EVALUATION OF MICROBIAL INOCULANTS FOR ENHANCING GRAIN LEGUME PRODUCTION IN THE SUDAN AND GUINEA SAVANNA ZONES OF NIGERIA

BY

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BSc. Agriculture (Hons)

MSc. Soil Science

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A Thesis submitted to the Department of Crop and Soil Sciences, Faculty of Agriculture, College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology Kumasi, Ghana, in partial fulfilment of the requirements for the award of degree of

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JUNE, 2019

DECLARATION

I Abdulkadir Nasiru Ahmad, hereby declare that this Thesis was written by me and that it is the record of my own research work towards the fulfilment of the requirement for PhD degree and to the best of my knowledge, it is neither in part nor in whole been presented for the award of another degree elsewhere, except where citations and due acknowledgement have been made in the text.

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DEDICATION

I dedicate this thesis to my late father Alh. Ahmad Abdulkadir, mother Haj. Zulaiha Imam and my entire household.

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ABSTRACT

This experiment was conducted in Kano (Sudan savanna) and Bauchi (Guinea savanna) states of Nigeria between 2015 to 2016 cropping seasons to assess microbial inoculants use for soybean and groundnut production in northern Nigeria. The experiment in each location was a randomized complete block design (RCBD) with seven treatments and replicated four times. Four rhizobia inoculants were tested on soybean (TGX 1835) and groundnut (SAMNUT 24) in the two agro-ecological zones to monitor their performance and their ability to establish symbiotic relationship and nodulate soybean and groundnut. The treatments for soybean were; Legume fix, Alosca, nitrogen, cattle manure, Legume fix + cattle manure, Alosca + cattle manure and control whilst those for groundnut comprised Histick, Biofix, nitrogen, cattle manure, Histick + cattle manure, Biofix + cattle manure and control. Most probable number (MPN) method was used to assess the number of rhizobia cells in the inoculants used for the field experiment. During the 2016 cropping season, maize (SAMMAZ 14) was planted to evaluate the residual effect of microbial inoculants and treatments on maize grain yield. Results showed that, the inoculants contained enough number of viable rhizobia strains to inoculate legumes. The study showed that in the Sudan and Guinea savanna agro-ecological zones, inoculation resulted in significant soyabean yield increments compared to the control. However, the application of nitrogen fertilizer had no significant effect ($P > 0.05$) on grain yield in both agroecologies. In the Sudan savanna, increased nitrogen fixation values of 173.90, 101.64, 56.16 and 40.40% were obtained for Legume fix, Alosca, Legume fix + cattle manure and Alosca + cattle manure over the control. The same nitrogen fixation trend was observed in the Guinea savanna zone. Combination of inoculants with 4 tonnes ha^{-1} cattle manure gave higher soybean grain yield than sole inoculant in the Sudan savanna. In the case of groundnut, it was observed that, inoculated plots produced higher grain yield, even though

not significantly different from the control. In soybean inoculants influenced BNF significantly ($P < 0.05$) when compared to control in both study locations. In the groundnut field, Biofix produced higher nitrogen fixed than all treatments in the Sudan savanna, while in the Guinea savanna no significant differences ($P = 0.67$) were observed between the treatments and the control. However, inoculated plots had higher nitrogen fixation than the control. Legume fix and Alosca performed well under soybean field while Histick and Biofix performance was low under groundnut fields in both locations. Economic analysis showed that, Legume fix was the most economically viable treatment having the highest net benefit in both locations under soybean, while under groundnut, the usage of inoculants was not economically viable due to low net benefit. It can thus be concluded that, application of Legume fix and Alosca in study both locations under soybean resulted in yield increment while the result in groundnut fields showed little response in Histick. Results of the second-year study showed that residual effect resulting from inoculation enhanced maize yield on the soybean and groundnut fields in both study locations. It is recommended that farmers should use inoculants in combination with cattle manure for better yield.

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CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

Legumes are the third largest group of angiosperms and the second largest group of food for human as well as feed for animals in the world (Akinbamijo *et al*., 2018). It includes various food crops like alfalfa, beans, peanut, cowpea, faba beans, bambara groundnut, clover, chick pea, soybean, etc. Legumes have potential to increase household food security, reduce climate change impact and mitigate of greenhouse gas (GHG) emission (Akinbamijo *et al*., 2018). They also contribute to farming systems through their ecological and diversified production patterns and provision of sustainable and viable livelihood-enhancing options (Akinbamijo *et al*., 2018). This means that, leguminous plants must occupy a special place in food security strategy and planning issues of African agriculture (Akinbamijo *et al*., 2018). Grain legumes further supply about one-third of processed vegetable oil and dietary protein nitrogen need (Graham and Vance, 2003; Sarra *et al*., 2015).

The legume-rhizobia symbiosis is the most important symbiotic association in terms of biological nitrogen fixation, producing about 200 million tons of nitrogen annually (Peoples *et al*., 1989; Graham and Vance, 2003). Legume inoculation is a popular agricultural practice which has been implemented for over a very long period. The practice involves inoculation of exotic rhizobia into the soil especially when a new crop is introduced. The yardstick for enhancing the performance of the rhizobia includes quality and efficiency of the introduced strain in N fixation with host plant species. Large numbers of rhizobia is an indication of quality (Amarger, 2001). When selecting an inoculant strain, the most important factor considered is the symbiotic effectiveness of the rhizobia (Date

2000; Stephens and Rask, 2000). Other factors worthy of consideration include the genetic stability and capability to persist in inoculant carrier as well as the newly introduced soil environment and also its capacity to compete in nodule formation with native soil rhizobia (Graham and Vance, 2003).

Nigeria was rated the major producer and consumer of soybean in sub Saharan Africa. Soybean was first introduced into Nigeria in 1908 (Fennel, 1966; Dugje *et al*., 2009). The average soybean grain production in Nigeria is 1 tonne per hectre (Ronner *et al.*, 2016). Soybean fixes between 44 and 300 kg N ha^{-1} besides the highly valuable grains that is produced. This for example makes a significant N contribution to intercropped and rotated cereal crops that are grown alongside with soybean crops.

In an effort to harness the benefit of biological nitrogen fixation through soybean rhizobia inoculation in Nigeria, farmers were involved in demonstration trials in 2011 and 2012 by N2 Africa in partnership with other local stakeholders. However, despite the positive results on the usage of inoculants, local availability of good quality inoculants remains a major challenge (Ronner *et al*., 2016). Research have shown that, the yield of soybean was observed to be inhibited when compatible rhizobia was absent in the soil. This was confirmed by Bintu *et al*. (2017) while working on soils from eastern Democratic Congo. Other researchers also confirmed the need for inoculation with the right rhizobia especially when a new legume crop is introduced to an area (Thuita *et al*., 2012; Zimmer *et al*., 2016; Heerwaardena *et al*., 2017). It is critical to understand that legumes recognize the right type of rhizobia before establishing interaction (Drew *et al*., 2012; Via *et al*., 2017).

Groundnut (*Arachis hypogaea* L.) or peanut is also one of the important legume crops of tropical and semi-arid tropical countries of the world, where it supplies edible oil and vegetable protein. In Nigeria, average yields are much lower (0.6 - 0.7 tons ha^{-1}).

Groundnut is an annual crop which grows best in light textured sandy loam soils with neutral pH. Best temperature for their growth and development ranges from 28 to 30 ºC and requires rainfall of about 500 - 600 mm. The main yield limiting factors in semiarid regions are drought and high temperature stress (Prasad *et al*., 2009). Inoculation of groundnut seed with the right rhizobia could further enhance nitrogen fixation (Prasad *et al*., 2009).

1.2 Problem Statement/ Justification

According to Giller (2001) and Laditi *et al*. (2012), certain microbial products exert considerable influence on specific plant growth and development. Nevertheless, there exists substantial proliferation of new products in the market of sub Saharan Africa which are claimed to increase yield of specific crops. In view of this, there is urgent need to vigorously scrutinize these products especially in different agro – ecologies to ascertain whether the claim of manufacturers is valid. This will protect farmers from the risk of purchasing poor quality products that can undermine their agricultural productivity and consequently their economic wellbeing. Furthermore, the use of microbial inoculants on soybean and groundnut productivity in Nigerian agriculture is an emerging science which needs adequate research to harness benefit associated with the use of microbial products.

1.3 Objectives

The overall objective of this study was to evaluate the benefit of microbial inoculants to enhance soybean and groundnut production in northern Nigeria.

The specific objectives were to:

i. assess the effects of some microbial inoculants on the growth and yield of soybean and groundnut;

ii. determine the influence of microbial inoculants, mineral nitrogen and cattle manure applications in enhancing the N_2-fixation and nutrient uptake of soybean and groundnut; iii. evaluate the residual effect of microbial inoculants, mineral nitrogen and cattle manure applications on the yield of maize, and iv. appraise the economic benefits of microbial inoculants use in soybean and groundnut production.

1.4 Hypotheses

The above specific objectives were based on the null hypotheses that:

i. inoculation with commercially produced microbial inoculants will improve nodulation of soybean and groundnut; ii. application of microbial inoculants, mineral nitrogen and cattle manure application will enhance N fixation and nutrient uptake in soybean and groundnut; iii. microbial inoculants, mineral nitrogen and cattle manure application will influence the performance and yield of subsequent maize crop and iv. microbial inoculants, mineral nitrogen and cattle manure will be of economic benefit for soybean and groundnut nodulation.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biofertilizer

Biofertilizer is usually an organic material that contains microorganisms which when applied to seed or soil will colonize the rhizosphere and promote the growth of plants in most cases increase nutrients through nitrogen fixation, solubilization of phosphorus and synthesis of growth promoting substances (Sudhir *et al*., 2014). Soil integrated management system will be achieved through the use of biofertilizer due to their cost effectiveness and environmentally friendly attributes. The soil natural nutrient cycle and organic matter is enhanced by the microorganisms contained in biofertilizer (Sudhir *et al*., 2014).

Pollution of agricultural land, water resources and soil salinisation due to the consequence of excess and inappropriate application of mineral nitrogen is a global phenomenon which affects all countries especially the developing ones. Therefore, in an attempt to reduce chemical inputs and raise soil quality and sustainability, natural biotechnological practices, such as the application of bacterial inoculants is receiving special attention in many countries globally (Sogut, 2006).

Inoculation of legume plants with effective rhizobial strains is an alternative to the continuous usage of chemical fertilizer which could be detrimental to ecosystems in the long run. The introduction of viable rhizobia strains into agricultural ecosystem can be achieved through “on-seed inoculation”, or by direct application into the soil, which is termed “in-furrow inoculation” (Bogino *et al*., 2011) . Inoculation will only stimulate growth and yield of a legume in the absence of effective native rhizobia species (Singleton and Tavares 1986; Streeter, 1994). The goal of inoculation is to provide sufficient numbers of viable and effective rhizobia to achieve rapid colonization of the rhizosphere, whereby

nodulation takes place after germination and produces optimal yields based on Biological Nitrogen Fixation (BNF). For an inoculant to be successful, its formulation and application must take into account the variables and principles that affect viability of the bacteria, even though, the user may not be familiar with these principles (Bogino *et al*., 2011).

2.2 Rhizobia

The family *Rhizobiaceae* is a classification of a group of genetically diverse and physiologically heterogeneous soil organisms collectively called rhizobia (Somasegaran and Hoben, 1994). Rhizobia is a common language used to identify a wide species of bacteria which comprises *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium* and *Azorhizobium* that are able to enter into symbiosis and nodulate members of the plant family (Sprent 2001; Sessitsch *et al*., 2002; Howieson and Ballard, 2004). German scientists Hellriegel and Wilfarth discovered legume- rhizobium relationship in 1886 (Franche *et al*., 2009). They revealed that legumes that contained root nodules could use molecular (gaseous) nitrogen. In 1888, Beijerinck, a Dutch microbiologist, succeeded in isolating a bacterial strain (*Rhizobium leguminosarum*) from the root nodules of a legume plant and pure cultures were shown to induce nodules when inoculated to the same host plan (Franche *et al*., 2009). He named these bacteria *Bacillus radicicola*. The generic name, *Rhizobium* was later adopted formally in 1962 by Buchaman (Giller, 2001).

According to Morel *et al*. (2012), the current taxonomy of rhizobia consists of several genera in the subclass alpha and beta proteobacteria: *Rhizobium*, *Mesorhizobium*, *Ensifer*, *Sinorhizobium*, *Azorhizobium*, *Methylobacterium*, *Bradyrhizobium*, *Phyllobacterium*, *Devosia* and *Ochrobactrum*. Rhizobia vary genetically in nature and they are physiologically a heterogeneous group of bacteria (Somasegaran and Hoben, 1994).

Rhizobia comprise of a major part of the soil microflora in free living state around the rhizosphere of legumes globally (Allen and Allen, 1981; Somasegaran and Hoben, 1994). They have special ability to form symbiotic association with plants belonging to the family *Fabaceae*. This is an exclusive characteristic related only to those bacteria belonging to the family *Rhizobiaceae* (Pepper and Upchurch, 1991). Rhizobium distinguished itself from most of other soil microorganisms by being free–living in soils and in symbiotic association inside host legumes (Fujihara, 2009). With the aid of a microscope, they appear rod shaped, (0.5 - 0.9 micrometers wide and 1.2 to 3 microwave long organisms). Oxygen is necessary for their metabolic need. Their propagation is achieved through cell division and not formation of spores. Their life cycle comprises of three phases, i.e. infective, symbiotic and saprophytic (Somasegaran and Hoben, 1994).

Native rhizobia have the capability to live in the soil without attaching to the host plant. They can live as saprophytes till when they establish symbiotic relationship with host legumes (Sanginga and Woomer, 2010). Native rhizobia could be very diverse in terms of their population with different types of strains. However, often the population of native rhizobia in the soil is low depending on legume host or sometimes the strains of rhizobial species which may not be very effective (Sanginga and Woomer, 2010). Under such a condition, there is need for inoculation with more efficient rhizobia (Brady and Weil, 2002). Even though, native rhizobia are well adopted to their soil environment, some factors could affect their persistence. Increase in moisture content of the soil improves rhizobial survival but may result in low pH in the long run which can be detrimental to the host plant (Woomer and Singleton, 1998).

2.2.1 Rhizobia diversity in soils

According to Ogutcu *et al*. (2008), the advent in rhizobia genetic diversity assessment, is contributing towards understanding the usefulness of rhizobial groups in fixing

atmospheric nitrogen through legume–rhizobium association, and shaping future agricultural productivity in a sustainable manner. It is eminent to note that population diversity of a particular native rhizobia will be high at a place where its host originates (Lie *et al*., 1987). Nevertheless, various rhizobial population can be found in symbiotic association with some legumes species which were introduced to a particular location (Sadowsky and Graham, 1998). Over half century, studies have been conducted on the nature, size and natural populations and behavior and survival of inoculant strains on different types of agricultural soils (Hirsch, 1996). Rahim and Hameed (2017) while working with soils from twenty-two different sites in Himalayan region of KashmirPakistan, observed a great influence of native rhizobia in enhancing the growth of soybean. However, they observed variability between sites probably due to microbial diversity across different sites (Rahim and Hameed, 2017).

The advent of modern molecular techniques has resulted in proposals of differing reliability to improve upon the study of symbiotic N_2 fixation. Rhizobial numbers vary depending on environment, soil type and type of soil cultivation. Hirsch (1996) also mentioned that since populations in bulk soil rarely exceed 106 cells g^{-1} soil, compared with an estimated 10–109 viable bacterial cells g^{-1} soil, the soil consistently contained a smaller group, despite much higher numbers around the vicinity of rhizosphere. The population of rhizobia is improved in agricultural soils, by the abundance of legumes that could be nodulated by rhizobia (Thies *et al.*, 1992; Sadowsky and Graham, 1998). Previously, *Rhizobium* species and biovars were generally distinguished based on the host plants which they infect nodulation with. However, with recent advancement of molecular techniques, DNA based taxonomy classification has been achieved (Hirsch, 1996; AlMujahidy *et al*., 2013). It was reported that when legumes were planted even before the

onset of nodulation, rhizobia population increased. This phenomenon signifies that rhizobia-host interaction is something beyond nodule formation alone (Hirsch, 1996).

2.2.2 Effect of environmental factors on rhizobia

A lot of attention was given to the factors that affect the ability to establish inoculant strains on a host that is growing in soil that already contains indigenous rhizobia. It was reported that different environmental factors affect competition of nodulation; the presence of indigenous rhizobia within the soil, soil temperature, soil type, soil moisture content, soil pH, nitrogen availability and microbial antagonists (Abaidoo *et al*., 1990; Desta *et al.,* 2015). Research has shown that rhizobium and *Bradyrhizobium* that can induce nodules on roots are more tolerant to salinity than their host legume plants. El-Sheikh and Wood (1995) in a trial using 340 mm NaCl with soybean and chickpea rhizobia, discovered that fast growing strains are more tolerant than slow growing ones. Salinity is a major stumbling block in arid and semi-arid regions agriculture and to correct this, small application of nitrate and very effective rhizobia is useful (Deshwal *et al*., 2013).

2.3 Symbiotic relationship of rhizobia with legumes

Symbiotic nitrogen fixation is a natural phenomenon which occurs between a compatible type of legume species and the right strain of nitrogen fixing bacteria. Biological nitrogen fixation occurs naturally and it is a basic life support mechanism in the ecosystem and is environmentally friendly (Salisbury and Ross, 1992). It is well known that nitrogen is very essential for plants and animals and nitrogen fixing organisms are the suppliers as well as inorganic nitrogen fertilizers.

Nitrogen fixation can be achieved either symbiotically or asymbiotically (Raven and Handley, 1992). Most of the time, symbiotic relationship is only achieved when a legume

is infected with the right type of rhizobia. Contrarily, some legume species are capable of nodulating with a wide range of rhizobia strains (Broughton *et al*., 2000; Graham and Vance 2000). The explicit mechanism involves the identification of the right rhizobium by the host legume through signal messages of compatibility which portrays differential gene appearance between the symbiotic partners (Broughton *et al*., 2000). Those legumes that nodulate with a wide range of rhizobia are called promiscuous varieties. Researchers at IITA were able to produce such legume species that could even establish symbiotic relationship with native rhizobia without necessarily using microbial inoculants (Sanginga *et al*., 1996a; Osunde et al., 2003).

2.4 Historical background of rhizobial inoculation

According to Abaidoo *et al.* (2013), history of legume inoculation in sub-Saharan Africa dates back to the 1950s. Researches concentrated mostly on forage legumes and soybean varieties. In Nigeria specifically, these research works commenced around 1970s in IITA, Ibadan. The emphasis then was more on soybean crop. Through their exceptional capacity to fix atmospheric N into plant usable form, bacteria establishes a symbiotic association with compatible legume species through the formation of nodules on the root of a host plant (Abaidoo *et al*., 2013). The nodule structure serves as a kind of natural factory to manufacture N for plant benefit. This natural way can be useful in increasing yields.

Knowledge on the selection and development of best inoculant strain is advancing due to inter-disciplinary research works going on especially in the area of molecular biology, environmental and genetic related issues that can influence nodulation and N_2 fixation (Sessitsch *et al.*, 2002). The N fixation benefit by legume rhizobia could be enhanced through integration of related data bases, models and related information that can contribute towards more understanding and making progress in the exploitation of benefits associated

with N fixation (Sessitsch *et al.*, 2002). Recent discoveries have revealed novel nodulating bacteria and its diversity. Historically, rhizobial diversity has been found to be associated with nodule isolates. A lot of research work are on-going to identify nodule bacteria that could be appropriate in nodulating various legume plants (Sessitsch *et al*., 2002). As such, current discoveries are geared toward identifying rhizobial strains that will best suit various locations in the sub-Saharan Africa (Sessitsch *et al*., 2002). Research have shown that when conditions are very favourable, grain legume plants can fix up to 200 kg N ha^{-1} which can significantly contribute to soil nitrogen pool and reduce drastically the need for inorganic nitrogen fertilizers (Giller, 2001; Mapfumo, 2011). Growing grain legumes on poorly fertile soil contribute to the net nitrogen of the soil, but growing legumes in fertile soil gives better result due to non-nitrogen benefits which increase stover and grain yield (Kermah *et al*., 2017).

