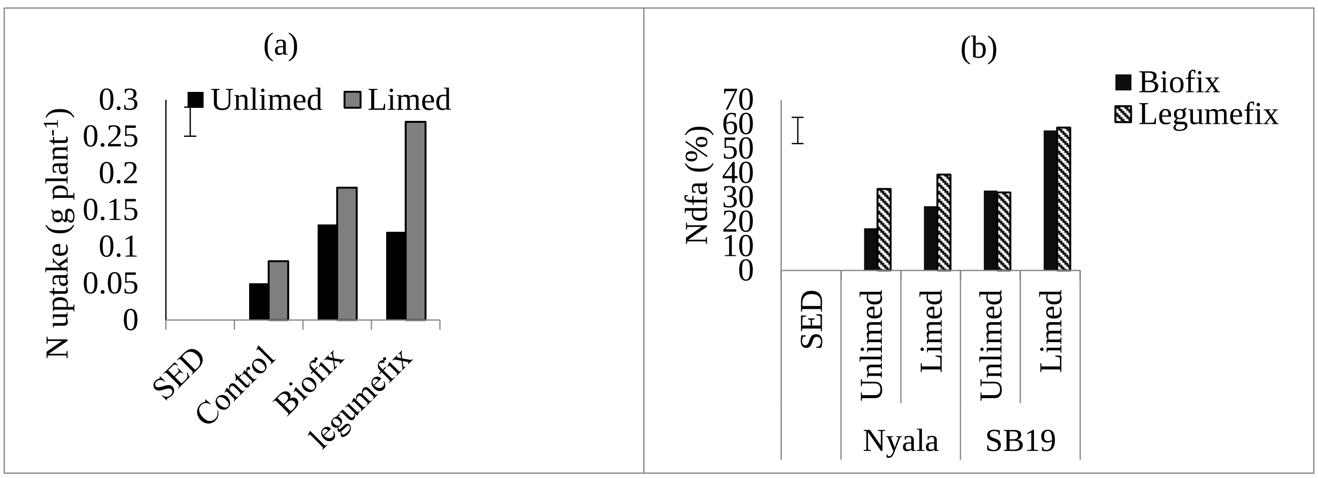
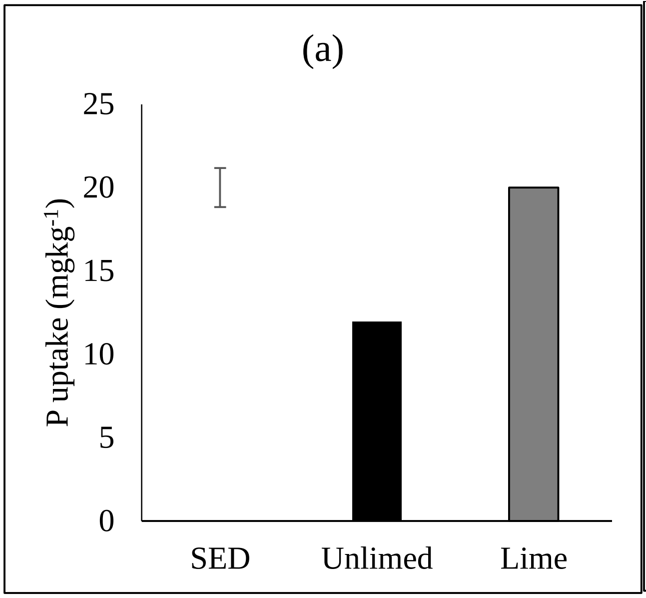


Figure 5.12: (a) Nitrogen uptake as influenced by liming and inoculation interaction and (b) percent Ndfa as influenced by interaction of lime, variety and inoculation in Kuresoi soil. The error bars indicate the standard error of differences of the means.