The impact of these recent discoveries is under examination to quantify how it will enhance the global nitrogen fixation and how it will provide effective strains of rhizobia for the manufacture of inoculants (Giller, 2012). With the advent of N2 Africa projects, attention is now towards supplying effective inoculants to the smallholder farming communities where N2 Africa is operating before it warrants the establishment of local inoculant industries (Giller, 2012). Another important aspect is the effort dedicated to capacity building in terms of training.

2.4.1 Importance of legume inoculation in African agriculture

According to Machido *et al*. (2011), soils in the semi-arid zone of Nigeria are predominantly sandy in nature characterized by moisture stress in most cases, low in inherent fertility status and low levels of crop production. This and many other factors warrant the importance of legume cultivation and soil inoculation to enhance the fertility status. Legumes like soybean

when inoculated can fix between 44 to 300 kg N ha^{-1}. This contributes significantly to N addition to the soil. Thus, subsequent cereals can benefit tremendously from the added N (Mutegi and Zingore, 2008).

The high diversity of rhizobia in soils of Africa affects nodulation significantly (Phanuel *et al*., 2016). Research conducted at University of Ghana, Legon revealed variability in the ability of *Bradyrhizobia* to nodulate promiscuous soybean variety (Phanuel *et al*., 2016). This was influenced significantly by the number of indigenous rhizobia in the soil (Phanuel *et al*., 2016). Majority of African farmers cannot purchase mineral fertilizers due to high costs as over 75% of it are imported (Chianu *et al*., 2011). In places where native rhizobia population is low, the need for inoculation with the right and effective rhizobia is necessary (Chianu *et al.*, 2011). This will help farmers increase the yield of their legumes with positive effect on their livelihoods.

Integration of legumes into cropping systems of northern guinea savanna of Nigeria (NGSN) has been considered as important resource management for reduction of energy usage cost and environmental pollution (Yusuf *et al*., 2009a). Despite this effort, herbaceous legumes only contribute 40 - 70 kg N ha^{-1} per season. This is just about 30% of the total nitrogen applied as crop residue. Despite the demonstration by various extension agents of the importance of green manuring and incorporation of legume residue to the soil, the adoption of this practice by west African farmers is limited (Sanginga, 2003).

2.4.2 Need for inoculation

When legumes are inoculated with proper strain of rhizobia, they can supply up to 90% of their own nitrogen. Shortly after the seed germinates, the rhizobia bacteria present in the soil penetrate the root hairs and move inside the root to form pink nodules that fix nitrogen

from the atmosphere into plant usable forms (Auburn, 1998). However, if legumes are not inoculated, yields often remain low, regardless of the amount of nitrogen applied. Nodules apparently help the plant use fertilizer nitrogen efficiently. It is worthy of understanding that, rhizobia are specific to the legumes they nodulate (Loynachan, 2005). Inoculation can be highly effective if synergistic interaction is established among rhizobia symbionts (Meghvansi *et al.*, 2008). The use of commercial inoculants is getting prominence in recent times due to the realized effect of global warming which have caused great concern to the ecosystem. Even though, the use of inoculants can be a supplement to mineral nitrogen fertilizer and yield increment in legumes, there is need to ensure that viable rhizobia are introduced into the field through successful seed inoculation (Bogino *et al*., 2011).

It is advisable to inoculate fields only when there are no specific rhizobia in the soil to nodulate a legume, hence, the major objective of inoculation is to maintain a high level of rhizobia on the seeds and the soil to hasten the colonization of rhizosphere and subsequent efficient nodulation with maximum yields (Albareda *et al*., 2009). Therefore, inoculants should be recommended to a soil when there is good reason to believe that population of bacteria is scarce. Successful inoculation must be achieved for the bacteria to come into contact with the seed and ultimately the roots. In most cases, the in-furrow method of application is more recommended as compared to either the use of sticking agents onto the seeds or ordinarily mixed with the seeds, because there is less stress on the bacteria in most in-furrow applications (Bogino *et al.*, 2011).

Experiment conducted by Albareda *et al*. (2009) showed that measured agronomic parameters were significantly higher when soybean was inoculated compared to the uninoculated. In their experiment, varying the concentration of rhizobia strain did not have significant effect on parameters and persistence of rhizobia strains depended on the type of soil and soil condition especially pH. Significant increase in agronomic parameters

including grain yield were also observed by Diaz *et al*. (2009) when soybean was inoculated with rhizobia. However, they opined that early season application of nitrogen yielded no benefit for soybean yield or quality. Inoculation of legumes with rhizobia improves significantly biological nitrogen fixation (Fernández-Luqueño *et al*., 2012).

2.4.3 Inoculants manufacture

Although commercial microbial inoculant production is increasing in many countries, their quality is a measure of the number of effective rhizobia in the product that can establish successful symbiotic relationship with host plant (Amarger, 2001). Even though, manufacturers of commercial microbial products always claim effectiveness and authenticity of their products, this cannot always be trusted and hence the need to carry out test to ascertain their claims (Aliyu *et al*., 2013; Asei *et al*., 2015). When choosing strains of inoculants, their symbiotic effectiveness must first be evaluated to determine whether they have enough number of compatible rhizobia in the carrier (Date, 2000; Stephens and Rask, 2000). Other characteristics to be considered are genetic characteristics and capacity of the rhizobia to endure in inoculant, to persevere in the soil and to compete in nodule occupancy with the already existing indigenous soil rhizobia (Graham and Vance, 2000).

Inoculant quality can be measured by estimating the number of viable rhizobia cell that it contains and how effective they can be (Hiltbold *et al*., 1980; Stephens and Rask, 2000). Many of the inoculants that exist in the market are of poor quality for one reason or the other especially in countries where quality control is not given much attention (Catroux *et al*., 2001). Inoculants are produced in powdered or liquid form. High quality inoculants can provide approximately 2×10^{11} to 4×10^{11} rhizobia. This is estimated to be about 1 %

of the native rhizobia population that could be found in the top 20 cm of soil in some locations (Catroux and Amarger, 1992).

Inoculants are produced in granular shape, slurry, powdered, lime or phosphate pelleting or in liquid forms (Rodriguez-Navarro *et al*., 2011). They can be applied as a dressing with seeds which is the most common method or applied to the soil as mineral granules (Wadoux, 1991), or incorporated straight into the seed bed (Haynes *et al*., 1995). The technique of inoculation is variable depending on the nature of legume and the inoculant form. Table 2.1. provides clue to some basic inoculation techniques.

2.4.4 Efficacy of inoculation

For inoculants to be effective, it must be able to compete with indigenous rhizobia strains that are present within the soil and capable of establishing nodules with a legume crop (Date, 2000) . The presence of specific rhizobial population in a soil that is well adopted to soil condition but poorly effective in terms of their nitrogen fixation ability can limit nodule occupancy and hence inoculation failure. Under these conditions, forecasting nodule occupancy by rhizobia introduced into different environments becomes a key factor for a more judicious use of rhizobial inoculants (Rodriguez-Navarro *et al*., 2011). Research in sub Saharan Africa showed that, the response of soybean to inoculation depend largely on the variety used (Heerwaardena *et al*., 2017). Side by side result revealed that promiscuous variety performance was higher than inoculated ones suggesting the effectiveness of the indigenous bacteria (Heerwaardena *et al*., 2017).

The selection of rhizobia strains have several phases:

(1) Acquisition of nitrogen-fixing bacteria. These can be found from: (i) nodules of fieldgrown plants, or plants inoculated with soil samples for which there is an interest in

obtaining the isolates (ii) collections from the isolates of other researchers (Lupyawi *et al*., 2000).

(2) Purification and certification of bacterial isolates. Authentication can be tested by inoculation of the plant concerned with the isolate, followed by re-isolation from a nodule. Nodule isolates should be tested for purity and the ability to nodulate. Isolates showing promising symbiotic capacities in this preliminary screening are stored for further analyses (Lupyawi *et al*., 2000).

(3) Evaluation of the symbiotic properties. The test for nodulation and effectiveness in N_2 fixation of the isolates is then mounted under greenhouse condition or under controlled environmental conditions. Legumes seedling are then lined in sterilized assemblies of test tubes or Leonard jars. Growing seedlings are then inoculated with the chosen rhizobia strain, there after plant responses to inoculation are related with those of uninoculated controls. The essence here is to show the capability of introduced strains to fix nitrogen and induce growth to inoculated legumes, but uninoculated control should also be included and must be ensured that the growth is not limited by any other factor apart from N (Date, 2000).

Table 2.1: Legume inoculation techniques.

Technique	Description
Seed inoculation	
Dusting	Peat inoculant is mixed with the seed without rewetting.
Slurry	Seed is mixed with a water solution of peat often with the addition of an adhesive.

Lime or phosphate pelleting	Seed is treated with a slurry peat inoculant followed by a coating of calcium carbonate (superfine limestone) or rock phosphate. Rhizobia is introduced into or beneath the seed coat under vacuum
Soil inoculation	
Liquid inoculation	Peat culture mixed with water or liquid inoculant applied to the seedbed at the time of sowing (liquid inoculants may also be applied to the seed).
Granular inoculation	Granules containing inoculum sown with seed in the seedbed.

Source**:** (Deaker *et al*., 2004)

2.5 Biological nitrogen fixation

Biological nitrogen fixation (BNF) denotes the ability of some bacteria to fix atmospheric nitrogen to a reduced form of ammonium. This is a distinct capacity of some microorganisms (bacteria**,** actinomycetes and cyanobacteria) to establish distinct mutual relationship with leguminous plants (Bhattacharjee *et al*., 2008). Nitrogen fixation is a function performed by gram negative type of bacteria called rhizobia (Kanonge-Mafaune *et al*., 2018). Several factors such as low available P, adverse soil moisture and temperature as well as presence of active native soil bacteria can limit the efficiency of N fixation by rhizobia (Kabahuma, 2013). Nitrogen fixation symbiosis and an ability to sustain high rates of nitrogen fixation is a delicate activity for legume plants. Legumes fix their nitrogen from the atmosphere through the help of enzyme nitrogenase which is supplied by bacteria (Sinclair and Vadez, 2014).

Biological nitrogen fixation is the whole idea of inoculation technique. Under high fertility soil condition, legumes satisfy their N requirement from the soil rather than BNF which account for less than 25% of the N concentration under this condition (Pederson *et al*., 2002). Biological nitrogen fixation coupled with photosynthesis are considered the basis

of all life on earth (Cheng, 2008). Nitrogen fixing abilities differs between legume species. Example soybean have N fixing ability better than cowpea (Cheng, 2008).

Nitrogen is a very important plant element. It is necessary for plant metabolic needs. All living plants need nitrogen in large quantity for their metabolic system and living cells, because it is major component of all proteins and nucleic acid (Sinclair and Vadez, 2014). Therefore, it is not surprising that shortage or lack of nitrogen in all plant systems results in stunted plant growth. Legumes are endowed with natural capability to fix nitrogen for their metabolic needs through symbiotic association with special type of bacteria (Sinclair and Vadez 2014). Due to symbiotic nitrogen fixation, legumes are guaranteed with nitrogen availability in the soil (Sinclair and Vadez 2014).

Biological N_2 fixation is regarded as a very safe natural method of supplying nitrogen to the plants through symbiotic association with special type of bacteria (Loynachan, 2005). According to Solomon *et al*. (2012) between 50 to 80% of soybean nitrogen need can be achieved through BNF. Sanginga *et al*. (2002) also reported that promiscuous soybean genotypes need more of nitrogen and therefore N_2 fixation alone cannot satisfy the nutrient requirement for its growth and yield. Furthermore, some species of plants other than legumes can also fix nitrogen biologically through some strains of free-living bacteria which can form symbiotic association with roots of some cereals. Biological nitrogen fixation can also happen in cereals like wheat, rice, sorghum and maize that were inoculated and colonized by other species of bacteria like *Azospirillium* and *Azotobacter*. The N fixation even though low as compared to that of leguminous plants but can be significant up to about 5 kg N ha^{-1} $year^{-1}$ in inoculated wheat sorghum and maize (Boddey and Dobereiner, 1994).

2.6 Importance of biological nitrogen fixation in agriculture

Biological nitrogen fixation plays a vital role within the complex nitrogen cycle which is considered one of the most important biochemical cycles controlling and maintaining balance for living things within the ecosystem. Without this important cycle, life will be difficult to control and manage in a healthy and effective manner (Mulongoy, 2015).

Plant-associated nitrogen fixation presently contributes about 50–70 million tonnes annually to the global agricultural N budget (Unkovich *et al*., 2008). This accounts for 40 to 70% of total global nitrogen input (Kahindi and Karanja, 2009). Studies have proved that quantification of nitrogen fixed by rhizobia inoculant in monetary value per hectare costs $3.0/ha as against $87.0 fertilizer N (Kahindi *et al*., 2000). Supplementing nitrogen supply through BNF is a very viable alternative for poor peasant farmers of sub-Saharan countries (Rattan, 1995). Biological nitrogen fixation is a free way of sourcing nitrogen for agriculture (Giller and Cadisch, 1995). Nitrogen-fixing systems provide an economically and ecologically friendly means of reducing external inputs and improving internal resources (Bohlool *et al*., 1992; Albareda *et al*., 2009).

Biological nitrogen fixation is a cheap source of N resource for poor farmers and also reduce the potential risks associated with the manufacture of mineral nitrogen. Some legume crops can fix between 30 to 150 kg of nitrogen per crop per hectare (Unkovich *et al*., 2008). Compared to inorganic N fertilizer, BNF is better economically and is environmentally friendly. Economically, it decreases costs of production. The use of inoculants as substitutes to N fertilizer will convert the eminent hazards of contaminating ground water though runoff and leaching of excess fertilizer. It has been confirmed by researchers that the continuous usage of nitrogenous fertilizer leads to increased soil acidity (Werner *et al*., 2005).

In recent years, global warming has been a serious concern and many research works have linked this serious issue with the production and usage of nitrogen fertilizers. The production of nitrogen fertilizers by industrial fixation generates large quantities of carbon dioxide, contributing to global warming (Ghaly and Ramakrishnan, 2015). The natural process of BNF offers an economic means of reducing environmental problems and improving internal resources (Kahindi and Karanja, 2009). A study has also shown that the use of biological fixation in flooded paddies can yield up to 50 kg ha^{-1} $crop^{-1}$ (Stoltzfus *et al*., 1997).

2.6.1 Recent advancement in biological nitrogen fixation

Fresh efforts are under way to influence cereal plants to nodulate with a view to fix nitrogen (Kramer, 2014). The recent discovery of signaling mechanisms between mycorrhization and nodulation, new discovery of cereal endophytes and advances in plastid physiology have paved away for the new effort (Kramer, 2014). However, the contribution of agronomic significant quantities of BNF through endophytes needs more rigorous effort in research. Recent advances in molecular biology and advancement in plant breeding has paved way for selecting high nodulating genotypes for the maximization of BNF. Also, recent progress includes the discovery of some rhizobia species, emphasis on study of climate change effect on environment and adaptation to various abiotic stresses like high temperature, drought and salinity effects on agriculture

(Kramer, 2014).

2.6.2 Factors affecting biological nitrogen fixation

There are so many factors that can have impact on the amount of N_2 fixed. These factors can be categorized into **e**daphic, climatic and biotic factors (Montanez, 2000; Liu *et al*., 2011). These factors can affect the microsymbiont, the host plant or both. Whatever the case, the decline in biological nitrogen fixation can significantly lead to reduction in yield.

Generally speaking, for a strong well-being and healthy functioning of host plant, there is need for all the factors to be in the proper form and right time (Weisany *et al*., 2013).

There are six main edaphic factors that can retard progress in biological nitrogen fixation process; excessive soil moisture, drought, P deficiency, soil acidity, excess mineral N and deficiency of Ca, Co, Mo and B (Ramos *et al*., 2003). It was reported in previous studies that moisture stress can harshly impede the nodulation process. The drought conditions can cause a decline in nitrogenase activity which can lead to a decrease in nodule weight. Exposing plants to moisture stress for ten days will cause the nodule cell wall to start desiccating which can lead to senescence (Ramos *et al*., 2003).

The nitrogenase enzyme activity is directly or indirectly controlled by the level of phosphorus contained in the soil (Liu *et al*., 2011). Phosphorus deficiency reduces nitrogen fixation indirectly by reducing nodule mass. Biological nitrogen fixation process needs a lot of energy in the form of ATP. Therefore, high amount of P is required during this important stage (Ali *et al*., 2010). Increments in grain yield of legumes as a result of increase in rate of phosphorus application has been reported by many researchers (ZamanAllah *et al*., 2007; Yakubu *et al*., 2010b; Islam *et al*., 2012; Ahiabor *et al*., 2014; AdjeiNsiah *et al*., 2018). The presence of excess amount of nitrogen makes the bacteria sluggish in nitrogen fixation capability or sometimes stopping the nitrogen fixing process completely (Reed *et al*., 2011). Availability of some micro nutrients in the soil can also enhance BNF capacity. According to Khan *et al*. (2014), application of Mo and Fe improves growth, yield and biological nitrogen fixation of chickpea.

For ensuring optimum fixation of atmospheric nitrogen, there must be availability of all other plant's nutrients except nitrogen. Thus, the availability of adequate supply of nitrogen in the soil can drastically reduce the amount of N_2 fixed in legume roots (Singh

and Usha, 2003). It has been observed by many researchers that when a plant has to choose between applied nitrogen in the soil and the symbiotic nitrogen fixation, it tends to choose the former. This is why in the presence of high nitrogen in the soil, legume nitrogen fixation is inhibited (Singh and Usha, 2003). Similar situation arises when for example, a legume is intercropped side by side with cereals, the former tends to compete for the applied nitrogen in the soil that was supposed for the cereal crops. Such competition may limit the productivity of the cereal (Singh and Usha, 2003).

There are two most influential climatic determinants hindering BNF; temperature and light. The chance for the inoculant bacteria survival diminishes under intense temperature condition, thus higher number of bacteria strains is necessary under this adverse situation (Keyser and Li, 1992; Siczek and Lipiec, 2011). Competition for light under dense canopies or due to shading in intercropped systems may limit the use of light and reduce photosynthesis which will lead to subsequent reduction in biological nitrogen fixation (Montanez, 2000). The plant's nitrogenase activity reduces dramatically due to the formation of ineffective nodules at high temperature of 40 ^{o}C (Hungria and Franco, 1993). The other limiting biotic factors are the excessive defoliation of host plant, crop competition and insects and nematodes (Keyser and Li, 1992; Mulongoy, 2015). Other workers have also reported that the process of N_2 fixation is also strongly related to the physiological states of the host plant (Zahran, 1999). Among biotic factors affecting N fixation are the absence of the required rhizobia species which is a major constraint in the N_2 fixation process. The severe environmental conditions such as salinity, unfavourable soil pH, nutrient deficiency, mineral toxicity, extreme temperature conditions, low or extremely high levels of soil moisture, inadequate photosynthates and disease conditions have direct effect on the growth and performance of crops, through hinderance of root infection by rhizobia strains and hence low N fixation. (Zahran, 1999; Montanez, 2000).

Salinity situation due to excess Na^+ accumulation hinders plant growth, nodule formation and ultimately BNF (Sousssi *et al*., 1998; Kouas *et al*., 2010). Extreme soil pH interferes with rhizobia colonization around the rhizosphere of legume. Nitrogen fixation has been reported to be inhibited by low soil pH (Van Jaarsveld *et al.*, 2002). High acidic soils (pH < 4) result in low level of calcium, phosphorous and molybdenum coupled along with aluminum and manganese toxicity, which affect both plant and rhizobia. These conditions severely affect nodulation and N_2 fixation. Most leguminous plants need pH near neutral or slightly acid for their growth activity, especially during symbiotic N_2 fixation process (Bordeeau and Prevost, 1994).