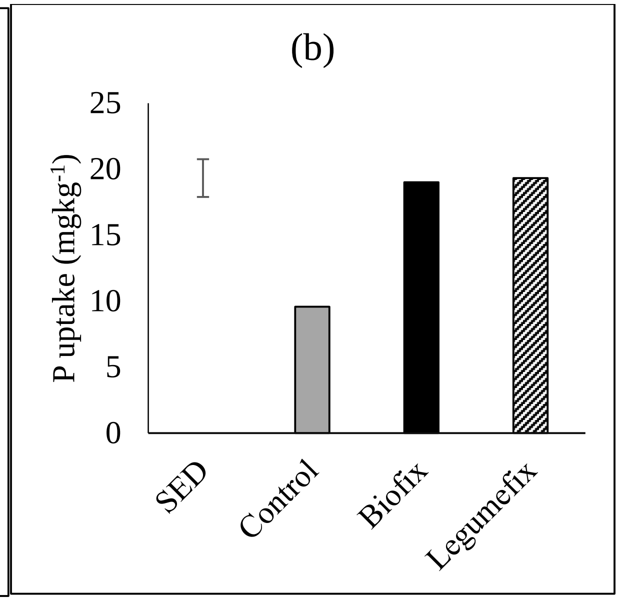


Figure 5.13: Shoot P as influenced (a) liming and (b) inoculation in Kuresoi soil. The error bars indicate the standard error of differences of the means.

5.5.7.2 Murang'a soil

Soil liming did not have significant influence on N uptake; however, inoculation and soybean variety were significant in influencing N uptake. Inoculation with Legumefix resulted in high N uptake (0.07g plant^{-1}) compared to Biofix (0.03g plant^{-1}). Soybean variety SB19 had high N uptake (0.04g plant^{-1}) compared to Nyala (0.02g plant^{-1}). The three-way interaction was not significant for N uptake; however, lime and variety interactions were significant at $p \leq 0.05$. Inoculation of Nyala and SB19 with Legumefix had the highest N uptake compared to inoculation with Biofix (figure 5.13a). Nyala inoculation with Legumefix had 0.08 g plant^{-1} N uptake while for SB19 was 0.07g plant^{-1}.

Soil liming had significant influence on Ndfa (nitrogen derived from atmosphere), with lime soil having 11.32% Ndfa while without-lime treatment had 8.53%. Inoculation with Legumefix resulted in high Ndfa (43.38%) compared to Biofix (22.34%). The three-way interaction was not significant, however only the two-way interaction of lime and inoculation were significant $p \leq 0.05$. Soybean inoculation in limed soil had high percentage Ndfa compared to without-lime (figure 5.14b). Planting soybean inoculated with Legumefix in limed soil resulted in high percentage Ndfa (62.35) compared Biofix (46.58). soil liming had significant influence on P uptake, plants in limed soil had average of 23.39mgkg^{-1} P while those in without-lime treatment had 16.88mgkg^{-1} P. Soybean Inoculation with Biofix and Legumefix produced had high average

P uptake of 25.75mgkg^{-1} P and 0.25 mgkg^{-1} P respectively above control 10.01 mgkg^{-1} P. The three-way and two-way interactions were not significant for P uptake in soybean.

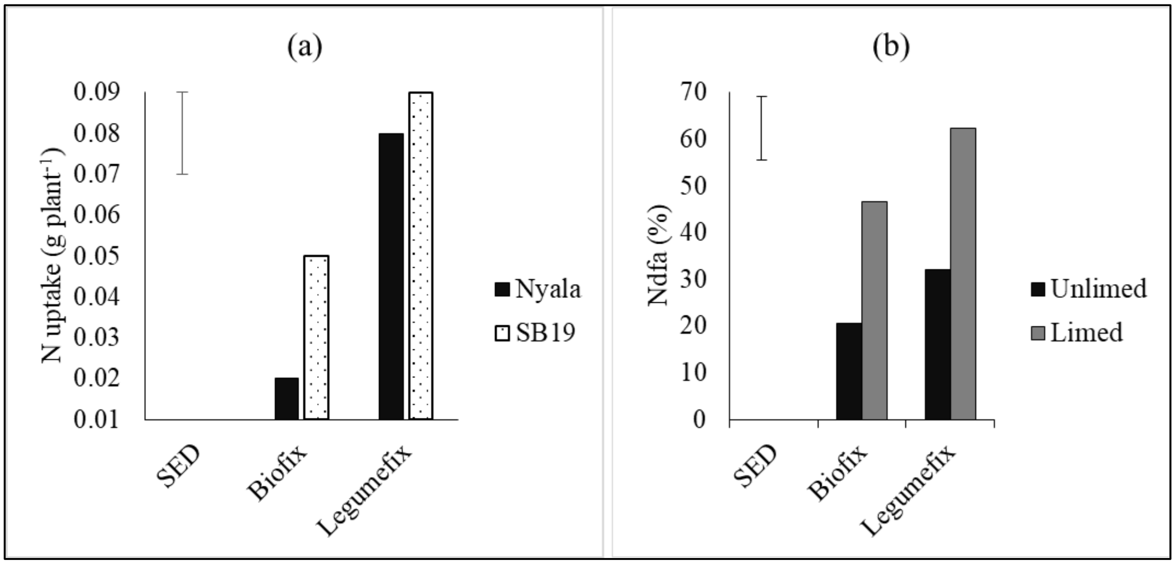


Figure 5.14: (a) Nitrogen uptake as influenced by variety and inoculation interaction and (b) Ndfa as influenced by interaction of lime and inoculation in Murang'a soil. The error bars indicate the standard error of differences of the means.

5.5.8 Ttest for pH at planting and pH at harvesting

The paired Ttest conducted to compared soil pH at planting and at harvesting showed significance difference between soil pH at planting (5.3±0.5) and pH at harvesting (5.9±0.7) at df (71), t=6.91 and p<0.0001

5.5.9 Nodule analysis

Nodules from SB19 inoculated with Legumefix in both lime and without-lime treatments were analyzed for Kuresoi and Murang'a soil (Table 5.6). There were no enough nodules from Nyala inoculated with Biofix in limed soil, Nyala and SB19 inoculated with Legumefix in without-lime treatment in Kuresoi soil for analysis. For Murang'a soil, Nyala inoculated with Legumefix in without-lime treatment, SB19 inoculated with Biofix in both lime, and without-lime treatments, lack enough nodules for analysis. Control plants treatments did not nodulate.

Table 5.2: Intergenic spacer region (percentage) from nodule analysis of soybean variety planted in limed and without-lime treatment

Soil		Inoculants	Intergenic spacer region (%)			
			Nyala		SB19	
			I	II	I	II
Kuresoi	Limed	Biofix	0	0	0	75
		Legumefix	62.5	0	50	0
	Without-lime	Biofix	0	75.00	0	0
		Legumefix	0	0	62.5	0
Murang'a	Limed	Biofix	0	100	0	0
		Legumefix	50	0	62.5	0
	Without-lime	Biofix	0	50	0	0
		Legumefix	0	0	62.5	0

5.6 Discussion

Soil acidity affects plants growth and development and in soybean nodulation and BNF is mostly impacted. Liming and inoculation resulted to high number of pods this may be attributed to the reduced soil acidity hence reduction in aluminum toxicity. This is in agreement with results found by Fageria *et al.* (2013) where after application of 18 T Ha^{-1} number of pods increased. Soil liming improves nutrients availability including P, Ca and Mg. Low number of pods in without-lime treatment can be attributed to low soil available P caused by P sorption by Al and Fe at low soil pH (Keino *et al.*, 2015) . Mesfin *et al.* (2014) also observed increase in numbers of pods in haricot beans in limed soil. Soil liming and soybean inoculation had a positive impact on nodules fresh weight and nodule effectiveness compared to soybean inoculation in without-lime treatments. This is has also been observed by Mullen *et al.* (2006) where there was poor nodulation in without-lime treatments and hence low BNF was observed. In another study, Meng-Han *et al.* (2012) recorded low nodule growth as soil pH decrease, and at soil pH <5.0 no nodules were found. Soil acidity limits shoot and root dry weight, in this study soil liming increased shoot and root dry weight. The high shoot and root biomass in limed soils has also been observed by Keino *et al.* (2015) and they attributed this increase in plant nutrient availability due to reduction in soil acidity.