2.7 Mechanism for BNF

Biological nitrogen fixation mechanisms involve conversation of strongly bounded atmospheric nitrogen (N_2) into ammonia. High amount of energy is needed for the bond to be broken as shown in equation (1) before they are transformed into a reactive form as shown in equation (2).

$$N_2 + 6\ H^+ + ATP \longrightarrow 2\ NH_3 + ADP + Pi.$$

$$N{\equiv}N + 8\ H^+ + 8e^- + 16\ ATP \longrightarrow 2\ NH_3 + H_2 + 16\ ADP + 16\ Pi$$

The process involves the breakage of the triple bond of N_2 and addition of three atoms of hydrogen to each of the nitrogen atoms for the ammonia to be formed. The energy source of nitrogen fixing bacteria to do the work of fixing molecular nitrogen (N_2) to (NH_3) comes from oxidation (burning) of carbohydrate. The amount of nitrogen fixed will be determined by the soil N availability, absence of yield limiting factors and compatibility between bacteria and legume host. Response to inoculation is influenced by the level of indigenous rhizobia, the N requirement and yield potential of the host plant and also availability of N

in the soil (Keyser and Li, 1992). If the native rhizobia can satisfy the growing legume N requirements, it is unlikely to have response in inoculation (KanongeMafaune *et al*., 2018). Thus, when growing newly introduced legume to an area, there is tendency for nodulation to fail due to the presence of native rhizobia which may not be compatible to the crop, so appropriate rhizobia culture is necessary to be applied (Solomon *et al.*, 2012). The presence of effective and highly competitive rhizobia is very vital for the success of nodulation and subsequent nitrogen fixation to occur.

There are contradicting reports on the role of mineral nitrogen fertilizer in nodule formation. Some workers reported negative influence of nitrogen fertilizer on nitrogen fixation (Sprent *et al*., 1988; Singh and Usha, 2003). Other researchers have reported the importance of supplementing mineral nitrogen in the establishment of vigorous plant biomass and general crop performance before nodulation commences (Keyser and Li, 1992; Hardarson and Danso, 1993; Cheema and Ahmad, 2000; Carsky *et al*., 2001; Ruark, 2009). Some workers reported that there is a need for reasonable amount of nitrogen for proper plant establishment before nodule formation occurs (Hardarson and Danso, 1993; Carsky *et al*., 2001; Youseif *et al*., 2017).

The process of effective nodule formation begins via signals between host and microsymbiont. Once the compatibility of the two partners is recognized, flavonoids are released from the root of the legume host which induce transcription of nodulation genes in compatible rhizobia, leading to the formation of lipochito oligosaccharide molecules that in turn send signal to the host plant to initiate nodulation on the root of the partner plant (Long, 1996; James and Nancy, 2000). Lipo-chito oligosaccharide nodulation factors (nod factors) which is produced by rhizobia will determine the host range. These factors play an essential role which leads to the signal exchange, infection and induction of symbiotic initiating responses in legumes and consequently nodule formation in which

rhizobia carry out the function of N_2 fixation (Reddy *et al*., 1998). Nodulation process is a very complex mechanism which involves multiple processes. Once the signal exchange is identified by the compatible partner, the rhizobia penetrate the root through an infection thread and multiply. The root cortex cells will proliferate to form nodule primordia and the rhizobia are then released into nodule cells and enveloped into host plasma membrane. The rhizobia now became bacteroids that can begin to fix nitrogen (James and Nancy, 2000). Nitrogenase is an enzyme which catalyzes the nitrogen fixation mechanism. This enzyme comprises two proteins namely; an iron protein and a molybdenum-iron protein. Immediately after signal initiation, multiplication of rhizobia in the root surface of the plant begin on the emerging legume plant. The point of entry into the root is root hairs. There are other channels of penetration apart from root hairs, for example, wound entry for groundnut. After about two weeks from infection, small bumps appear on the roots. These bumps gradually enlarge, grow and mature into fully functional nodules. The nodules serve as a manufacturing house for producing nitrogen to the legume throughout the growing period with its peak activity usually around the blooming stage (Singleton *et al*., 1990). Rhizobia strains vary in their tolerance to harsh environmental conditions. Adverse environmental conditions like extreme temperature, pH and drought situation will have detrimental effect on legume-rhizobia symbiosis and consequently nodule formation and nitrogen fixation. Therefore, there is need to focus on selection of strains that can resist harsh environmental conditions (Mabrouk and Belhadj, 2012; Abd-Alla *et al*., 2014).

2.7.1 Methods for estimating BNF

Presently, there is no reliable perfect method for estimating the exact amount of nitrogen fixed biologically. Each method has its merits and demerits, but a method can be used based on the level of technology and equipment available in an area (Peoples *et al*., 1989;

Farooq and Azam, 2003).

2.7.1.1 Nitrogen difference method

The N difference method is based on the assumption that equal amount of native soil N are made available to the legume plants regardless of their genetic make-up and native origin (whether they are leguminous or non-leguminous) (Njira *et al*., 2012). However, depending on the rooting zone some plants can have advantage over others in extracting soil N. The N difference method involves the use of Kjeldahl method of N analysis to analyse plant samples collected. The amount of N_2 fixed is computed using the formula: $\text{N2 fixed} = \text{N uptake by legume} - \text{N uptake by reference crop (non legume)}$ (Peoples *et al*., 1989).

In this method, it is assumed that legume and non-legume plants have equal access to the soil available nitrogen. This method does not consider the genetic variability of plant that can affect mineralization and nitrogen availability (Peoples *et al*., 1989). The method does not take into consideration, rooting depth and soil characteristic such as soil volume and depth. This further weakens the validity of this estimate. It has been established that N uptake is directly correlated with plant types (Peoples *et al*., 1989; Hardarson and Danso, 1993; Farooq and Azam, 2003).

2.7.1.2 ^{15}N isotope method

Probably, this method is the most reliable for quantifying nitrogen fixed in a given environment (James and Nancy, 2000). The isotope method allows for the estimate of different sources of nitrogen either from soil or from the atmosphere unlike other methods which hardly differentiate the sources using N isotope (^{15}N). In this method, there is no

need for control non-fixing plant in estimating the fertilizer and soil contribution of nitrogen. This method involves addition of small quantity of labeled nitrate or ammonium to the soil where both N fixing and non-fixing plants are growing. The N difference and ^{15}N isotope were reported by some workers to give almost similar results (Loges *et al*., 2000). According to Peoples *et al*. (1989), the major constraint that makes this system unpopular and unpracticable in the developing countries is the sophisticated equipment and the high cost of ^{15}N labelled material needed (Peoples *et al*., 1989).

2.7.1.3 Xylem sap method

In legumes, N containing compounds originating from BNF (ammonia) are incorporated into glutamine and glutamate via glutamine synthesis and glutamine oxoglutarate aminotransferase pathway, respectively (Peoples *et al*., 1989). These compounds are transformed to form aspartate and other amino acids compounds. Some of the amino acid being produced are combined into purines, which are oxidized and restructured to yield ureide compounds. These newly formed compounds chemically differ from those that can be obtained from soil nitrogen and are then translocated to the plant aerial parts through xylem sap. Through vacuum extraction, sample of the sap can be extracted. Estimate on how much of the plant nitrogen comes from BNF can be determined from laboratory analysis of the collected sap. Generally, actively functioning BNF system export amide or ureides from the roots to the shoot, while those depending mainly on soil N have xylem sap rich in NO_3^- because of negligible NO_3^- reductase activity at the root level.

Relative concentrations of the ureides and NO_3^- have been used to estimate N_2 fixation capacity of a legume. The method is an estimate of tolerant level of NO_3^- of the BNF system, which is very important especially when legumes are grown side by side with cereals and some chemical fertilizers applied to the soil. The major limitations to this

method are: (i) minimum NO_3 reductase should occur at the root level to reduce misleading result. However, practically such conditions are hard to meet because of genotypic differences in the level of nitrate reductase (ii) it only allows for a short-term result. Therefore, where seasonal fixation measurement is required, there is need to repeat sequential sampling of crop (Peoples *et al*., 1989; Hardarson and Danso, 1993; Farooq and Azam, 2003).

2.7.1.4 Acetylene reduction method

This method is rapid, sensitive, cheap and accurate method for estimating N_2 fixed. It is based on the activity of nitrogenase. (Hardarson and Danso, 1993; Farooq and Azam, 2003). This method employs the technique of reducing acetylene (C_2H_2) to ethylene (C_2H_4). The method can be used in estimating the N_2 fixed at any time. This is achieved by frequent measurement of enzyme activity and sampling of the air stream containing small concentrations of C_2H_4 passing over the nodules followed by measurement of C_2H_2. The technique involves incubation of nodules detached from the plants with acetylene of known quantity in a vacuum container upon which the released acetylene is measured. The major limitation is that, some nodules may not be recovered from the plants. The enzyme activity frequency remain unchanged (Hardarson and Danso, 1993; Farooq and Azam, 2003).

2.8 Soil microorganisms as indices of a fertile soil

Soil microorganisms through their different activities enhance soil fertility status of a particular soil environment (Zhu *et al*., 2003). They help in organic litter decomposition which result in release and subsequent enhancement of soil structure and increased water holding capacity and aeration in the soil (Chen *et al*., 2001). According to Bending *et al*.

(2004), presence of large microbial biomass population is a clue that a soil is fertile and healthy and can support crop production. Bending *et al*. (2004) observed that the size of the microbial biomass can be considered as an index of soil fertility and indicator of soil quality, which depends primarily on the rate of nutrient fluxes. At a gross level, an increase in microbial biomass is considered beneficial to the fertility status of a soil, while its decline may be considered detrimental (Partey *et al*., 2014).

2.9 Organic manure

Organic manure simply means any material applied to the soil that is either of plant or animal origin that can enhance the soil structure and fertility status. Application of organic manures similarly, has positive effects on soil physical and biochemical properties (Abdullahi *et al*., 2014). It lowers soil bulk density, increases water holding capacity and CEC of the soil, builds up beneficial soil microbes, improves good soil structure and enhance stable soil aggregates (Abdullahi *et al*., 2014). Organic manure has been proven to have a desirable effect on soil through the enhancement of physical, biological and chemical conditions which in turn results in favourable plant growth (Farhad *et al*., 2011). Organic manure including farm residues, cattle manure, sheep manure and poultry manures have been found to be an excellent replacement for mineral nitrogen which can be hazardous to the environment through ground water pollution (Farhad *et al*., 2011).

The term bulky organic manure is used mostly for cattle manure, farmyard manure and compost due to the large quantity applied in relation to their nutrient contents. Comparably, organic manure such as slaughter house wastes, fishmeal and poultry manure are higher in NPK (FAO, 2006). The C:N ratio below 25:1 is needed for microbial decomposition and avoidance of N deficiency to the growing crops. Higher C:N ratio causes immobilization

of nutrients. In general, low C:N ratio enhances the release of nutrients while high C:N ratio delays release of nutrients.

The use of materials having high lignin content greater than 15% will result in low decomposition and N immobilization due to difficulty in degrading such aromatic complex compounds by microorganisms (Madejon *et al*., 2001; Odhiambo, 2010; Rezig *et al*., 2014). Low level of polyphenol of 3 to 4% is also a quality factor of any organic manure in order to avoid N immobilization. High amount of polyphenol results in binding of nitrogen in crop residue thereby forming decomposition resistant compounds (Palm and Sanchez, 1991; Verkaik *et al*., 2006).

2.9.1 Cattle manure

Research conducted by Detailed and Epr (2003) showed that application of cattle manure in combination with chemical fertilizer (30 kg N ha^{-1}) produced higher maize yield compared to sole application of 60 kg N ha^{-1} mineral fertilizer. Organic manure not only result in enhancement of beneficial activities of microorganisms but also decreases N losses through enhancement of CEC of the soil and improvement of soil structure (Abdullahi *et al*., 2014). In an experiment to observe the effect of organic manure on growth and nutrient content of pearl millet conducted in northern Nigeria Sahelian region, it was observed that application of biofertilizer in combination with organic manure enhanced millet crop growth and performance. However, it was also observed that application of 10 tonnes of cattle manure alone did not result in high millet yield and was not even significantly different from the control (Abdullahi *et al*., 2014). Cattle manure is environmentally friendly and is cheap compared to chemical fertilizer (Chinthapalli *et al*., 2015). Combined application of biofertilizer + poultry manure and single application of poultry manure yielded a better result than bio-fertilizer + cattle manure or cattle manure

singly. This can be due to differences in chemical constituents of the material and the high C/N ratio of cattle manure relative to poultry manure (Abdullahi *et al*., 2014). Despite the fact that many workers proved the beneficial effect of organic manure on crop performance, its bulkiness, low contents and slow release of nutrients especially when the material is of wide C/N causes a major challenge to its usage.

2.10 Summary of literature review

Nitrogen is mostly low in the soils of Sub Saharan Africa. This in no doubt, results in low productivity of such soils. Most farmers in sub-Saharan Africa are resource-poor to purchase expensive chemical fertilizers. In view of this, the rhizobia inoculation technique may help in improving soil fertility through biological nitrogen fixation which is natural and hazardous free to ecosystem. Atmospheric air comprises approximately 80% of nitrogen gas which is in inert form. The whole idea of biological nitrogen fixation is the conversion of this inert gas into plant usable form. This is achieved through legume association with special type of bacteria. Biological nitrogen fixation (BNF) is an important component of nitrogen cycle which is considered one of the most important biochemical cycle controlling and maintaining balance for living activities within the ecosystem.

Many studies were done on inoculation in Nigeria, but most focused on cowpea hence this research aimed to bridge this gap by focusing on soybean and groundnut which are also important legumes. Legume inoculation is a new technology in African farming systems hence there is a need to give it the needed attention in Nigeria especially in smallholder systems to improve livelihoods. There were many contradictory reports on response of legumes to inoculation. These necessitate the need to properly assess the quality of inoculants at the point of use, but not just to rely on the claim of manufacturers. Combined application

of rhizobia inoculant with organic manure may also be of more benefit to smallholder farmers through enhancement of soil structure, soil water holding capacity and the fertility status in a natural and environmentally safer manner to attain yield increments. In recent research works, a lot of attention was given to the evaluation of treatments to examine their economic viability not only mere yield increment.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of experimental sites

This study was conducted in two agro-ecological zones of northern Nigeria: the Sudan and Guinea savanna agro-ecologies. Kwari *et al*. (1999) described the soil at both experimental sites as Alfisols, formed on wide range of parent material including aeolian, alluvial and colluvial deposits. This soil is characterized with high base saturation at some depth and moderate clay content with lower content in top soil (FAO, 2014). According to FAO (2014), this soil is commonly found on gently sloping area under warm environment with distinct wet and dry seasons. The soil has suitable large agricultural uses and supports a wide range of crops.

3.1.1 Sudan savanna

The first field experiment was set up at the Kano University of Science and Technology Research farm in the Sudan savanna agro-ecological zone, Kano State. The location lies on latitude 11^{o} 37. 409’ N and longitude 08^{o} 22. 994’ E (Figure 3.1). The relief of the site is 481 meters (1580 feet) above sea level. The area is characterized by high temperatures throughout the year. There are seasonal temperature changes, indicating a gradual increase from January to April where maximum value reaches as high as 43 ^{o}C (Ibrahim, 2011). The mean monthly temperature is generally warm, with mean value of about 26 °C and a diurnal range of about 10 - 13 °C in September. The mean annual rainfall is about 600 mm and starts usually in May and ends in September. Great temporal variation occurs in the amount of rainfall received and no two consecutive years record the same amount. Soil type of the study area is predominantly sandy loam with low in nutrient status (Olofin,

1987). The cropping history of the experimental area revealed wide range of crop production ranging from cereals to vegetables for research purposes. The crops included maize, sun flower, groundnut, cowpea and vegetable crops for three years. A year before this experiment the area was under maize cultivation and sunflower cultivation.

3.1.2 Guinea savanna

The second field experiment was established at the Abubakar Tafawa Balewa University Research field (Figure 3.1). It is located on latitude 10°27. 985' N and longitude 9°49.768' E. The area is situated at about 666.5 m (2187 feet) above sea level in the northern Guinea savanna agro-ecology of northern Nigeria (Giwa *et al*., 2017). The Climate of the area is marked by two seasons; dry and wet seasons lasting from October to March and April to September, respectively. The soil is mostly sandy loam and low in inherent fertility (Vonchir *et al*., 2006).

The cropping history of the area being a research station, revealed that a wide range of agricultural crops are being produced yearly for research purposes. The crops include groundnut, cowpea, Bambara groundnut cereals and vegetable crops for some years. A year before the onset of this experiment the area was under cereals and cowpea cultivation.

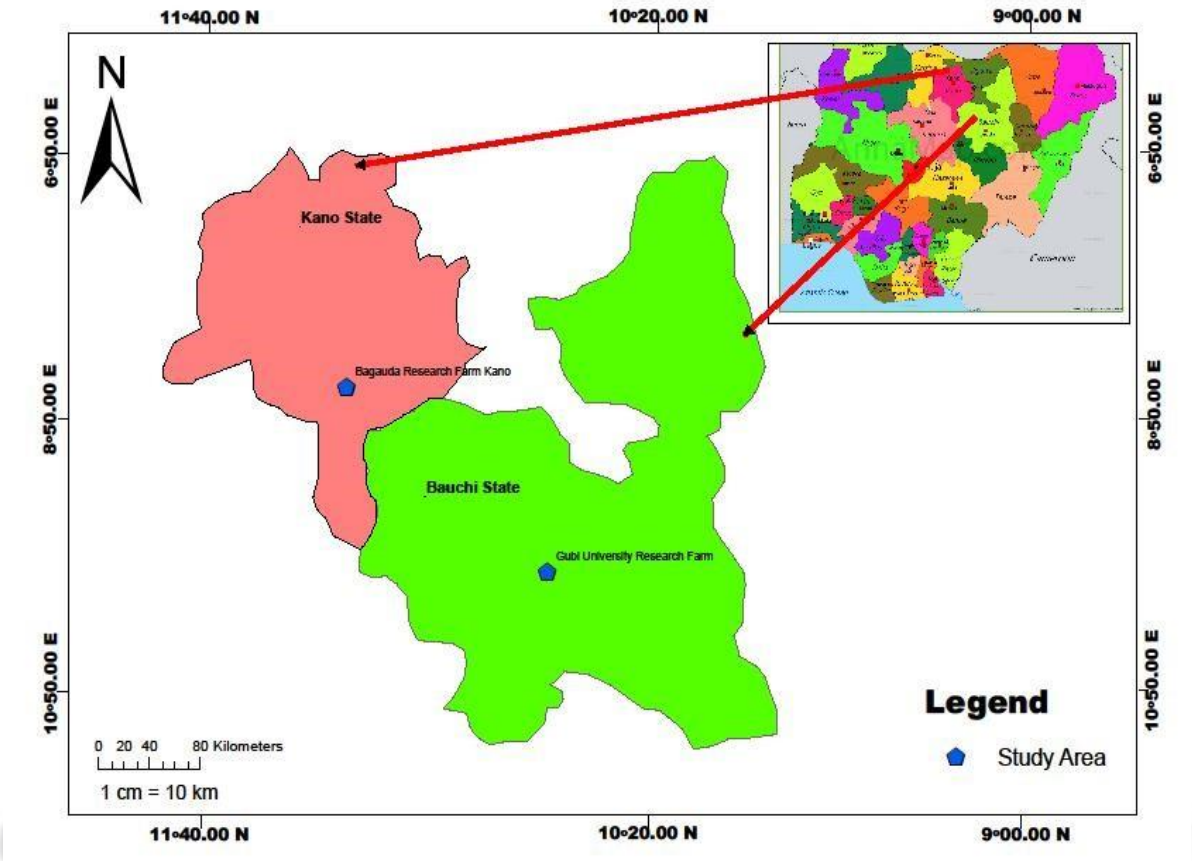


Figure 3.1: Location of the study areas.