Acidic soils have low level of Ca hence impairing roots development (Whalen *et al.,* 2001). Cations K, Mg and Ca are prone to leaching in high rainfall areas leading to their deficiency and soil liming increases cations saturations in soil solution increasing their uptake hence increase in shoot and root dry weight (Keino *et al.,* 2015). Poor roots development in acidic soils is attributed Ca deficiency. Calcium plays a role in cell growth both at terminal and root tip and in low Ca soils, browning and dying of the root tip causes poor root system development (Keino *et al.*, 2015). Magnesium plays a role in enzyme activation in protein synthesis hence plant growth and development. The nutrients deficiencies contribute to low root dry weight, poor nodulation, retarded growth with low shoot dry weight and poor pod formation due limited nutrients uptake. Soybean variety SB19 had high shoot dry weight compared to Nyala variety. This result is in agreement with Thuita *et al.* (2011) where SB19 had much higher shoot dry weight compared to Nyala and its was attributed to high N fixing ability of the promiscuous soybean varieties contributing to high biomass and pod formation. Moreira *et al*, (2015) also observed increase in shoot dry weight with liming on different soybean varieties. The study concluded that different soybean varieties adapted to tropical and subtropical soils respond differently to soil acidity. Soil liming improved N and P uptake compared to without-lime treatment, and this is in agreement with study by Fageria *et al.* (2013) and he concluded that N and P uptake increased with soil liming. Whalen *et al*. (2001) also observed that soil liming improved plant nutrients concentration in canola and wheat. Benvido *et al.* (2018) observed that application of lime increased N and P uptake but its combination with P fertilizers and organic manure had significant influence; this contrasts this study since use of lime alone improved P uptake. The impact of liming in improving P availability is debatable and its ability to increase P availability is high dependent on lime rates. At high lime rates, soil pH raises to >6.5 and soluble P forms complex with Ca and at low lime rate there is toxicity effects of Al and Fe. A study by Keino *et al.* (2015) are in agreement with current study where soil liming increase nutrient uptake in soybean. Increase in soil pH after liming had a positive effect on BNF. At soil pH above 5.5, there is increasing in nitrogenase activity hence high rate on N fixation due to easy uptake of Mo and K.

Various researchers have observed the effect of liming in the consecutive planting season. At the end of this study, the soil pH in limed soil was 6.0 and this indicated that replanting will translated in much higher response in nodulation, podding, biomass and generally BNF. Mullen *et al.* (2006) reported that soil liming supported subsequent planting of wheat and canola for up to 9 years. Soil liming has support soybean growth for subsequent 3 years after application of 18T Ha^{-1} during the first season (Fageria *et al.*, 2013).

5.7 Conclusion

Inoculated soybean grown in limed soils compared to without-lime treatment has significance influence on number of pods, shoot and root biomass, nodule fresh weight, BNF and nutrients uptake (N and P). The liming rates used in this study were relatively higher compared to what other authors recommended thus, there is need for a more convenient method of lime requirement determination to ensure maximum economic yield for soybean production.