3.2 Field preparation, treatments, layout and experimental design

This study was laid out in a Randomized Complete Block Design (RCBD) replicated four times for each crop. Each plot size measured 4 m by 4.5 m. There were seven plots per block. The inter row spacing was 0.75 m. Weeding was done with hoe regularly. No herbicide was applied throughout the growing period.

Treatments for soybean were: T_1 = Legume fix, T_2 = Alosca, T_3 = Nitrogen (Urea at 50 kg N ha^{-1}), T_4 = cattle manure (4 tonnes/ha), T_5 = Legumefix + cattle manure (4 tonnes/ha), T_6 = Alosca + cattle manure (4 tonnes/ha), T_7 = Control. Treatments for groundnut were: T_1 = Histick, T_2 = Biofix, T_3 = Nitrogen (Urea at 50 kg N ha^{-1}, T_4 = cattle manure (4 tonnes /ha), T_5 = Histick + cattle manure (4 tonnes/ha), T_6 = Biofix + cattle manure (4 tonnes/ha),

T_7 = Control. Soybean (*Glycine max* (L)) and groundnut (*Arachis hypogaea*) seeds were sown in July, 2015. Maize was sown at a spacing of 75 cm by 30 cm on fields which were previously cropped with soybean and groundnut preceding year. Basal application of 20 kg N ha^{-1} and 30 kg N ha^{-1} were applied at 4 and 8 weeks after planting, respectively.

3.3 Soil sampling and laboratory analyses

Twelve core soil samples from a depth of 0 - 15 cm were collected from each plot from the two experimental sites following a 'W' design for initial characterization before planting. Composite sample was obtained after careful mix of the soil and air dried before sieving through a 2 mm mesh sieve. The collected samples were packaged into clean ziplock bags prior to transport to the laboratory. The final soil sampling was done after harvesting the crops. Twelve soils samples were collected from each plot and a composite sample was obtained. After mixing, labelling and packaging in clean ziplock bags the soils was then taken to the laboratory for analysis.

3.4 Soil physical properties

3.4.1 Particle size analysis

Particle size distribution of soil sampled was determined using the Bouyoucos hydrometer method (Bouyoucos, 1962). Fifty-one grams of soil sample was weighed into a 1.0 litre screw cap shaking bottle. Hundred millimetres of distilled water was added and swirled gently until it mixed thoroughly. Twenty millilitres of 30% H_2O_2 was then added, followed by 50 ml of 5% sodium hexametaphosphate and drops of amyl alcohol and stirred gently. The content was then shaken on a mechanical shaker for 2 hours. The mixture was then transferred into a 1.0 L cylinder to allow the sediments to settle down. After 40 seconds, the first hydrometer reading was taken and the first temperature was read with the aid of a

thermometer. The content of the sedimentation cylinder was left undisturbed for 3 hours and second hydrometer and temperature readings produced.

Calculation:

$$\% \, Sand = 100 - \{H1 + 0.2(T1 - 20) - 2\} \; 2$$

$$\% \, Clay = \{H2 + 0.2(T2 - 20) - 2\} \; 2$$

$$\% \, Silt = 100 - (\% \, Sand + \% \, Clay)$$

where:

H_1 = 1st hydrometer reading

T_1 = 1st temperature reading

T_2 = 2nd temperature reading

H_2 = 2nd hydrometer reading

-2 = Salt correction factor to be added to hydrometer reading

0.2 (T – 20) = Temperature correction

The textural triangle was used to determine the soil textural distribution in percentage.

3.5 Soil chemical properties

The soil chemical properties that were determined included: total nitrogen, available phosphorus, exchangeable cations, total exchangeable acidity, cation exchange capacity, organic carbon and pH.

3.5.1 Soil pH

Soil pH was determined using an electrometric method (Page *et al*., 1982) in a 1: 2.5 soil: water solution ratio. Ten grams of air-dried soil was weighed into a 50 ml plastic beaker and followed by 25 ml of distilled water. The suspension was stirred vigorously using glass

stirring rod for 20 minutes. The solution was allowed to settle for 30 minutes before measuring the pH by inserting carefully the glass electrode of the pH meter. Before the measurement was done, the glass electrode of the pH meter was calibrated with blank solution at pH of 4 and 7, respectively.

3.5.2 Soil organic carbon

Soil organic carbon was determined using the Walkey-Black method as described by Nelson and Sommers (1982). This method involves chemical or wet oxidation followed by the measurement of expelled CO_2. Excess dichromate was determined by titrating with standard ferrous sulphate solution. One gram of the soil sample was weighed into 500 ml Erlenmeyer flask and mixed thoroughly. Reference sample was also used as a blank during titration. Precisely, 10 ml of one equivalent (0.167 *M*) $K_2Cr_2O_7$ solution was pipetted and 20 ml of concentrated H_2SO_4 was also added to facilitate the reaction through generated heat. The mixture was mixed gently and then allowed to settle for 30 minutes on an asbestos sheet to allow for cooling. Thereafter, 200 ml of distilled water was added, followed by addition of 10 ml of orthophosphoric acid and diphenylamine indicator. Ferrous sulphate solution (1*N* Fe_2SO_4) was used to titrate the mixture till the colour changed to dark blue and then finally to the green end point. The titre value was then produced as the volume of the Fe_2SO_4 used.

Calculation:

The organic carbon content of soil was calculated as:

$$\%C = \frac{M \text{ x } 0.39 \text{ x mcf } (V_1 - V_2)}{W}$$

where:

M = molarity of Fe_2SO_4

V_1 = amount of Fe_2SO_4 solution (ml) required for blank

V_2 = amount of Fe_2SO_4 solution (ml) required for sample mcf =

moisture correction factor (100 + % moisture content) / 100 w =

weight of air - dry sample in gram

0.39 = 3 × 0.001 × 100 % × 1.3 (3 = carbon equivalent weight)

1.3 = correction factor to neutralize incomplete oxidation of the organic carbon).

3.5.3 Total nitrogen

Soil total nitrogen content was determined using the Macro – Kjeldahl digestion and distillation method. The method involved digestion, distillation and titration as described by Bremner and Mulvaney (1982). Soil sample (0.5 g) was digested in 5 ml of concentrated sulphuric acid (H_2SO_4), followed by the addition of few drops of 30% hydrogen peroxide (H_2O_2) to the solution. This procedure ensured the conversion of organic nitrogen to ammonium sulphate. The resultant solution was made alkaline by the addition of 5 ml of 40% sodium hydroxide (NaOH). Ammonia was distilled into 2% boric acid and titrated against standard hydrochloric acid (0.02 *N* HCl). Blank distillation was then run to cater for nitrogen traces present in the reagents and the water that was used.

Calculation:

14g of N contained in one molecule of NH_3

$$Weight\ of\ N\ in\ the\ soil = \frac{14 \text{ x } (\text{A} - \text{B}) \text{ x } N}{1000}$$

where:

A = volume of standard HCl used in the sample titration

B = volume of standard HCl used in the blank titration

N = Normality of standard HCl

3.5.4 Available phosphorus

Bray No 1 method was used to extract the readily acid soluble forms of phosphorus from the soil as described by hydrometer. Five grams of soil was weighed into a 100 ml extraction bottle and 35 ml of Bray 1 solution (0.03 *M* NH_4F and 0.025 *N* HCl) was added and the reciprocal shaker was used to shake the soil solution for 10 minutes and then allowed to settle for two minutes and filtered through a Whatman No 42-filter paper. After that, an aliquot of 5 ml of the filtrate was then pipetted into 25 ml centrifuge conical flask and then 10 ml of colouring reagent (ammonium molybdate) was added and mixed thoroughly. A 2 ml of ascorbic acid solution was added and allowed to stand for 15 minutes until it developed a deep blue colour. The colour intensity was measured at 660 nm wavelength using a 2 I D spectrophotometer. Standard curve was used to estimate the available phosphorus content of the soil. Standard series of 0, 1.2, 2.4, 3.6, 4.8 and 6.0 mg P/L concentration was prepared by pipetting 0, 10, 20, 30, 40 and 50 ml of 12.0 mg P/L concentration, respectively in 100 ml volumetric flask, using distilled water to make the volume.

Calculation:

$$\mathrm{P(mg/kg)} = \frac{(a - b) \times 35 \times 15 \times mcf}{g}$$

where:

a = mg P/L in the sample extract

b = mg P/L in the blank g =

sample weight in grams mcf =

moisture correction factor

35 = volume of extraction solution

15 = final volume of sample solution

3.5.5 Exchangeable cations

Potassium, sodium, magnesium and calcium in the soil sample were determined using 1.0 *M* ammonium acetate (NH_4OAc) extract. A 10 g soil sample was weighed into a leaching tube and leached with 250 ml 1.0 *M* (NH_4OAc) solution adjusted to pH 7. The solution was then diluted with distilled water to 1.0 litre in a volumetric flask.

3.5.5.1 Exchangeable K and Na

The exchangeable K and Na concentration in the soil solution was determined by flame photometry as described by Cottenie (1980). Standard solutions of potassium and sodium concentrations were prepared by dissolving 2.5 g sodium chloride and 1.9 g potassium chloride each into a separate 200 ml of distilled water. The two solutions were mixed and made up to volume of 1000 ml with distilled water. Then 50 ml solution was drawn from the stock solution and diluted to 1000 ml each for K + Na. Portions of 0, 4, 8, 12, 16 and 20 ml of the 50 mg L^{-1} standard solution was placed into separate 200 ml volumetric flasks. To each of the flasks, 100 ml of one equivalent of ammonium acetate solution was added and using distilled water, made to volume (200 ml). The standard series obtained was 0, 2, 4, 6, 8 and 10 mg L^{-1} for potassium plus sodium (K + Na). A 100 ml of one equivalent of ammonium acetate solution was added into 10 g of soil in the extraction bottle and shaken on a mechanical shaker for 2 hours. The soil solution was then filtered through Whatman filter paper No. 42 and a 10 ml aliquot was pipetted and read for K and Na contents with the flame photometer at a wavelength of 766.5 and 589.0 nm, respectively. The reading was used to determine the concentration of potassium and or sodium in the soil extract.

Calculations:

$$\text{Exchangeable K } (\text{cmol}_{(+)}/\text{kg soil}) = \frac{250 \times (a - b)\ \text{mcf}}{10 \times 39.1 \times s}$$

$$\text{Exchangeable Na } (\text{cmol}_{(+)}/\text{kg soil}) = \frac{250\ x\ (c - d) \times\ \text{mcf}}{10 \times 22.9 \times s}$$

where:

a = mg K/Lin the sample b = mg K/ Lin the blank sample c = mg Na/Lin the sample d = mg Na/L in the blank sample

mcf = moisture content correcting factor s = weight of air-dried soil sample in grams

3.5.5.2 Exchangeable Ca and Mg

The concentration of calcium and magnesium were determined using titration method with ethylene diamine tetra acetic acid (EDTA) solution (Moss, 1961). A 10 ml aliquot of the sample solution extracted with 1.0 *N* ammonium acetate and filtrate was transferred into a 100 ml conical flask. Ten millilitres of 10 % potassium hydroxide solution was added and then followed by 1 ml of 30 % triethanolamine. Potassium cyanide solution (10 %) was added in three drops followed by a few crystals of cal-red indicator and the mixture was vigorously shaken to get it uniform. The mixture was titrated with 0.02 *N* EDTA solution from a red to blue end-point and the titre value of calcium was then produced.

A 10 ml aliquot of the same sample solution extracted with 1.0 *N* ammonium acetate and filtered as described above were transferred into a 100 ml conical flask. Five millilitres of ammonium chloride-ammonium hydroxide buffer solution was added followed by 1 ml of triethanolamine. Three drops of 10% potassium cyanide solution were added and also a few drops of Eriochrome Black T (EBT) indicator solution and the mixture was vigorously shaken to get it uniform. The mixture was titrated with 0.02 *N* EDTA solution from a red

to blue end-point. The titre value was produced again and to get the titre value for Mg, the titre value of Ca was subtracted from this value.

Calculation:

$$\text{Concentration of Ca + Mg } (\text{cmol}_{(+)}/\text{kg soil} = \frac{0.02 \text{ x } (V_a - V_b) \text{ x } 1000}{0.1 \text{ x w}}$$

where:

W = weight (g) of air-dry soil

V_a = volume (ml) of 0.02 *N* EDTA used in the sample titration

V_b = volume (ml) of 0.02 *N* EDTA used in the blank titration

0.02 = concentration of EDTA used

NB: Ca = Titre value of Ca × 2 in cmol/kg or Meq/100 g soil

3.5.5.3 Exchangeable acidity

Extraction of hydrogen and aluminum anions was done by titration method (Mclean 1965). For the hydrogen, five grams of air-dried soil sample which was sieved through 2 a mm mesh sieve was weighed into a 100 ml plastic bottle and shaken for 2 hours after adding 50 ml of 1.0 *N* potassium chloride solution. The solution was filtered through a Whatman No. 42 filter paper into an Erlenmeyer flask and 6 drops of phenolphthalein indicator solution added and titrated with 0.05 *N* sodium hydroxide until pink end point was reached. The value of sodium hydroxide was produced.

For the aluminium, 4 ml of 3.0 *N* sodium fluoride (NaF) was added to the titrated extract. The mixture was titrated with hydrochloric acid (0.05 *N*) until a colourless end point was reached. The volume (ml) of HCl used was produced.

Calculation:

$$\text{Exchangeable aluminium } \left(\frac{\text{meq}}{100\text{g}}\right) = \text{v x } 0.05 \text{ x} \frac{100}{\text{w}}$$

where:

v = volume of NaOH used (ml)

w = weight soil sample used (5 g)

0.05 = normality of NaOH solution

$$\text{Exchangeable aluminium } \left(\frac{\text{meq}}{100\text{g}}\right) = \text{v x } 0.05 \text{ x} \frac{100}{\text{w}} = \text{v x1}$$

where:

v = titre volume of hydrochloric acid (HCl) used (ml)

0.05 = normality of HCl w = weight

of soil sample used (5 g)

3.5.5.4 Effective cation exchange capacity

The ECEC was calculated by summation of exchangeable cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) and exchangeable acidity (Al^{3+} and H^{+}).

3.6 Laboratory analysis of cattle manure and plant samples

Cattle manure was analyzed after drying at room temperature, grinding and sieving through a 1 mm mesh. Total nitrogen, phosphorus, potassium, organic carbon and magnesium contents were determined (Motsara and Roy, 2008). Determination of polyphenol and lignin contents for cattle manure were undertaken using protocols as outlined by Anderson and Ingram (1998).

At 50% flowering stage (i.e. peak of reproductive stage), 10 plant samples were randomly collected from the two inner rows of each plot. Root and shoot were then separated, weighed and oven dried at 60 ^{o}C for 72 hours, then crushed and milled using grinding machine.

The laboratory analyses of plant samples were done to determine the amount of nitrogen fixed, potassium and phosphorus estimates.

3.6.1 Organic carbon

Dichromate acid oxidation method was used to determine the organic carbon content of cattle manure and plant samples. Ten millilitres each of concentrated sulphuric acid solution (H_2SO_4) and 1.0 *N* potassium dichromate solution ($K_2Cr_2O_7$) were added to 0.1 g of a powdered manure specimen in an Erlenmeyer flask. The solution was kept to settle for 30 minutes and 300 ml of distilled water added, followed by 15 ml of orthophosphoric acid and 3 ml of diphenylamine indicator. Titration was done with 0.5 *N* Fe_2SO_4 solution until the color changed to dark blue and then to a green end point. The titre value for the blank and the solution were then produced.

The organic carbon content of the cattle manure was calculated from the equation:

$$\%C = \frac{N\ x\ (a-b) x\ 100\ x\ 1.33}{W}$$

where:

N = normality of ferrous sulfate (from blank titration)

a = ml of ferrous sulphate solution for blank titration

b = ml ferrous sulphate solution for sample titration

w = weight of oven dried soil sample

0.003 = milli- equivalent weight of carbon

1.33 = incomplete combustion correction factor

3.6.2 Total nitrogen

Total nitrogen of the cattle manure and plant samples was determined by the Kjeldahl distillation method as described by Bremner and Mulvaney (1982). Twenty grammes of

oven-dried samples was ground in a stainless-steel mill and passed through a 1 mm mesh sieve. A 0.5 g portion of the sample was digested in a 10 ml concentrated sulphuric acid and selenium mixture was added to catalyse the reaction. The resulting clear digest was transferred into a 100 ml volumetric flask and made to volume with distilled water. A 5 ml aliquot of the sample and a blank were pipetted into the Kjeldahl distillation apparatus separately and 10 ml of 40 % NaOH solution was added followed by distillation. The resultant ammonia gas was trapped in 15 ml of 2 % boric acid. The distillate was then titrated with 0.1 *M* HCl with bromocresol green and methyl red indicator solution and N calculated as follows.

Calculation:

$$\%N = \frac{(a - b) \text{ x } M \text{ x } 1.4}{w}$$

where:

a = HCl volume for titration (ml) b = HCl

for volume for blank titration (ml) m =

molarity of HCl

1.4 = 14 x 0.001 x 100% (14= atomic weight of N) w

= weight of sample

3.6.3 Total phosphorus

Total phosphorus in the samples was determined using the spectrophotometric vanadium phosphomolybdate method as described by Motsara and Roy (2008). This method involves the addition of 1 g of plant tissue sample into a digestion tube, followed by addition of 1 ml of digestion mixture ($HClO_4.HNO_3$). The digestion of the content was made after making up the volume to 500 ml in a volumetric flask. Ten millilitres of the digest was then transferred into 50 mL volumetric flask followed by the addition of 10 millilitres of

vanadomolybdate solution. The content was then mixed with distilled water, shaken well and then allowed to settle for about 30 minutes until colour development and read finally on an atomic absorption spectrophotometer. Percentage transmittance was then determined as well as the absorbance and the P content after plotting a standard curve of absorbance against transmittance.

3.6.4 Polyphenol

Polyphenol content of the cattle manure was determined using the procedure of Folin - Denis (Anderson and Ingram, 1998). One gramme cattle manure was weighed into 50 ml clean conical flasks. Twenty milliliters of ethanol were then added to the sample and heated to 60 ^{o}C to extract the polyphenol. The extraction procedure was repeated after the alcohol extract was poured into another flask. The volume of the extract was made up to 50 ml by adding ethanol after the third extraction was made. Standard solutions of tannic acid (with concentrations of 0, 15, 30, 60 and 90 mg tannic acid per liter) were prepared. The sample and tannic acid standards were subjected to color development. Spectrophotometer (UV 752) was used to read absorbance values of the standard and sample solutions at a wavelength of 760 nm. A standard curve was used to determine the sample solutions by plotting absorbance values against concentrations of the prepared standard solutions.

Calculation:

Polyphenol (mg kg^{-1}) = graph reading × dilution of aliquot × dilution of sample where:

$$Sample\ dilution = \frac{final\ volume\ of\ sample}{weight}$$

3.6.5 Lignin

The lignin content of the manure was determined through an acid detergent fiber procedure described by Anderson and Ingram (1998). After obtaining the extract from the addition

of sulphuric acid and alcohol, 2 ml of 75% HCl acid was added and shaken for 4 hours. The solution was then transferred into an Erlenmeyer flask and 40 ml of distilled water added and boiled for two hours, followed by filtration. Sugar content was determined in the hydrolysate. The residue was then washed with water, dried at 65 ^{o}C for 48 hours, weighed and then ashed in muffle furnace at 550 ^{o}C for 4 hours. The lignin content of the manure was then measured as the loss in weight on ignition.