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CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

Soil acidity limits nutrients uptake in soybean affecting nodulation, biomass, and nitrogen fixation. The measured number of pods was highest in soils S5-S10 and low in S1-S4. The observed difference is due to the effects of soil acidity on soybean grown in soils of pH < 5. Acidic soils have high concentration of Al, Fe and H ions limiting root development, hence poor development of the roots in turn cause poor nutrients uptake which are already limiting in these soils. Inoculation in acidic soils did not increase the measured parameters; this is attributable to soil acidity effects on the rhizobium and the soybean variety itself. The root and shoot biomass in soils S1-S4 (pH <5) was low and this was attributed to the toxic effects of soil acidity on plant roots, impairing nutrients uptake and in turn contributing to poor plant growth.

There was no nodulation in extreme acidic soils, soils S1-S4 (pH<5.0) and control plants except for soil S9 and S10. Nodulation in the control plants in soil S9 and S10 is due to the presence of native rhizobia, however, the nodules fresh weight and nodules effectiveness was lower than that of inoculated plants indicating that the inoculants were effective in increasing nodulation. Inoculation ineffectiveness is mainly due to rhizobia sensitivity to low soil pH, toxicity effects of Al and Fe on the rooting system of the plants affecting root and nodules formation and functioning. Soil acidity causes binding of P by Al ions, causing low levels of P in soil solution. This impairs plants growth, nodulation since P play a major role in increasing the size, and number of nodules, the amount of N fixed and in turn increases the amount of nitrogen uptake by the host legume and improves the density of Rhizobia bacteria in the soil surrounding the root. Inoculation of soybean in soil pH >5.5 had a high level of nitrogen fixation and uptake compared to soils with pH <5.5; this is attributed to poor roots development and poor nodulation in acidic soils. Soybean variety SB19 had a high level of Ndfa and N uptake after inoculation with Biofix in soil S7 and inoculation with Legumefix in soil S6. These soils had pH >5.5 hence high nutrients availability including K which is crucial in N fixation. Nodules effectiveness and BNF are directly related to the number of nodules per plant, hence affected directly as a result of soil pH and nutrient content. Calcium also plays a major role in rhizobia-legume symbioses, it’s used for adhesion by rhizobia hence its deficiency in acidic soils affects rhizobia attachment, infection and infection thread formation thus nitrogen fixation is negatively affected.

Soil liming increases P, Ca, Mg, K, Mo availability in soil and reduces the toxic effects of Al, Fe and H ions in soil, hence increased nutrients uptake by plant increasing nodulation. Application of agricultural lime increased soil pH-to-pH 6.0 in both acidic soils S2 and S4 compared to Mijingu phosphate rock (MPR). The difference in changes in soil pH between the two is due to the high calcium carbonate equivalent (CCE) and fineness of agricultural lime compared to MPR. Estimation of lime requirement using the Shoe-maker, McLean and Pratt method resulted in change of soil pH to target pH 6.0 while exchangeable acidity method did not change soil pH to >5. Exchangeable acidity underestimates the liming needs of soil since it does not consider the soil buffering capacity. Soil limed with MPR had high amounts of available P compared to liming with agricultural lime, this is due to initial levels of P in MPR.

Soil liming with agricultural lime increased the initial soil pH of soil S2 and S4 to target pH 6.0 and co-application of inoculants and lime increased the number of pods, nodules fresh weight, root and shoot dry weight, N fixation and N and P uptake. Inoculation with Legumefix in limed soils increased nodules fresh weight compared to inoculation with Biofix. Rhizobia inoculants are highly sensitive to low soil pH (<5.5) and application of lime increases soil pH to a favorable level hence increasing rhizobia efficiency. Soil liming and inoculation of Nyala with Legumefix resulted in high nodules fresh weight and nodules effectiveness. The difference in nodulation for the two varieties is due to their maturity and nutrient uptake ability hence influence other parameters such as the number of pods, shoot dry weight and nitrogen fixation.

Soil liming increased shoot dry weight and this is due to increased plant nutrient availability due to reduction in soil acidity. Cations K, Mg and Ca are prone to leaching in high rainfall areas leading to their deficiency and soil liming increases cations saturations in soil solution increasing their uptake hence increase in shoot dry weight. Soybean variety SB19 had high shoot dry weight compared to Nyala variety and this is due to high N fixing ability of the promiscuous soybean varieties contributing to high biomass and pod formation.