3.7 Determination of rhizobia population at the experimental sites

The plant infection counts also called the most-probable number (MPN) was used to determine the number of viable rhizobia in the presence of other microorganisms as described by Somasegaran and Hoben (1994). This indirect method is commonly used to determine the quality of inoculants produced from non-sterile carrier materials. Soil samples from a depth of 0 - 15 cm were collected, bulked and sub samples (composite) obtained after mixing the soils these were sealed and stored at 4 ^{o}C for the evaluation of rhizobia population. The soil was used to inoculate soybean and groundnut in a growth pouch using serial dilutions 10^{-1} to 10^{-5} and replicated five times in each case.

After successful germination, the seedlings were carefully placed into growth pouches with the radicle pointing downwards. A nutrient solution free of N was prepared and addition was done regularly. A serial dilution was prepared by taking 100 g of the soil and diluting it with 400 mL of distilled water. A fivefold serial dilution was made 10^{-1} to 10^{-5} while growth pouches were replicated five times and set in the greenhouse and inoculated with 1 mL dilution. The plants were observed for 4 weeks. Thereafter, the nodules were observed and counted. Number of rhizobia were determined using the following formula:

$$X = \frac{m\ x\ d}{v}$$

where:

m = likely number from the MPN table for the lowest dilution of the series

d = lowest dilution (first unit) v = volume of aliquot applied to plant

3.8 Estimation of nitrogen fixation

The nitrogen difference method was used to estimate the amount of nitrogen fixed by soybean and groundnut. Nitrogen content from the reference crop (maize) was then subtracted from that of the legume crop (Peoples *et al*., 1989).

$$\text{BNF (kg/ha)} = \text{N uptake in legumes - N uptake reference crop}$$

Nitrogen uptake (kg/ha) was thus obtained as follows:

$$\text{N, P, K uptake (kg/ha)} = \frac{\text{dry matter (kg/ha)} \times \%\ \text{(N, P, K)}}{100}$$ Sharma et al. (2012).

3.9 Assessment of the quality of microbial inoculants used

In this study, a total of four microbial inoculants were used; two for soybean and two for groundnut. The soybean inoculants were Legume fix and Alosca while the groundnut inoculants were Histick and Biofix. Experiment was set up to estimate the number of rhizobia in the inoculants to be used for soybean and groundnut experiments using the Most Probable Number method (Vincent, 1970). Cowpea seeds were used for this experiment after several attempts to raise healthy soybean seedlings failed due to poor seed viability. The seeds were sterilized with 95% ethanol for 10 seconds followed by addition of 3% hydrogen peroxide for 2 - 3 minutes and rinsed 5 to 6 times with distilled water. The seeds were lined on moist tissue paper and left for 4 hours to imbibe water in petri dishes and then kept in the incubator for 48 hours to germinate.

Healthy seedlings were then selected and the radicles carefully inserted into the growth pouches using clean forceps. The pouches were then arranged on wooden racks and kept

in the greenhouse before inoculation. For inoculation with *Bradyrhizobium*, ten-fold serial dilution was prepared for inoculant samples. Serial dilution of 10^{-1} to 10^{-10} were used to inoculate the seedlings. Each growth pouch was inoculated with 1 ml of the dilutions, which was replicated four times for each of the dilution series, starting from the highest dilution to prevent contamination. Periodic watering with N free solution were done (Broughton and Dilworth, 1971). Assessment of nodules was done after twenty-eight days. The total number of pouches that nodulated for each dilution replicated unit was produced and used to determine the number of rhizobia using charts generated by MPNES software (Woomer *et al*., 1990).

3.9.1 Viable cell count

Viable cells in the inoculants were determined through the spread plated technique employed by Zuberer (1994). Saline solution (0.85% NaCl) was used as a diluent for all tenfold dilution series. A 10-fold serial dilution from 10^{-1} to 10^{-10} was prepared with each inoculant for which 100 ul of each dilution was spread onto Congo red yeast extract mannitol agar. Each dilution plate culture was replicated two times. The plates were then kept for 2 - 7 days at a temperature of 28 ºC in an incubator using dilution levels having colony counts ranging from 30 – 300. The number of colonies forming units was determined from the relationship below:

$$\text{Colonies forming units (Cfu)} = \text{Number of colonies counted x } \frac{\text{dilution factor}}{\text{aliquot plated}}$$

3.9.2 Determination of pH

This was determined using a glass electrode (Jenway 3610) pH meter in a 1:2.5 inoculant to deionized water ratio (refer to 3.4.1 for details).

3.9.3 Moisture content

The moisture content of all the inoculants were determined according to the procedure described in the American Association of Cereal Chemists (A A C C) (2000). Five grams of the inoculant was weighed into open glass dish. The inoculant was then dried in the oven for 3 hours at a temperature of 105 $\pm$ 5 ^{0}C. The dish was then covered and transferred to desiccators and weighed quickly as soon as the dish was cooled. The heating and weighing procedures were repeated several times until successive weights did not differ by more than one milligram. The moisture content was determined on the weight loss of sample before and after drying using the relation below:

$$\text{Moisture (\%)} = \frac{\text{weight loss}}{\text{weightof sample}} \text{ x } 100$$

3.10 Inoculation and sowing

Soybean TGX 1835 (Tropical Glycine Cross) obtained from Institute of Agricultural Research, Ahmadu Bello University (ABU) Zaria was used in the experiment in all the locations. Three seeds were sown per hill at a depth of three centimeters at a spacing of 10 cm intra row. Two weeks after emergence, the seedlings were thinned to two per hill. The inter row spacing was 75 cm whilst spacing between individual plots was 150 cm. The inoculants used for soybean were Legume fix and Alosca which contained *Bradyrhizobium* strains. Legume fix was in the form of black powdery peat while Alosca contained *Bradyrhizobium* in ash fine granular form. Fifty grams each of Alosca and

Legume fix inoculants were added to 1.0 kg of seed after adding little water and gum Arabic to ensure stickiness. The inoculated soybean seeds were allowed to air dry under shade for 30 minutes before sowing.

For groundnut seed, the variety SAMNUT 24 obtained from Institute of Agricultural Research Zaria was used. Two seeds were sown per hole at a spacing of 10 cm intra row

and 2 weeks after emergence, the seedlings were thinned to one per stand. The inoculants used for groundnut were Histick and Biofix. Histick and Biofix contained *Bradyrhizobium* strains in a black powdery peat. Fifty grams per 1.0 kg seed each of these inoculants were used. Inoculants were coated with gum Arabic and water to ensure stickiness to the seed. The coated seeds were left to dry under a shade for 30 minutes before sowing.

For maize, the variety SAMMAZ 14 was used. This is white maize seed early maturing variety obtained from Institute of Agricultural Research (IAR) Zaria, Nigeria.

3.11 Residual effect experiment

A residual effect experiment was conducted during the second-year field trial in 2016 on fields that were used for soybean and groundnut the previous year in both agro-ecological zones. The same cultural practice was maintained as in first year experiment and no herbicide was applied throughout the experiment. The data collected were stover yield, plant total N, P and K contents, grain yield and harvest index.

3.12 Agronomic data

3.12.1 Nodule number

Ten plant samples were randomly selected and then carefully uprooted from the two inner rows of each plot at peak flowering stage (8 weeks after planting) to assess nodulation. A spade was used to uproot plants from the soil. Care was taken to collect all the nodules including the detached ones and kept inside ziplock bags, labelled and taken to the laboratory where they were carefully washed and counted. Ten nodules were randomly selected and sharp razor blade was used to cut open the nodules longitudinally to examine their colour and appearance for number of effective nodules. Nodules that appeared

reddish or pink in colour were regarded as effective. Percent effective nodules was calculated as the ratio of active nodules to total number of nodules per plant.

3.12.2 Nodule weight

Fresh nodules were collected from 10 plants in each plot and weighed accordingly and produced. The nodules of each of the treatments were subjected to oven drying at 75 ^{o}C for 48 hours and the weight was produced to obtain nodule dry weight and expressed in mg for each treatment.

3.12.3 Mean 100 seed weight

The mean 100 seed weight was computed by counting 100 seeds three times separately from each of the treatments. The mean weights were then obtained and produced for each treatment.

3.12.4 Number of pods

Number of pods per plant was obtained after computing the average number of pods from ten plants.

3.12.5 Grain yield

The two inner rows of each plot were harvested at plant physiological maturity for yield estimate, and the grains were oven - dried at 60 ℃ for 72 hours. The grain dry weights for each of the plot were then produced and used to determine grain yield per hectare (Okogun *et al*., 2005).

3.12.6 Shoot and root dry weights

Ten plants were randomly harvested from the second row of each plot at peak flowering stage. The plants were washed and separated into shoot and roots and subjected to oven drying at 65 ℃ for 72 hours. Dry weights of shoot and root were taken separately and produced.

For maize, five plants were harvested from the second rows at middle silking stage and put into A3-sized envelops and oven dried at 60 ℃ for 48 hours.

3.12.7 Plant sampling

At 50 % flowering stage, 10 plants each of soybean and groundnut were harvested from the two outer rows and put in large envelops, air-dried, ground and sieved with 2 millimeter steel mesh sieve and then taken to the laboratory for the N, P and K chemical analysis.

3.12.8 Grain N, P and K determination

Sample grains of each treatment after harvesting were also ground and analysed for N, P and K contents.

3.12.9 Harvest index

This is the ratio of the economic portion to the non-economic portion of the plant and was obtained by dividing the cob yield by the total biomass yield of each plot.

$$HI = \frac{CY}{TY}$$

where:

HI = harvest index

TY = total yield

CY= cob yield

3.13 Economic analysis

The cost and benefit of the treatments used were expressed in monetary terms to measure their economic viability. The indices used to compute economic viability were gross revenue, net profit and marginal cost (CIMMYT, 1998). Marginal net benefits and marginal costs were computed to determine the percentage marginal rate of return (%) using the following relation:

$$\text{Marginal rate of return (\%)} = \frac{\text{Marginal net benefit}}{\text{Marginal cost}} \text{ x } 100$$

3.14 Statistical analysis

The data generated from the experiment were subjected to analysis of variance (ANOVA) using Genstat 12th edition statistical software. Separate analyses were done for soybean, groundnut and maize plant to separate means of different treatments. Fishers protected least significant differences (LSD) was used to detect significant differences among the treatment means. Percentage cumulative variation was also used to show the extent of variability within treatments units. Pearson correlation matrix was used to measure the degree of relationship between some growth parameters such as shoot dry weight, root dry weight, nodule dry weight, 100 seed weight and grain yield of soybean and groundnut.

CHAPTER FOUR

4.0 RESULTS

4.1 Physico-chemical properties of soils at the experimental sites

The results of the initial soil properties of the study sites are shown in Table 4.1. The soil in both study sites was sandy loam in texture. Total nitrogen content was 0.11 % in both locations. Available phosphorus values in the Sudan and Guinea savannas were 8.05 mg kg^{-1} and 4.03 mg kg^{-1} respectively, while soil pH values (1:2.5 H_2O) were 5.90 and 6.50 for Sudan and Guinea savanna agro - ecologies, respectively. The soil exchangeable potassium values were 0.09 and 0.11 cmol $_{(+)}$ kg^{-1} in the Sudan savanna and Guinea savanna zones, respectively. Soils of Sudan and Guinea savannas had inherent K value below the critical level of 0.15 cmol $_{(+)}$ kg^{-1}. Organic carbon values in the Sudan and Guinea savanna agro – ecological zones were 0.41 and 0.37%, respectively. The indigenous rhizobia population (IRP) were found to be 1.02 x 10^1 and 2.20 x 10^1 cells g^{-1} of soil in the Sudan and Guinea savanna agro - ecologies, respectively.

Table 4.1: Initial physico-chemical properties and indigenous rhizobia counts of the experimental sites

Parameters	Sudan savanna (Bagauda location)	Guinea savanna (Gubi location)
pH (1:2.5 H_2O)	5.90	6.50
Organic carbon (%)	0.41	0.37
Total nitrogen (%)	0.11	0.11
Available P (mg kg^{-1})	8.05	4.03
Exchangeable cations ($cmol_{(+)}\ kg^{-1}$)		
Ca^{2+}	5.20	3.63
Mg^{2+}	1.04	0.43
K^{+}	0.09	0.11
Na^{+}	0.08	0.12
Total exchangeable acidity ($Al^{3+} + H^{+}$) ($cmol_{(+)}\ kg^{-1}$)	0.80	0.40
ECEC	7.60	5.30
Sand (%)	66.00	68.00
Silt (%)	22.00	18.00
Clay (%)	12.00	14.00
Texture	Sandy loam	Sandy loam
IRP (cells g^{-1} of soil)	1.02×10^1	2.20×10^1

IRP - Indigenous rhizobia populations

4.2 Lignin and polyphenol contents of cattle manure used

Analyses of the lignin and polyphenol contents of the cattle manure used is as shown in Table 4.2. Lignin and polyphenol contents were 10.73 and 2.97%, respectively.

Table 4.2: Chemical analysis of cattle manure

Parameters	Lignin	Polyphenol (%)	pH	N	P	K	C	C/N ratio	Fe	Mn
	←	→		←	(%)		→		← (mg kg^{-1})	→
Values	10.73	2.97	7.1	1.35	0.48	0.73	26.95	19.96	2.82	1.27

4.3 Quality assessment of microbial inoculants used

4.3.1 Most probable number (MPN) method

The results of MPN is presented in Table 4.3. The four inoculants had sufficient number of rhizobia to inoculate the seeds. Inoculants containing strains 10^5 and above gave a better result. Legume fix had the highest number of rhizobia while the least number was observed in Histick. The pH and moisture content of inoculants used were favourable for the survival of rhizobia strains.

Table 4.3: Quality of inoculants used

Inoculants	Form	pH	Moisture content (%)	MPN estimate (cell g^{-1} inoculant)
Legume fix	Peat powder	6.86	47.75	1.7×10^6
Alosca	Granular	6.78	17.25	3.1×10^5
Histick	Peat powder	7.21	49.75	5.8×10^3
Biofix	Peat powder	6.64	63.50	5.5×10^4

4.4 Study 1: Effect of some microbial inoculants on the growth and yield of soybean and groundnut in the Sudan and Guinea savanna zones

4.4.1 Nodule number, number of effective nodules and nodule dry weight of soybean

The results of nodule number, number of effective nodules and nodule dry weight of soybean in the Guinea savanna and Sudan savanna are presented in Table 4.4. In the Sudan savanna, Legume fix + cattle manure gave the highest nodule number, which was statistically different from the control which had the least value of 23. The results further showed that all treatments produced nodule numbers which were significantly higher than the control. Percent number of effective nodules was affected by inoculation. Legume fix gave the highest percent (85%) effective nodules while nitrogen (50 kg ha^{-1}) gave the least

(32%). Legume fix + cattle manure and Alosca + cattle manure gave percent effective nodule of 80 and 77%, respectively.

In the Guinea savanna, significant differences ($P \leq 0.001$) were observed among some treatments in soybean nodule number (Table 4.4). Alosca + cattle manure gave the highest nodule number followed by Legume fix + cattle manure. Nitrogen (50 kg ha^{-1}) gave the least nodule number although not significantly different from the control. Treatment combinations of inoculants and cattle manure (Alosca + cattle manure and Legume fix + cattle manure) gave relatively higher number of nodules. All other treatments gave relatively higher nodule numbers compared to the control. Percentage number of effective nodules showed that Legume fix + cattle manure gave the highest value (87%) while the least was obtained under sole nitrogen (25%). Legume fix and Alosca gave number of effective nodules values of 85 and 77%, respectively.

In the Sudan savanna, the least nodule dry weight (80 mg $plant^{-1}$) was produced under 50 kg N ha^{-1}, which differed significantly ($P \leq 0.001$) from all other treatments. The highest was produced under Legume fix + cattle manure (295 mg $plant^{-1}$) which differed significantly from other treatments (Table 4.4). The results of nodule dry weight of soybean in the Guinea savanna showed significant differences ($P \leq 0.001$) among the treatments. Highest value of 287 mg $plant^{-1}$ was observed in Alosca + cattle manure, which was statistically different from all other treatments except Legume fix + cattle manure. Least value of nodule dry weight (80 mg $plant^{-1}$) was also observed on 50 kg N ha^{-1} treatment which differ significantly from the rest of treatments.

Table 4.4: Nodule number, number of effective nodules and nodule dry matter weight of soybean

Treatments	Nodule number ($plant^{-1}$)	Effective nodules (%)	Nodule dry weight (mg $plant^{-1}$)

	Sudan savanna	Guinea savanna	Sudan savanna	Guinea savanna	Sudan savanna	Guinea savanna
Legume fix	44.98 ^{ab}	28.15 ^{bc}	85 ^{a}	85 ^{a}	192 ^{b}	187 ^{b}
Alosca	35.13 ^{cd}	29.50 ^{b}	70 ^{a}	77 ^{a}	213 ^{b}	177 ^{b}
Nitrogen*	30.55 ^{d}	16.38 ^{d}	32 ^{b}	25 ^{c}	80 ^{c}	80 ^{c}
Cattle manure**	40.98 ^{bc}	26.55 ^{bc}	35 ^{b}	45 ^{b}	180 ^{b}	177 ^{b}
Legume fix + cattle manure	48.40 ^{a}	30.38 ^{b}	80 $_{a}$	87 ^{a}	295 ^{a}	262 ^{a}
Alosca + cattle manure	40.40 ^{bc}	39.32 ^{a}	77 ^{a}	80 ^{a}	187 ^{b}	287 ^{a}
Control	23.30 ^{e}	22.12 ^{cd}	37 ^{b}	27 ^{bc}	155 ^{bc}	162 ^{b}
F pr.	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
CV (%)	11.8	10.4	19.4	21.1	27.3	23.7

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.4.2 Nodule number, number of effective nodules and nodule dry weight of groundnut

Nodule number, number of effective nodules and nodule dry weight of groundnut is as shown in Table 4.5. In the Sudan savanna, cattle manure produced the highest number of nodules (28) but was not significantly different ($P \leq 0.001$) from that of Histick + cattle manure and Biofix + cattle manure. Histick and Biofix treatments were not significantly ($P \leq 0.001$) different from each other. Among the inoculants, Histick produced the least number of nodules (21). In all, 50 kg N ha^{-1} produced the least number of nodules (13) which differed significantly ($P \leq 0.001$) among all the other treatments. In Sudan savanna, percent number of effective nodules showed significant difference exists among some treatments. However, results showed that among the sole inoculants, Biofix gave the highest number of effective nodules (72%) which differed statistically ($P \leq 0.001$) from Histick, while nitrogen gave the least (30%) differs significantly with all treatments.

In the Guinea savanna, nodule number was highest under Biofix (48) (Table 4.5). Cattle manure produced nodule number (46) which was not significantly different from the

control (44). Histick and Biofix produced nodule numbers of 35 and 48, respectively. Furthermore, it was observed that 50 kg N ha^{-1} produced the least number of nodules (30). The highest percentage effective nodules (77%) was observed under Biofix which was not statistically significant from Histick (70%). The least was obtained on plots fertilized with 50 kg N ha^{-1} (32%). Cattle manure produced nodules which were 55 % effective but not statistically ($P \leq 0.001$) different from combined inoculant and cattle manure and the control treatments.

Results for nodule dry weight in the Sudan savanna showed that Biofix + cattle manure had the highest nodule dry weight (42 mg $plant^{-1}$) which differed statistically from all other treatments except cattle manure (Table 4.5). The sole inoculants even though produced nodule dry weights which were slightly higher than the control however not statistically different. The control produced least nodule dry weight (23 mg $palnt^{-1}$) which was significantly different from Biofix + cattle manure and cattle manure. The rest treatments were not statistically different.

In Guinea savanna, Biofix + cattle manure gave the highest nodule dry weight (36 mg $plant^{1}$) which was not significantly ($P \leq 0.001$) different from all treatments except 50 kg N ha^{-1} and control. The least value (19 mg $plant^{-1}$) was under the 50 kg N ha^{-1} which differed significantly from all the other treatments (Table 4.5).