Soil liming and soybean inoculation had a significant influence on nodulation. Soybean inoculation in limed soils had high nodulation compared to unlimed treatments this is due to increased nutrients availability in limed soil hence improved root hair development increasing inoculants efficiency. Inoculation of SB19 with Legumefix in limed soil increased nitrogen fixation and uptake. SB19 is a promiscuous variety with longer growth period and high ability to nodulate hence higher percentage Ndfa compared to Nyala variety. Soil liming improved nitrogen fixation and uptake compared to without-lime treatment indicating that N and P uptake increased with soil liming. The increase in soil pH after liming had a positive effect on BNF due to reduced toxic effects of Al and Fe and increased availability of Ca and Mo necessary for nitrogen fixation. At soil pH above 5.5, there is an increase in nitrogenase activity hence high rate on N fixation due to easy uptake of Mo and K.

6.2 Conclusions

Declining soil fertility is one of the limiting factor to crop production and contributing to food insecurity. Soil acidity limits soybean yields due to toxicity effects of Al, Fe and H ions and nutrients deficiency of P, Ca, Mg, Mo, and K affecting soybean growth and the rhizobia strain in the inoculants. This results in poor nodulation, nitrogen fixation, and hence low yield. The following conclusions were made from the study;

i) Biological nitrogen fixation and nodulation is effective at soil pH range 5.5-6.3 and soybean inoculation should be at this pH range.
ii) Soils of pH range 5.5-6.3 have sufficient nutrients supply for effective nodulation and BNF.
iii) Nodulation and BNF was more effective in soybean variety SB19 than in Nyala.
iv) Legumefix inoculants is more effective in increasing nodulation and BNF compared to Biofix inoculant.
v) Agricultural lime has shown to be most effective lime material to raise soil pH compared to MPR and lime estimation using SMP method resulted in a greater change in soil pH compared to exchangeable acidity.
vi) Co-application of liming and inoculation improved nodulation and BNF in acidic soil.

6.3 Recommendations

From this study, the following recommendations can be made;

i) Soybean should be planted in soil pH 5.5-6.3 were there are sufficient nutrients supply.
ii) TGx1740-2F (SB19) soybean variety is recommended due to its high response to inoculation and high nodulation and BNF.
iii) Agricultural lime is a recommended lime material in amelioration of acidic soils.

iv) There is need for further study on field calibration of SMP method in Kenyan acidic soils.

v) Documentation of lime rates for Kenyan acidic soils is crucial.

APPENDICES

Appendix 1: Nutrients salts and application rates

Nutrients	Salt	Rate of application ($mgkg^{-1}$)
P	KH_2PO_4	300
Cu	$CuSO_4.5H_2O$	0.06
Zn	$ZnCl_2$	0.2
B	$Na_2B_4O_7.10H_2O$	0.04
Mo	$Na_2MoO_4.2H_2O$	0.008

Appendix 2: Mean square table for the factors affecting soybean growth parameters

	DF	No. of pods	Nodule fresh weight (g plant^{-1})	Effective nodules (%)	Shoot dry weight (g plant^{-1})	Roots dry weight (g plant^{-1})	N uptake (g plant^{-1})	Ndfa (%)	P uptake (mg kg^{-1})
Soil (S)	9	3822.52***	99.79***	4333.78***	2776.65***	68.37***	1.30***	8235.41***	1190.49***
Varieties (V)	1	2706.69***	18.94^{ns}	493.10^{ns}	20.41^{ns}	29.22*	0.08^{ns}	1022.05^{ns}	0.94^{ns}
Inoculation (I)	2	15849.91***	430.94***	44279.22***	3542.87*	243.2***	4.92***	50317.18**	2140.35***
S×V	9	346.29**	6.03^{ns}	275.73^{ns}	443.94^{ns}	9.50^{ns}	0.04^{ns}	433.87^{ns}	34.83^{ns}
S×I	18	971.85***	28.37**	1446.67**	835.36^{ns}	15.15**	0.31***	2484.63***	157.02^{ns}
V×I	2	910.97**	9.76^{ns}	1337.07*	1099.55^{ns}	1.33^{ns}	0.01^{ns}	132.24^{ns}	43.12^{ns}
S×V×I	18	257.41**	4.54^{ns}	740.92*	941.40^{ns}	9.05*	0.07*	646.05^{ns}	52.70^{ns}
CV (%)		70.14	68.14	53.56	92.13	71.16	48.25	62.00	47.47
Mean		3.33	4.33	37.06	16.99	3.23	0.38	34.79	21.62

*, **, *** and ns indicate significant at $p<0.05$, 0.01, 0.0001 and not significant respectively

Appendix 3: Mean square for soil pH in the soil-lime incubation

Source of variation	Df	pH
Soil (S)	1	4.45^{***}
Method (M)	1	19.25^{***}
Lime (L)	2	18.90^{***}
Week (W)	5	0.26^{***}
S×M	1	0.73^{***}
S×L	2	0.77^{***}
S×W	5	0.12^{ns}
M×L	2	4.84^{***}
M×W	5	0.01^{ns}
L×W	10	0.05^{***}
S×M×L	2	0.21^{***}
S×M×W	5	0.01^{ns}
S×L×W	10	0.01^{ns}
M×L×W	10	0.01^{ns}
S×M×L×W	10	0.01^{ns}
CV (%)		1.76
Mean		5.24

*, **, *** and ns indicate significant at p<0.05, 0.01, 0.0001 and not significant respectively

Appendix 4: Mean squares for soil available phosphorus for Mehlich and Olsen P

	DF	Mehlich P (mg/100g soil)	Olsen P (mg/100g soil)
Soil (S)	1	3802.78^{*}	2320.03^{***}
Method (M)	1	10000^{**}	156.25^{ns}
Lime (L)	2	18611.11^{***}	641.08^{**}
S×M	1	11.11^{ns}	393.36*
S×L	2	1011.11^{*}	479.19^{ns}
M×L	2	11033.33^{***}	39.58^{ns}
S×M×L	2	77.78^{***}	130.03^{ns}
CV (%)		47.50	24.52
Mean		51.39	42.92

*, **, *** and ns indicate significant at $p<0.05$, 0.01, 0.0001 and not significant respectively

Appendix 5: Mean square for the factors affecting soybean growth parameters (Kuresoi Soil)

	DF	No. of pods	Nodule fresh weight (g plant^{-1})	Effective nodules (%)	Shoot dry weight (g plant^{-1})	Roots dry weight (g plant^{-1})	N uptake (g plant^{-1})	Ndfa (%)	P uptake (mg kg^{-1})
Lime (L)	1	1995.11**	12.26**	3.33^{ns}	690.55*	19.1***	0.002*	1019.22^{ns}	4.69*
Variety (V)	1	693.44*	6.24^{ns}	6693.09***	0.49^{ns}	0.63^{ns}	0.02^{ns}	558.63***	584.03^{ns}
Inoculation (I)	2	1803**	74.50***	21232.39***	596.15***	32.84***	0.03***	5583.74***	367.69***
L×V	1	1.00^{ns}	2.85^{ns}	373.68*	2.25^{ns}	0.84^{ns}	0.00^{ns}	752.22^{ns**}	26.69^{ns}
L×I	2	370.11^{ns}	3.03*	309.73**	75.6*	2.92*	0.00*	296^{ns*}	20.86^{ns}
V×I	2	311.11^{ns}	14.84***	5297.63***	3.76***	0.04^{ns}	0.001^{ns}	1017.36***	52.03^{ns}
L×V×I	2	156^{ns}	2.91***	99.23^{ns}	4.83^{ns}	1.02^{ns}	0.01^{ns}	24.63^{ns}	8.58^{ns}
CV (%)		54.06	42.86	17.64	28.58	19.98	49.00	37.6	31.08
Mean		24.33	2.19	40.61	14.91	3.63	0.07	24.8	15.97