Table 4.5: Nodule number, number of effective nodules and nodule dry matter weight of groundnut

Treatments	Nodule number ($plant^{-1}$)		Effective nodules (%)		Nodule dry weight (mg $plant^{-1}$)	
	Sudan savanna	Guinea savanna	Sudan savanna	Guinea savanna	Sudan savanna	Guinea savanna
Histick	21 ^{c}	35 ^{c}	47 ^{b}	70 ^{ab}	27 ^{c}	35 ^{a}

Biofix	23 [bc]	48 [a]	72 [a]	77 [a]	27 [c]	30 [ab]
Nitrogen*	13 [d]	30 [d]	30 [c]	32 [c]	24 [c]	19 [c]
Cattle manure**	28 [a]	46 [ab]	50 [b]	55 [b]	36 [ab]	35 [a]
Histick + cattle manure	25 [ab]	44 [ab]	60 [ab]	52 [bc]	30 [bc]	33 [ab]
Biofix + cattle manure	26 [ab]	31 [d]	62 [ab]	50 [bc]	42 [a]	36 [a]
Control	24[b]	44 [b]	60 [ab]	52 [bc]	23 [c]	29 [b]
F pr.	<0.001	<0.001	<0.001	0.008	0.003	<0.001
CV (%)	10.6	7.5	19.4	25.3	19.9	13.9

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.4.3 Shoot biomass nitrogen uptake of soybean at 50% flowering stage

The result for shoot biomass N uptake is presented in Table 4.6. Results in the Sudan savanna showed significant (P≤ 0.001) differences between treatments. Increased shoot biomass N uptake of 79, 44, 57.41 and 53% for Legume fix + cattle manure, Alosca + cattle manure, cattle manure and Legume fix, respectively over the control were observed. The N uptake (31.0 kg ha^{-1}) of the control was not significantly different from Alosca (35.2 kg ha^{1}) and 50 kg N ha^{-1} (32.9 kg ha^{-1}). In the Guinea savanna, results showed that, significant differences existed between treatments. Legume fix significantly outperformed Alosca in N uptake. However, the highest N uptake value at 50% flowering stage (47.2 kg N ha^{-1}) was obtained by Legume fix + cattle manure while the least value (24.3 kg N ha^{-1}) was produced under the control.

Table 4.6: Shoot biomass nitrogen uptake of soybean at 50% flowering stage

Treatments	**N uptake (kg ha^{-1})**	
	Sudan savanna	**Guinea savanna**
Legume fix	47.5 [abc]	43.3 [a]
Alosca	35.2 [cde]	31.3 [cd]
Nitrogen*	32.9 [de]	40.7 [ab]
Cattle manure**	48.8 [ab]	33.5 [bc]

Legume fix + cattle manure	55.5 [a]	47.2 [a]
Alosca + cattle manure	41.1 [bcd]	39.7 [abc]
Control	31.0 [e]	24.3 [d]
F pr.	<0.001	<0.001
CV (%)	15.3	15.7

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.4.4 Shoot biomass nitrogen uptake of groundnut at 50% flowering stage Results in Table 4.7 show the response of the various inoculants on groundnut shoot biomass N uptake at 50% flowering stage. In the Sudan savanna, the highest uptake was observed under Biofix + cattle manure (51.5 kg N ha^{-1}) which was not significantly different (P≤ 0.001) from Histick + cattle manure (47.5 kg N ha^{-1}) but differed from all other treatments. Furthermore, 50 kg N ha^{-1} and cattle manure gave N uptake values of 36.6 kg ha^{-1} and 37.7 kg ha^{-1}, respectively, which were not statistically different. However, 50 kg N ha^{-1} did not differ significantly from all the treatments except Biofix + cattle manure and Histick + cattle manure (P≤ 0.001).

In the Guinea savanna, the highest (43.6 kg ha^{-1}) N uptake was observed under 50 kg N ha^{-1} which was significantly different (P≤ 0.001) from all other treatments (Table 4.7). Sole cattle manure gave the least N uptake (24.9 kg N ha^{-1}). Histick, Biofix, Histick + cattle manure, Biofix + cattle manure and control were not significantly (P≤ 0.001) different in their roles in N uptake in groundnut.

Table

4.7: Shoot biomass nitrogen uptake of groundnut at 50% flowering stage

Treatments	N uptake (kg ha^{-1})	
	Sudan savanna	**Guinea savanna**
Histick	38.6 ^{cd}	31.2 ^{b}
Biofix	42.7 ^{bcd}	33.9 ^{b}
Nitrogen*	36.6 ^{d}	43.6 ^{a}
Cattle manure**	37.7 ^{d}	24.9 ^{c}
Histick + cattle manure	47.5 $_{ab}$	30.8 ^{b}
Biofix + cattle manure	51.5 ^{a}	34.6 ^{b}
Control	35.6 ^{d}	31.1 ^{b}
F pr.	<0.001	<0.001
CV (%)	9.4	12.0

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.4.5 Shoot biomass phosphorus uptake of soybean at 50% flowering stage Table 4.8 shows the results of shoot biomass P uptake of soybean. In the Sudan savanna, significant differences existed among the various treatment combinations (P= 0.007). Phosphorus uptake was highest under Legume fix + cattle manure (5.8 kg ha^{-1}) which differed significantly with other treatments except cattle manure. The control gave the least P uptake value (3.1 kg ha^{-1}), which differed significantly only from Legume fix + cattle manure and sole cattle manure.

In the Guinea savanna, shoot biomass P uptake was generally less than that of the Sudan savanna (Table 4.8). However, significant differences (P ≤ 0.001) occurred among the treatments. Legume fix gave the highest shoot P uptake (4.0 kg ha^{-1}) which differed significantly with all other treatments. Legume fix + cattle manure gave the second highest value of P uptake (3.1 kg ha^{-1}). Control had the least value (2.0 kg ha^{-1}) which differed significantly with Legume fix, cattle manure and Legume fix + cattle manure.

Table

4.8: Shoot biomass phosphorus uptake of soybean at 50% flowering stage

Treatments	P uptake (kg ha^{-1})	
	Sudan savanna	**Guinea savanna**
Legume fix	4.3 ^{bc}	4.0 ^{a}
Alosca	3.6 ^{bc}	2.5 ^{bc}
Nitrogen*	3.1 ^{c}	2.5 ^{bc}
Cattle manure**	4.8 ^{ab}	3.0 ^{b}
Legume fix + cattle manure	5.8 ^{a}	3.1 ^{b}
Alosca + cattle manure	3.8 ^{bc}	2.6 ^{bc}
Control	3.1 ^{c}	2.0 ^{c}
F pr.	0.007	<0.001
CV (%)	23.0	17.1

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.4.6 Shoot biomass phosphorus (P) uptake of groundnut at 50 % flowering stage The result of shoot biomass P uptake of groundnut is presented in Table 4.9. In the Sudan savanna, the highest groundnut shoot P uptake at 50% flowering stage was observed under Biofix (3.5 kg ha^{-1}) but was not significantly different (P≤0.001) from Biofix + cattle manure (3.4 kg ha^{-1}) and Histick + cattle manure (3.2 kg ha^{-1}). The control produced the least P uptake (2.5 kg ha^{-1}) which was not significantly different from 50 kg N ha^{-1}, cattle manure and Histick treatments.

In the Guinea savanna, results showed that 50 kg N ha^{-1} produced the highest shoot biomass P uptake (3.1 kg^{-1}) which was 37% higher than the control (Table 4.9). It was observed that 50 kg N ha^{-1} and Histick were not significantly different from each other. However, significant differences (P≤ 0.001) existed among Histick, cattle manure and Histick + cattle manure (Table 4.9).

Table

4.9: Shoot biomass phosphorus uptake of groundnut at 50% flowering stage

Treatments	P uptake (kg ha^{-1})	
	Sudan savanna	**Guinea savanna**
Histick	2.8 ^{bc}	2.8 ^{ab}
Biofix	3.5 ^{a}	2.1 ^{de}
Nitrogen*	2.5 ^{c}	3.1 ^{a}
Cattle manure**	2.7 ^{c}	1.9 ^{e}
Histick + cattle manure	3.2 ^{ab}	2.4 ^{cd}
Biofix + cattle manure	3.4 ^{a}	2.6 ^{bc}
Control	2.5 ^{c}	2.3 ^{d}
F pr.	<0.002	<0.001
CV (%)	11.9	8.8

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.4.7 Shoot biomass potassium (K) uptake of soybean at 50% flowering stage

In the Sudan savanna, all the treatments gave higher K uptake values over the control (Table 4.10). The highest K uptake (32.1 kg ha $^{-1}$) was obtained under Legume fix + cattle manure. The control differed significantly from Legume fix + cattle manure and cattle manure alone (P≤0.001). Generally, the Sudan savanna produced relatively higher shoot K uptake values than the Guinea savanna.

In the Guinea savanna, results showed that Legume fix + cattle manure enhanced shoot biomass K uptake (21.8 kg ha^{-1}) significantly relative to the other treatments, except sole cattle manure (Table 4.10). The control gave the least shoot biomass K uptake which was not significantly different from 50 kg N ha^{-1} and Alosca treatments (P= 0.002).

Table

4.10: Shoot biomass potassium uptake of soybean at 50% flowering stage

Treatments	K uptake (kg ha^{-1})	
	Sudan savanna	**Guinea savanna**
Legume fix	26.9 ^{abc}	20.1 ^{ab}
Alosca	22.8 ^{bc}	15.1 ^{cd}
Nitrogen*	20.0 ^{c}	14.2 ^{cd}
Cattle manure**	30.1 ^{ab}	17.7 ^{abc}
Legume fix + cattle manure	32.1 ^{a}	21.8 ^{a}
Alosca + cattle manure	23.7 ^{bc}	16.3 ^{bc}
Control	19.6 ^{c}	10.9 ^{d}
F pr.	0.031	0.002
CV (%)	22.2	18.6

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.4.8 Shoot biomass potassium uptake of groundnut at 50 % flowering stage

The results of rhizobia inoculation on groundnut shoot biomass K uptake is as shown in Table 4.11. In the Sudan savanna, there were significant differences among some of the treatments. The highest K uptake (23.8 kg ha^{-1}) was obtained under Biofix + cattle manure which was not significantly different (P = 0.16) from that of Histick + cattle manure and sole Biofix. The least K uptake (14.6 kg ha^{-1}) was obtained under the control which was not significantly different from cattle manure, Histick, Histick + cattle and 50 kg N ha^{-1}. In the Guinea savanna, the highest shoot K uptake (21.4 kg K ha^{-1}) was observed under 50 kg N ha^{-1} which was not significantly different (P= 0.013) from that of Histick and Biofix + cattle manure. All other treatments did not differ significantly from the control. Cattle manure gave the least value (14.0 kg K ha^{-1}) of groundnut K uptake which was not significantly different from the control, Biofix and Histick + cattle manure.

Table

Table 4.11: Shoot biomass potassium uptake of groundnut at 50% flowering stage

Treatments	K uptake (kg ha^{-1})	
	Sudan savanna	**Guinea savanna**
Histick	16.7 ^{bc}	18.8 ^{a}
Biofix	20.5 ^{ab}	17.0 ^{bc}
Nitrogen*	17.8 ^{bc}	21.4 ^{a}
Cattle manure**	15.5 ^{c}	14.0 ^{c}
Histick + cattle manure	19.2 ^{abc}	16.8 ^{bc}
Biofix + cattle manure	23.8 ^{a}	19.5 ^{ab}
Control	14.6 ^{c}	15.8 ^{bc}
F pr.	0.016	0.013
CV (%)	18.1	14.5

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.4.9 Grain yield and harvest index of soybean in the Sudan savanna zone There were significant differences (P≤ 0.001) in grain yield and harvest index between some treatments (Table 4.12). In the Sudan savanna, yield increases of 70, 62, 36 and 30% were produced for Alosca + cattle manure, Legume fix, Legume fix + cattle manure and Alosca, respectively over the control. Legume fix differed significantly (P≤ 0.001) in harvest index from all other treatments with the highest value of 0.75. Legume fix, Legume fix + cattle manure, Alosca + cattle manure and Alosca gave harvest index increases of 177, 118, 74 and 67%, respectively over the control.

Table 4.12: Grain yield and harvest index of soybean in the Sudan savanna

Treatments	Grain yield (kg ha^{-1})	Harvest index
Legume fix	1366 ^{a}	0.75 ^{a}
Alosca	1099 ^{b}	0.45 ^{c}
Nitrogen*	873 ^{c}	0.53 ^{bc}
Cattle manure**	891 ^{c}	0.27 ^{d}
Legume fix + cattle manure	1145 ^{b}	0.59 ^{b}
Alosca + cattle manure	1436 ^{a}	0.47 ^{bc}
Control	843 ^{c}	0.27 ^{d}
F pr.	< 0.001	< 0.001

CV (%)	10.3	19.0

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.4.10 Grain yield and harvest index of soybean in the Guinea savanna

Table 4.13 shows the results of the effect of inoculation on grain yield and harvest index of soybean in the Guinea savanna. Some treatments differed significantly (P≤ 0.001). Legume fix produced highest grain yield (1342 kg ha^{-1}). Yield increases of 51, 41, 33, 26 and 21% were observed over the control for Legume fix, Legume fix + cattle manure, cattle manure, Alosca + cattle manure and Alosca, respectively. Grain yield value for 50 kg N ha^{-1} (997 kg ha^{-1}) was not significantly different from that of the control (890 kg ha^{1}). Harvest index in the Guinea savanna was generally higher than that of the Sudan savanna (Table 4.13). Legume fix gave the highest value (0.82 kg ha^{-1}) which differed significantly from all other treatments except 50 kg N ha^{-1} treatment. The control produced the least harvest index (0.52). Legume fix, 50 kg N ha^{-1} and cattle manure gave higher harvest index values of 57, 42 and 21%, respectively than the control. However, Legume fix + cattle manure, Alosca + cattle manure, cattle manure and Alosca were not significantly different from the control (P= 0.012).

Table 4.13: Grain yield and harvest index of soybean in the Guinea savanna

Treatments	Grain yield (kg ha^{-1})	Harvest index
Legume fix	1342 ^{a}	0.82 ^{a}
Alosca	1082 ^{bc}	0.53 ^{c}
Nitrogen*	997 ^{cd}	0.74 ^{ab}
Cattle manure**	1188 ^{ab}	0.63 ^{bc}
Legume fix + cattle manure	1257 ^{ab}	0.55 ^{c}
Alosca + cattle manure	1125 ^{bc}	0.59 ^{bc}
Control	890 ^{d}	0.52 ^{c}
F pr.	< 0.001	0.012

CV (%)	10.8	18.8

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.4.11 Grain yield and harvest index of groundnut in the Sudan savanna

Table 4.14 shows results of the effect of inoculation on grain yield and harvest index of groundnut in the Sudan savanna. Biofix gave the highest grain yield (506 kg ha^{-1}) which was not significantly higher than that of Histick (452 kg ha^{-1}), Biofix + cattle manure (445 kg ha^{-1}) and the control (432 kg ha^{-1}). Grain yields of cattle manure, 50 kg N ha^{-1} and Histick + cattle manure were not significantly different ($P \leq 0.001$) from each other. The least grain yield (335 kg ha^{-1}) was observed under 50 kg N ha^{-1}. Harvest index values showed no significant differences ($P = 0.95$) among all treatments.

Table 4.14: Grain yield and harvest index of groundnut in the Sudan savanna

Treatments	Grain yield (kg ha^{-1})	Harvest index
Histick	452 ^{ab}	0.19 ^{a}
Biofix	506 ^{a}	0.21 ^{a}
Nitrogen*	335 ^{c}	0.19 ^{a}
Cattle manure**	339 ^{c}	0.21 ^{a}
Histick + cattle manure	373 ^{bc}	0.20 ^{a}
Biofix + cattle manure	445 ^{ab}	0.20 ^{a}
Control	432 ^{a}	0.16 ^{a}
F pr.	< 0.003	0.60
CV (%)	13.7	20.3

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.4.12 Grain yield and harvest index of groundnut in the Guinea savanna

Inoculation of groundnut did not impact significantly on grain yield in the Guinea savanna (Table 4.14). However, there were yield increments of 10, 5, 4 and 3 % over the control for Histick, Biofix + cattle manure, Biofix and Alosca, respectively. The least yield

response was obtained under 50 g N ha^{-1} treatment. Harvest index showed no significant difference (p = 0.236) among all the treatments.

Table 4.15: Grain yield and harvest index of groundnut in the Guinea savanna

Treatments	Grain yield (kg ha^{-1})	Harvest Index
Histick	621 [a]	0.27 [a]
Biofix	590 [a]	0.24 [a]
Nitrogen*	554 [a]	0.24 [a]
Cattle manure**	558 [a]	0.23 [a]
Histick + cattle manure	580 [a]	0.23 [a]
Biofix + cattle manure	592 [a]	0.25 [a]
Control	562 [a]	0.25 [a]
F pr.	0.47	0.24
CV (%)	8.5	9.7

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.4.13 Soybean root: shoot ratio

The results of soybean root: shoot ratio showed no significant differences among the treatments (P= 3.24) in both the Sudan savanna and the Guinea savanna (Table 4.16).

Table 4.16: Soybean root : shoot ratio

Treatments	Root: shoot ratio	
	Sudan savanna	Guinea savanna
Legume fix	0.16 [a]	0.20 [a]
Alosca	0.16 [a]	0.22 [a]
Nitrogen*	0.13 [a]	0.16 [a]
Cattle manure**	0.17 [a]	0.21 [a]
Legume fix + cattle manure	0.16 [a]	0.18 [a]
Alosca + cattle manure	0.18 [a]	0.21 [a]
Control	0.16 [a]	0.22 [a]
F pr.	0.32	0.10
CV (%)	16.9	15.4

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.4.14 Groundnut root: shoot ratio

Cattle manure and 50 kg N ha^{-1} treatments gave the highest root: shoot ratio (0.13) which were significantly different from the control (0.10) (Table 4.17). Root: shoot ratio for Biofix + cattle manure, Biofix, cattle manure, 50 kg N ha^{-1} and Biofix were not significantly different from each other. However, Histick + cattle manure gave the least root: shoot ratio (0.06) which was significantly different from other treatments. In the Guinea savanna, significant differences were observed in groundnut root: shoot ratio for the various treatments (P= 0.016) (Table 4.17). Furthermore, cattle manure gave the highest root: shoot ratio (0.10) which was not significantly different from that of Histick + cattle manure. However, root: shoot ratio for control, Biofix + cattle manure, Histick + cattle manure, Biofix and Histick were not significantly different from each other (Table 4.17).

Table 4.17: Groundnut root: shoot ratio

Treatments	Root: shoot ratio	
	Sudan savanna	**Guinea savanna**
Histick	0.10 ^{d}	0.07 ^{bc}
Biofix	0.11 ^{bc}	0.08 ^{bc}
Nitrogen*	0.13 ^{ab}	0.06 ^{c}
Cattle manure**	0.13 ^{a}	0.10 ^{a}
Histick + cattle manure	0.06 ^{e}	0.08 ^{ab}
Biofix + cattle manure	0.12 ^{ab}	0.07 ^{bc}
Control	0.10 ^{cd}	0.08 ^{bc}
F pr.	<0.001	0.016
CV (%)	10.3	16.9

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.4.15 Relationships between growth and yield parameters of soybean and groundnut in the Sudan and Guinea savanna zones

A simple correlation was computed to ascertain the degree of relationship between the various growth and yield parameters of soybean and groundnut at the study locations (Tables 4.17 - 4.20). At the soybean field in the Sudan savanna, positive significant (P< 0.05) correlations were observed between shoot dry weight and root dry weight, shoot dry weight and nodule dry weight, shoot dry weight and 100 seed weight, shoot dry weight and grain yield (Table 4.18). Significant positive correlations were also observed between root dry weight and 100 seed weight, root dry weight and grain yield and nodule weight and 100 seed weight (Table 4.18). For groundnut, significant (P < 0.05) positive correlations were observed between shoot dry weight and nodule dry weight, shoot dry weight and grain yield and nodule dry weight and grain yield (Table 4.19).

For soybean field in the Guinea savanna, positive significant correlations (P < 0.05) were observed between shoot dry weight and nodule dry weight, shoot dry weight and grain yield, root dry weight and nodule dry weight and nodule dry weight and grain yield (Table 4.20). However, significant negative correlations were observed between shoot dry weight and 100 seed weight and 100 seed weight and grain yield (Table 4.20). At the groundnut experimental field in the Guinea savanna, only root dry weight against 100 seed weight showed a significant (P < 0.05) positive correlation (0.447**) among the growth and yield parameters measured (Table 4.21).

Table 4.18: Pearson correlation matrix of some measured growth and yield parameters of soybean in the Sudan savanna

	B D W	**R D W**	**N D W**	**100 S W**	**G Y**
B D W		0.466**	0.382**	0.505**	0.948**
R D W	0.466**		0.580**	0.416**	0.546**

N D W	0.382**	0.580**		0.521**	0.367*
100 S W	0.505**	0.416**	0.521**		0.657**
G Y	0.948**	0.546*	0.367*	0.657**	

*Non-significance at P > 0.05, ** Significance at P < 0.05. SDW = Shoot dry weight, RDW = Root dry weight, NDW = Nodule dry weight, SW = Seed weight, GY = Grain yield

Table 4.19: Pearson correlation matrix of some growth and yield parameters of groundnut in the Sudan savanna

	B D W	**R D W**	**N D W**	**100 S W**	**G Y**
B D W		-0.091*	0.396**	0.258*	0.794**
R D W	-0.091*		0.296*	0.057*	0.065*
N D W	0.396**	0.296*		-0.175*	0.435**
100 S W	0.258*	0.057*	-0.175*		-0.107*
G Y	0.794**	0.065*	0.435**	-0.107*	

* Non-significance at P > 0.05, ** Significance at P < 0.05. SDW = Shoot dry weight, RDW = Root dry weight, NDW = Nodule dry weight, SW = Seed weight, GY = Grain yield

Table 4.20: Pearson correlation matrix of some growth and yield parameters of soybean in the Guinea savanna

	B D W	**R D W**	**N D W**	**100 S W**	**G Y**
B D W		0.168*	0.428**	0.419**	0.863**
R D W	0.168*		0.542**	-0.2418*	0.156*
N D W	0.428**	0.542**		0.357*	0.474**
100 S W	0.419**	-0.241*	0.357*		-0.281**
G Y	0.863**	0.156*	0.474**	-0.281**	

* Non-significance at P > 0.05, ** Significance at P < 0.05. SDW = Shoot dry weight, RDW = Root dry weight, NDW = Nodule dry weight, SW = Seed weight, GY = Grain yield

Table 4.21: Pearson correlation matrix of some growth and yield parameters of groundnut in the Guinea savanna

	B D W	**R D W**	**N D W**	**100 S W**	**G Y**
B D W		0.138*	0.199*	0.039*	0.368*
R D W	0.138*		0.130*	0.447**	0.201*
N D W	0.199*	0.130*		-0.020*	0.231*
100 S W	0.130*	0.447**	-0.021*		0.234*
G Y	0.368*	0.201*	0.231*	0.234*	

* Non-significance at P > 0.05, ** Significance at P < 0.05. SDW = Shoot dry weight, RDW = Root dry weight, NDW = Nodule dry weight, SW = Seed weight, GY = Grain yield

4.5 Study 2: Influence of microbial inoculants, inorganic nitrogen, cattle manure and their complementary application in enhancing nitrogen fixation and uptake of major plant nutrients

4.5.1 Grain N uptake of soybean in the Sudan and Guinea savanna zones

Results for grain N uptake of soybean in the Sudan and Guinea zones are as shown in Figure 4.1. Legume fix had the highest grain N uptake (90.51 kg ha^{-1}) which differed significantly ($P \leq 0.001$) from the rest of the treatments. Increases of 74, 44, 26 and 21% over the control were obtained for Legume fix, Alosca, Legume fix + cattle manure and Alosca + cattle manure, respectively.

In the Guinea savanna, Legume fix gave the highest N uptake value (95.53 kg ha^{-1}) which was significantly different from all treatments except Legume fix + cattle manure. Grain N uptake increases of 58, 57, 21, 14 and 13% over the control were observed in Legume fix, Legume fix + cattle manure, Alosca + cattle manure, Alosca and cattle manure treatments, respectively. However, 50 kg N ha^{-1} gave the least grain N uptake (51.79 kg ha^{-1}) which was relatively lower than the control and differed significantly from the rest of the treatments ($P \leq 0.001$).

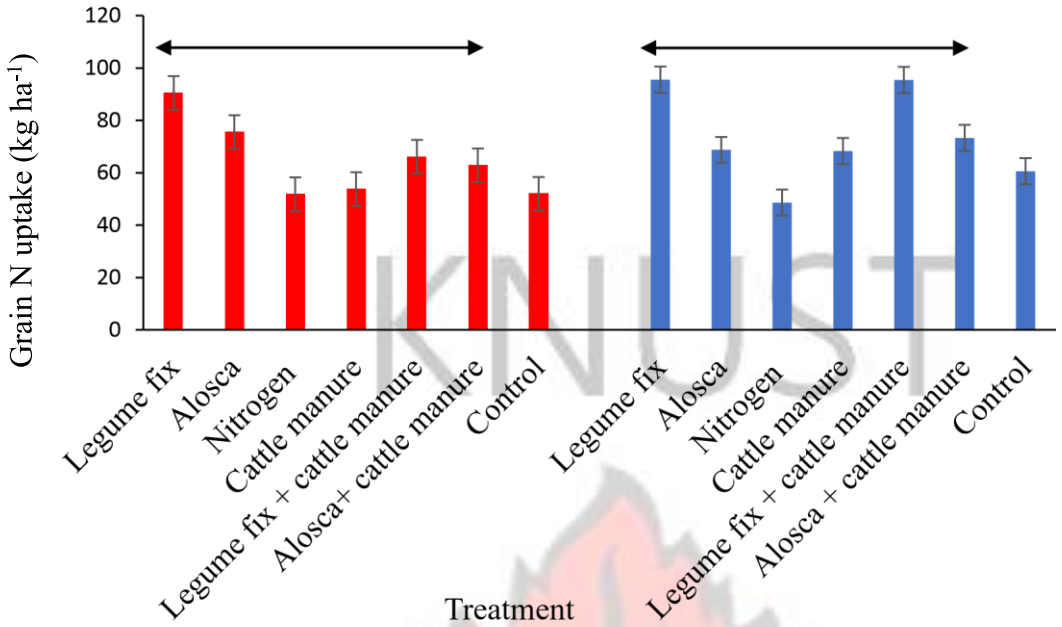


Figure 4.1: Grain N uptake of soybean in the Sudan and Guinea savanna agroecologies

4.5.2 Grain N uptake of groundnut in the Sudan and Guinea savanna agro-ecologies

Figure 4.2 shows results of grain N uptake of groundnut in the Sudan and Guinea savanna agro-ecological zones. In the Sudan savanna, no significant differences were observed among the treatments. However, increases in uptake of 23, 7 and 6 % over the control were observed in Biofix, Biofix + cattle manure and Histick, respectively. Similarly, results in the Guinea savanna did not show any significant differences (P = 0.79) among the treatments.

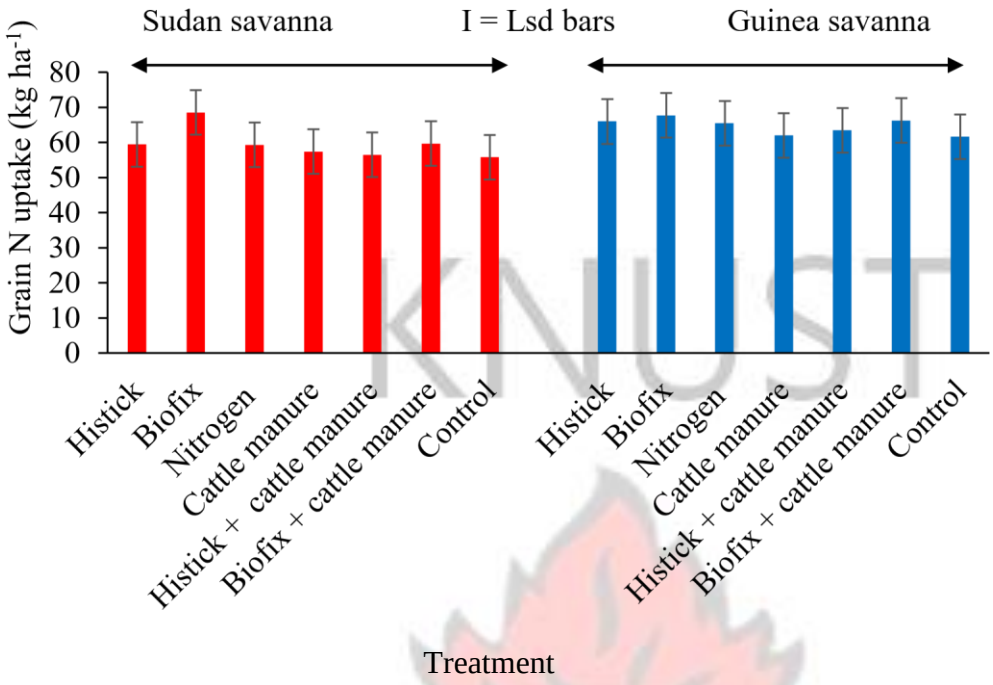


Figure 4.2: Grain N uptake of groundnut in the Sudan and Guinea savanna agroecologies

4.5.3 Grain P uptake of soybean in the Sudan and Guinea savanna agro-ecologies

Significant differences were observed among some treatments in n grain P uptake ($P \leq 0.001$) (Figure 4.3). Alosca + cattle manure gave the highest P uptake (11.18 kg ha^{-1}) while the control produced the least (6.34 kg ha^{-1}). Increases in P uptake of 76, 66, 49 and 34% over the control were observed under Alosca + cattle manure, Legume fix + cattle manure and Alosca, respectively. Furthermore, these treatments differed significantly from the control ($P \leq 0.001$). Cattle manure and 50 kg N ha^{-1} treatments produced higher P uptake values of 11 and 10%, respectively over the control (Figure 4.3).

In the Guinea savanna zone, there existed significant differences among treatments ($P \leq 0.001$) (Figure 4.3). Legume fix gave the highest P uptake (9.66 kg ha^{-1}) which significantly differed from all other treatments. However, all other treatments did not differ significantly from the control.

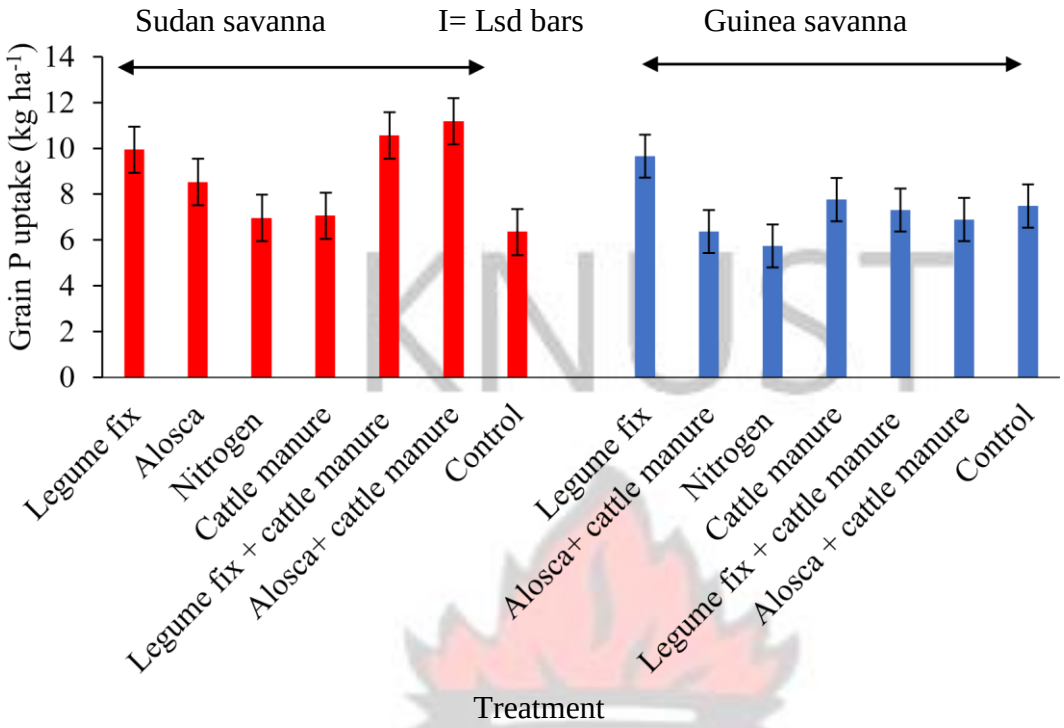


Figure 4.3: Grain P uptake of soybean in the Sudan and Guinea savanna agro- ecologies

4.5.4 Grain P uptake of groundnut in the Sudan and Guinea savanna agro-ecologies

Figure 4.4 shows the results for grain P uptake of groundnut. In the Sudan savanna, significant differences existed among the treatments (P = 0.001). Biofix gave the highest grain P uptake (6.99 kg ha^{-1}) which was significantly different from the control. All other treatments were statistically at par. In the Guinea savanna zone, the results showed significant differences (P= 0.029) among the treatments. The control produced the least P uptake (5.28 kg ha^{-1}) while Biofix produced the highest uptake (7.32 kg ha^{-1}).

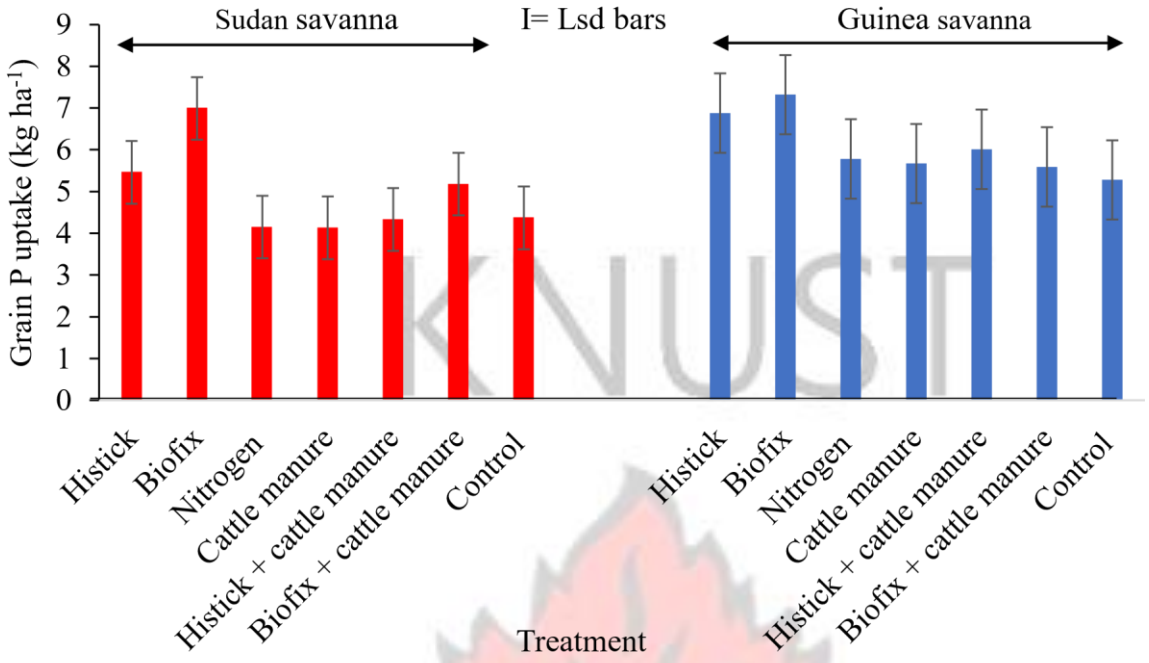


Figure 4.4: Grain P uptake of groundnut in the Sudan and Guinea savanna agroecologies

4.5.5 Grain K uptake of soybean in the Sudan and Guinea savanna agro-ecologies

Figure 4.5 shows the results for soybean grain K uptake. Significant differences ($P \leq 0.001$) occurred among some of the treatments in the Sudan savanna zone. Alosca + cattle manure enhanced K uptake (49.99 kg ha^{-1}) which was statistically at par with Legume fix. The control gave the least K uptake (29.01 kg ha^{-1}) which was not significantly different from that of cattle manure and 50 kg N ha^{-1} treatments. Significant K uptake increments of 72.14, 46.16, 40.47 and 33.92% over the control were produced for Alosca + cattle manure, Legume fix, Legume fix + cattle manure and Alosca, respectively.

In the Guinea savanna, the highest grain K uptake (41.32 kg ha^{-1}) was observed under Legume fix which was not significantly different from that of Legume fix + cattle manure and sole cattle manure. Grain K uptake increments of 49.28, 48.99, 37.93, 11.27 and

10.33% were obtained over the control for Legume fix, Legume fix + cattle manure, cattle manure, Alosca + cattle manure and Alosca, respectively.

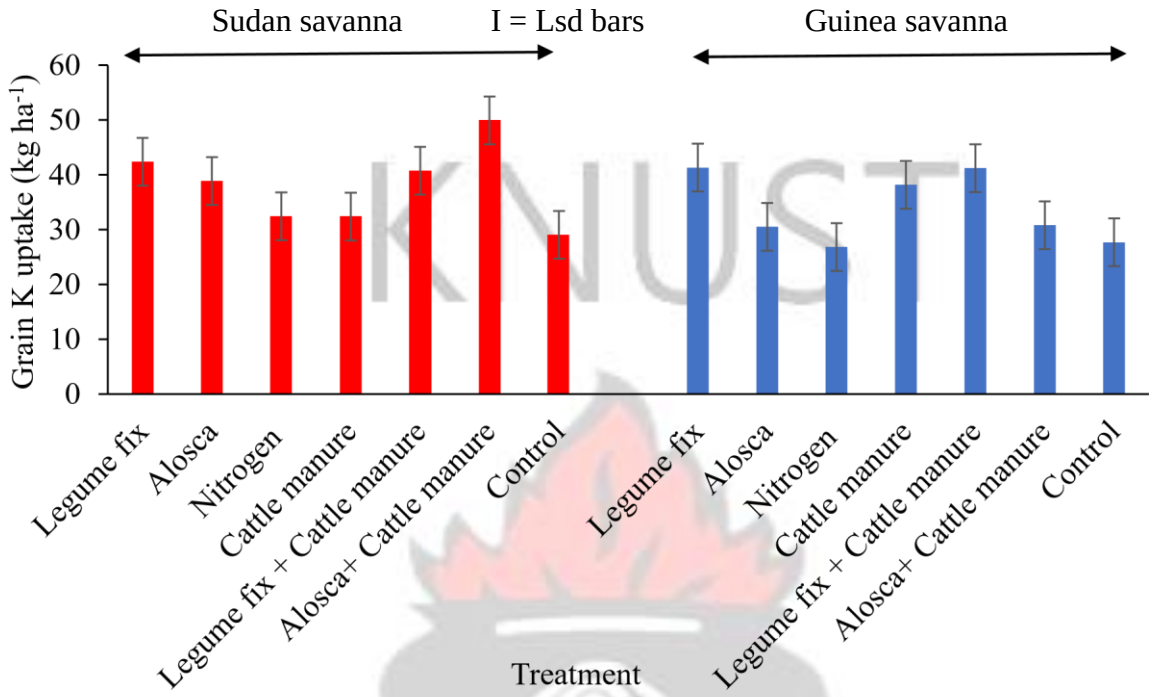


Figure 4.5: Grain K uptake soybean in the Sudan and Guinea savanna agroecologies

4.5.6 Grain K uptake of groundnut in the Sudan and Guinea savanna zones Figure 4.6 shows the results of grain K uptake for groundnut. In the Sudan savanna, significant differences occurred among the treatments ($P \leq 0.001$). Biofix enhanced the highest K uptake (22.48 kg ha^{-1}) which was not significantly different from that of Histick, 50 kg N ha^{-1}, and Biofix = cattle manure. No significant differences between Histick, cattle manure, Histick + cattle manure and the control were observed. Furthermore, in the Guinea savanna zone, grain K uptake did not show any significant differences among the treatments ($P = 0.14$). However, increase of 19 and 13 % over the control were observed for Histick and Biofix, respectively.