*, **, *** and ns indicate significant at p<0.05, 0.01, 0.0001 and not significant respectively

Appendix 6: Mean square for the factors affecting soybean growth parameters (Murang'a Soil)

	DF	No. of pods	Nodule fresh weight (g plant^{-1})	Effective nodules (%)	Shoot dry weight (g plant^{-1})	Roots dry weight (g plant^{-1})	N uptake (g plant^{-1})	Ndfa (%)	P uptake (mg kg^{-1})
Lime (L)	1	25^{ns}	0.71^{ns}	4546.05^{***}	0.37^{ns}	0.81^{ns}	0.01^{ns}	497.34^{*}	10.03^{ns}
Variety (V)	1	32.11^{ns}	1.48^{ns}	566.64^{ns}	120.89^{*}	1.87^{*}	0.02^{*}	4.64^{ns}	380.25^{*}
Inoculation (I)	2	1806.19^{***}	62.06^{***}	20552.17^{***}	120.75^{***}	2.72^{**}	0.02^{***}	5393.54^{***}	928.69^{***}
L×V	1	25^{ns}	3.89^{*}	57.11^{ns}	11.30^{ns}	0.18^{ns}	0.03^{ns}	522.88^{**}	66.69^{ns}
L×I	2	85.75^{*}	14.51^{***}	3618.81^{***}	2.76^{ns}	0.24^{ns}	0.001^{*}	349.22^{*}	135.53^{*}
V×I	2	211.26^{**}	3.15^{*}	995.5^{***}	0.08^{ns}	0.05^{ns}	0.001^{ns}	318.94^{ns}	14.25^{ns}
L×V×I	2	84.25^{*}	1.18^{ns}	2008.4^{**}	3.31^{ns}	0.56^{ns}	0.001^{ns}	223.68^{ns}	15.86^{ns}
CV (%)		23.39	40.83	28.35	16.93	14.45	66.64	44.07	27.77
Mean		20.77	2.09	56.53	14.63	4.31	0.04	21.60	20.14

*, **, *** and ns indicate significant at $p<0.05$, 0.01, 0.0001 and not significant respectively.

Appendix 7: List of publication

ACTA AGRICULTURAE SCANDINAVICA, SECTION B — SOIL & PLANT SCIENCE
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Impact of soil acidity and liming on soybean (*Glycine max*) nodulation and nitrogen fixation in Kenyan soils

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ABSTRACT

There is a wide application of rhizobia inoculants to legume crops in Africa, irrespective of the soil acidity, though the latter limits the effectiveness of inoculants. Two trials were conducted in a controlled environment to determine suitable soil pH and impact of liming on soybean nodulation and nitrogen fixation to inform proper application of the rhizobia-inoculant technology on acid soils. In the first trial; soil, variety and inoculation had significant influence ($p < 0.05$) on weighed nodule effectiveness (WNE) and N fixation. Strongly acidic soils recorded low WNE and N fixation. In the second trial, WNE and N fixation significantly increased with co-application of lime and inoculation ($p < 0.05$). The results showed that soybean inoculation is effective in increasing nodulation and N fixation in moderate acidic soils, contrarily to strongly acidic soils. Interestingly, co-application of lime and inoculation has potential of increasing nodulation and N fixation in strongly acidic soils. The WNE is recommended as a robust formula to report nodule effectiveness, compared to the current percentage method.

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Acidic soils; nodule effectiveness; biological nitrogen fixation; inoculation; nutrients; soybean; soil liming

Appendix 8: Research Permit

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