Sudan savanna I =Lsd brs Guinea savanna

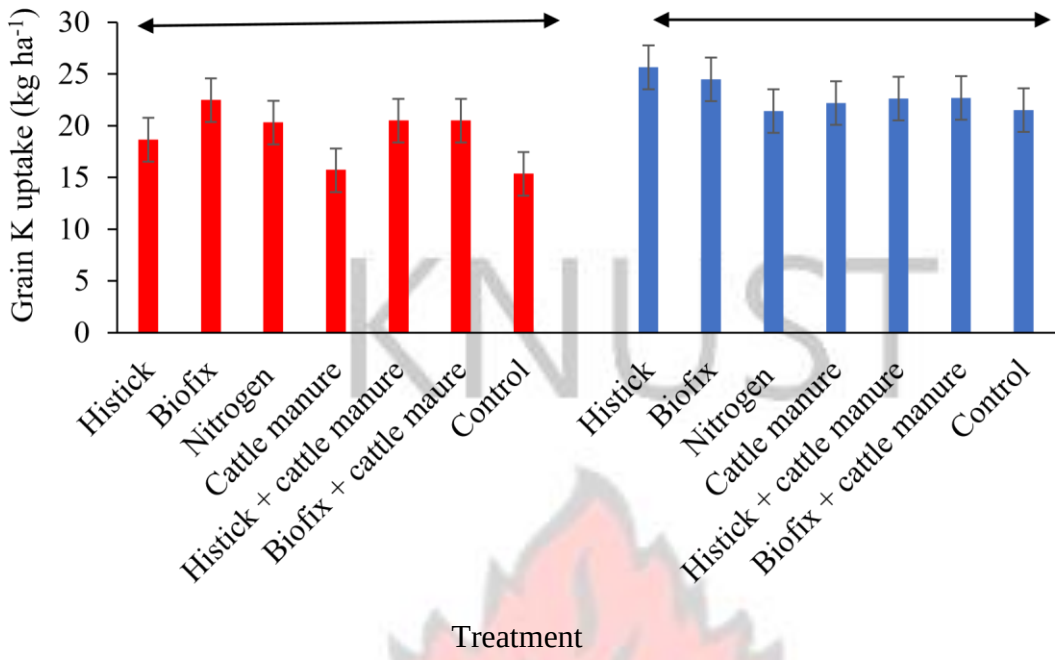


Figure 4.6: Grain K uptake of groundnut in the Sudan and Guinea savanna agroecologies

4.5.7 Nitrogen fixation of soybean in the Sudan and Guinea savanna zones

Results of nitrogen fixation for soybean at the two study locations are presented in Table 4.22. The results in the Sudan savanna experimental site revealed significant differences ($P \leq 0.001$) between the treatments and the control. Legume fix produced the highest nitrogen fixation (56.67 kg ha^{-1}) while the control had the least (20.69 kg ha^{-1}). In the Sudan savanna, increases of 173.90, 101.64, 56.16 and 40.40% for nitrogen fixation were obtained for Legume fix, Alosca, Legume fix + cattle manure and Alosca + cattle manure over the control. Furthermore, the control only differed significantly from Legume fix and Alosca treatments.

In the Guinea savanna, Legume fix + cattle manure gave the highest nitrogen fixation (58.53 kg ha^{-1}). All treatments were significantly higher than the control except 50 kg N ha^{-1} which produced the least nitrogen fixation (17.34 kg ha^{-1}). Nitrogen fixation

increments of 163.29, 141.38, 63.83, 50.38 and 41.38% were obtained for Legume fix + cattle manure, Legume fix, Alosca + cattle manure, Alosca and cattle manure, respectively.

Table 4.22: Nitrogen fixation in soybean

Treatments	Nitrogen fixation (kg ha^{-1})	
	Sudan savanna	**Guinea savanna**
Legume fix	56.67 ^{a}	53.66 ^{a}
Alosca	41.72 ^{b}	33.57 ^{b}
Nitrogen*	21.67 ^{c}	17.34 ^{c}
Cattle manure**	21.88 ^{c}	31.43 ^{b}
Legume fix + cattle manure	32.31 ^{bc}	58.53 ^{a}
Alosca + cattle manure	29.05 ^{c}	36.42 ^{b}
Control	20.69 ^{c}	22.23 ^{cd}
F pr.	<0.001	<0.001
CV (%)	24.8	20.2

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.5.8 Nitrogen fixation in groundnut in the Sudan and Guinea savanna zones

The highest nitrogen fixed (11.58 kg ha^{-1}) was observed under Biofix whilst the least (7.41 kg ha^{-1}) was observed under cattle manure (Table 4.23). Biofix, Biofix + cattle manure and control were statistically similar in N fixed. In the Guinea savanna, there was no significant differences among all the treatments observed (P= 0.67). However, there was an increase of 17.7 and 16.6 % N Fixed over the control in Histick and Biofix, respectively.

Table 4.23: Nitrogen fixation in groundnut

Treatments	Nitrogen fixation (kg ha^{-1})	
	Sudan savanna	**Guinea savanna**
Histick	10.12 ^{ab}	16.95 ^{a}
Biofix	11.58 ^{a}	16.80 ^{a}
Nitrogen*	7.76 ^{c}	15.35 ^{a}
Cattle manure**	7.41 ^{c}	15.50 ^{a}
Histick + cattle manure	8.59 ^{bc}	15.85 ^{a}
Biofix + cattle manure	10.08 ^{ab}	15.33 ^{a}

Control	9.90 [ab]	14.40 [a]
F pr.	<0.006	0.67
CV (%)	15.0	13.7

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.6 Study 3: Legume residues effect on the growth and yield of subsequent maize crop

This study was conducted during the 2016 cropping season to evaluate the residual effects of the legume residues on maize growth and yield in the two agro-ecological zones.

4.6.1 Maize grain yield and harvest index

Table 4.24 shows results of grain yield and harvest index of maize grown on soybean experimental fields at the two study locations. Results from the Sudan savanna showed no significant differences among the treatments in maize grain yield (P = 0.123). Plots previously amended with Legume fix + cattle manure gave the highest harvest index (0.45) which was not significantly different from that of cattle manure and 50 kg N ha^{-1} plots. Maize grain yield in the Guinea savanna showed significant differences among the treatments (P= 0.007). It was noted that 50 kg N ha^{-1} amended plots gave the highest maize grain yield (2175 kg ha^{-1}) which was not significantly different from Alosca, Legume fix + cattle manure and Alosca + cattle manure amended plots. The control plot gave the least grain yield (1438 kg ha^{-1}) which was significantly different from all other treatments except sole cattle manure. Maize grain yield increments of 51.25, 44.85, 32.13, 29.48, 25.17 and 22.25% over the control were produced for plots amended with 50 kg N ha^{-1}, Alosca, Legume fix + cattle manure, Alosca + cattle manure, Legume fix and cattle manure, respectively. However, harvest index among treatments in the Guinea savanna zone showed no significant differences among the treatments (P = 0.321).

Table 4.24: Grain yield and harvest index of maize grown on soybean experimental fields

	Sudan savanna		Guinea savanna	
Treatments	**Grain yield (kg ha^{-1})**	**Harvest index**	**Grain yield (kg ha^{-1})**	**Harvest index**
Legume fix	2229 ^{a}	0.30 ^{c}	1800 ^{b}	0.43 ^{a}
Alosca	2042 ^{a}	0.35 ^{bc}	2083 ^{ab}	0.58 ^{a}
Nitrogen*	2232 ^{a}	0.39 ^{ab}	2175 ^{a}	0.54 ^{a}
Cattle manure**	2192 ^{a}	0.38 ^{abc}	1758 ^{bc}	0.39 ^{a}
Legume fix + cattle manure	1904 ^{a}	0.45 ^{a}	1900 ^{ab}	0.51 ^{a}
Alosca + cattle manure	2438 ^{a}	0.30 ^{c}	1862 ^{ab}	0.67 ^{a}
Control	1821 ^{a}	0.36 ^{bc}	1438 ^{c}	0.33 ^{a}
F pr.	0.123	0.023	0.007	0.321
CV (%)	21.1	16.2	12.3	14.7

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

Table 4.25 shows results of grain yield and harvest index of maize grown on groundnut fields at the two study locations. In the Sudan savanna, 50 kg N ha^{-1} fertilized plots produced the highest maize grain yield (2167 kg ha^{-1}). However, it did not differ significantly from yield produced under Histick, Biofix, cattle manure and Biofix + cattle manure (Table 4.25). The control produced the least grain yield (1575 kg ha^{-1}), which was not significantly different from that of Biofix, cattle manure and Histick + cattle manure amended plots. The highest harvest index was observed under 50 kg N ha^{-1} (0.47) which differed significantly from all the other treatments except Histick + cattle manure. In the Guinea savanna zone, there were no significant differences in maize grain yield and harvest index among the treatments (P= 0.125) (Table 4.25).

Table 4.25: Grain yield and harvest index of maize grown on groundnut experimental fields

	Sudan savanna		Guinea savanna	
	Grain yield (kg ha^{-1})	**Harvest Index**	**Grain yield (kg ha^{-1})**	**Harvest index**

Histick	2042 [ab]	0.26 [b]	1896 [a]	0.40 [a]
Biofix	1875 [abc]	0.29 [b]	2250 [a]	0.38 [a]
Nitrogen[*]	2167 [a]	0.47 [a]	1979 [a]	0.36 [a]
Cattle manure[**]	1875 [abc]	0.34 [b]	1708 [a]	0.34 [a]
Histick + cattle manure	1708 [bc]	0.45 [a]	2000 [a]	0.37 [a]
Biofix + cattle manure	1958 [ab]	0.30 [b]	2083 [a]	0.39 [a]
Control	1575 [c]	0.22 [b]	1613 [a]	0.34 [a]
F pr.	0.049	0.002	0.125	0.709
CV (%)	16.6	31.0	16.1	16.3

[*]Nitrogen = 50 kg N ha^{-1}; [**]Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.6.2 Maize stover yield

Residue results for maize stover yield in the soybean experimental field in the Sudan savanna is presented in Table 4.26. There were significant differences among the treatments (P = 0.06). Generally, maize stover yield in the Sudan savanna zone was higher than those in the Guinea savanna. Legume fix inoculated plots gave the highest maize stover yield (280 kg ha^{-1}) which only differed significantly from 50 kg N ha^{-1} fertilized plots which produced the least (166 kg ha^{-1}). In the Guinea savanna zone, maize stover yield was generally low compared to the Sudan savanna. The control produced the highest maize stover yield (220 kg ha^{-1}), which was not significantly different from that of cattle manure and Legume fix plots. Alosca + cattle manure gave the least stover yield (105 kg ha^{-1}) (Table 4.26).

Table 4.27 shows results of maize stover yield on the groundnut experimental fields in the Sudan savanna zone. The results showed a significant difference among the treatments (P ≤ 0.001). Histick previously inoculated plots produced the highest maize stover yield (262 kg ha^{-1}). Whilst Histick + cattle manure amended plots produced the least stover yield. In the Guinea savanna, highest stover yield (239 kg ha^{-1}) was observed on Biofix inoculated plots while the least (168 kg ha^{-1}) was recorded on cattle manure amended plots. Stover

yield produced by sole Biofix inoculated plants differed significantly from cattle manure amended plots. All other treatments did not show any significant differences in stover yield from the control.

Table 4.26: Maize stover yield (Soybean experimental fields)

Treatments	Stover yield (kg ha^{-1})	
	Sudan savanna	**Guinea savanna**
Legume fix	280 ^{a}	180 ^{ab}
Alosca	223 ^{ab}	125 ^{bc}
Nitrogen*	166 ^{bc}	120 ^{bc}
Cattle manure**	222 ^{ab}	204 ^{a}
Legume fix + cattle manure	219 ^{ab}	136 ^{bc}
Alosca + cattle manure	269 ^{a}	105 ^{c}
Control	252 ^{a}	220 ^{a}
F pr.	0.006	0.007
CV (%)	24.8	27.8

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

Table

4.27: Maize stover yield (Groundnut experimental fields)

Treatments	Stover yield (kg ha^{-1})	
	Sudan savanna	**Guinea savanna**
Histick	262 ^{a}	198 ^{ab}
Biofix	215 ^{a}	239 ^{a}
Nitrogen*	141 ^{b}	224 ^{ab}
Cattle manure**	157 ^{b}	168 ^{b}
Histick + cattle manure	121 ^{b}	204 ^{ab}
Biofix + cattle manure	223 ^{a}	208 ^{ab}
Control	213 ^{a}	193 ^{ab}
F pr.	<0.001	0.007
CV (%)	19.6	23.2

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.6.3 Maize shoot biomass N, P and K uptake

Tables 4.28 – 4.33 show results for shoot N, P and K uptake. Shoot N uptake for maize grown on soybean fields in the Guinea savanna were generally higher than that of the Sudan savanna. Maize shoot nitrogen and phosphorus uptake in both the soybean and groundnut experimental fields at both study locations showed similar trends where statistical differences were not produced among the treatments. Furthermore, potassium uptake of maize under both soybean and groundnut fields in all the locations also followed a similar pattern (Table 4.30).

4.28: Maize shoot N uptake (Soybean experimental fields)

Treatments	Shoot N uptake (kg ha^{-1})	
	Sudan savanna	**Guinea savanna**
Legume fix	0.92 ^{a}	0.86 ^{a}
Alosca	0.63 ^{a}	0.97 ^{a}
Nitrogen[1]	0.60 $_{a}$	0.79 ^{a}

[1] Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

Table

Cattle manure*2	0.55 a	0.98 a
Legume fix + cattle manure	0.68 a	1.06 a
Alosca + cattle manure	0.78 a	1.00 a
Control	0.66 a	0.86 a
F pr.	0.48	0.75
CV (%)	28.2	27.5

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

Table 4.29: Maize shoot N uptake (Groundnut fields) Treatments

Shoot N uptake (kg ha^{-1})

	Sudan savanna	Guinea savanna
Histick	0.54 a	0.64 a
Biofix	0.56 a	0.65 a
Nitrogen*	0.47 a	0.89 a
Cattle manure**	0.45 a	0.74 a
Histick + cattle manure	0.60 a	0.58 a

Table 4.30: Maize shoot P uptake (Soybean experimental fields)

Treatments	Shoot P uptake (kg ha^{-1})	
	Sudan savanna	**Guinea savanna**
Legume fix	0.08 a	0.07 a
Alosca	0.08 a	0.07 a
Nitrogen*	0.08 a	0.07 a
Cattle manure**	0.07 a	0.08 a
Legume fix + cattle manure	0.08 a	0.08 a
Alosca + cattle manure	0.08 a	0.08 a
Control	0.06 a	0.07 a
F pr.	0.66	0.79
CV (%)	23.1	28.2

[2] Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

Table

Biofix + cattle manure	0.61 [a]	0.83 [a]
Control	0.56 [a]	0.56 [a]
F pr.	0.73	0.39
CV (%)	28.0	31.8

4.31: Maize shoot P uptake (Groundnut experimental fields) Treatments

	Shoot P uptake (kg ha^{-1})	
	Sudan savanna	**Guinea savanna**
Histick	0.06 [a]	0.06 [a]
Biofix	0.07 [a]	0.07 [a]
Nitrogen*	0.07 [a]	0.07 [a]
Cattle manure**	0.07 [a]	0.06 [a]
Histick + cattle manure	0.07 [a]	0.05 [a]
Biofix + cattle manure	0.08 [a]	0.05 [a]
Control	0.07 [a]	0.05 [a]
F pr.	0.62	0.87
CV (%)	22.7	31.3

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

Table 4.32: Maize shoot K uptake (Soybean experimental fields)

Treatments	Shoot K uptake (kg ha^{-1})	
	Sudan savanna	**Guinea savanna**
Legume fix	1.07 [a]	0.87 [a]
Alosca	1.11 [a]	0.90 [a]
Nitrogen*	1.03 [a]	0.79 [a]
Cattle manure**	0.89 [a]	0.94 [a]
Legume fix + cattle manure	0.95 [a]	1.04 [a]
Alosca + cattle manure	0.99 [a]	0.91 [a]
Control	0.94 [a]	0.82 [a]
F pr.	0.81	0.81
CV (%)	21.9	25.9

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

Table 4.33: Maize shoot K uptake (Groundnut experimental fields) Treatments

	Shoot P uptake (kg ha^{-1})	
	Sudan savanna	**Guinea savanna**
Histick	0.94 [a]	1.03 [a]
Biofix	1.05 [a]	1.12 [a]

Table

Nitrogen*	1.18 [a]	1.10 [a]
Cattle manure**	1.00 [a]	0.95 [a]
Histick + cattle manure	1.03 [a]	0.85 [a]
Biofix + cattle manure	1.14 [a]	1.00 [a]
Control	1.18 [a]	0.89 [a]
F pr.	0.61	0.92
CV (%)	20.2	32.9

*Nitrogen = 50 kg N ha^{-1}; **Cattle manure = 4 tonnes ha^{-1}; Means with same letters in same column are not significantly different at 5%.

4.7 Economic returns following soybean and groundnut inoculation

4.7.1 Net benefits

The result for partial cost-benefit analyses on soybean and groundnut is as shown in Tables 4.34 - 4.37. In the Sudan savanna, Legume fix gave the highest net benefit ($ 879) relative to the rest of the treatments (Table 4.34). The net benefit of all treatments increased over the control except 50 kg N ha^{-1} and cattle manure with net benefit values of $ 520 and $ 505, respectively. Sole inoculation using Legume fix gave higher net benefit ratio relative to Alosca (Table 4.34).

Results for partial cost-benefit analysis of soybean in the Guinea savanna zone showed that Legume fix gave the highest net benefit with an increase of $294 over the control, hence gave the highest profitability index (Table 4.35). Combination of Legume fix with cattle manure increased net benefit ratio ($ 200) over the control.

Partial cost-benefit analysis for groundnut in the Sudan savanna zone is presented in Table 4.36. The highest net benefit ($424) was obtained under the control. The least net benefit ($193) was produced in the 50 kg N ha^{-1} treatment. Furthermore, there was wide variability in net benefit between the fertilizer treatment and the control (Table 4.36). Biofix + cattle manure generated the highest net benefit compared to histic + cattle manure.

Partial net benefit analysis for groundnut in the Guinea savanna revealed that the control performed better than inoculated plants, 50 kg N ha^{-1} fertilizer and combinations of inoculant and cattle manure (Histick + cattle manure and Biofix + cattle manure) (Table 4.37). There was a decrease in net benefit of $ 63 and $ 90 over the control for Histick and Biofix inoculants. It was observed that 50 kg N ha^{-1} fertilizer also resulted in decreased net benefit of $ 134 and $ 141 for cattle manure and 50 kg N ha^{-1} over the control, respectively.

Similarly, decreases of $ 120 and $ 144 net benefit over the control were observed for the Biofix + cattle manure and Histick + cattle manure, respectively. Furthermore, in the groundnut field at the Guinea savanna, the control had the highest net benefit which was generally more profitable relative to the other treatments.

Marginal rates of return (MRR) for soybean and groundnut are as presented in Figures 4.7 - 4.10. In the soybean experimental field (Sudan savanna), inoculated plants had higher MRR relative to the control. Legume fix and Alosca inoculants had MRRs of 1077 and 514%, respectively. Furthermore, 50 kg N ha^{-1} and cattle manure had MRRs of -40 and 46%, respectively. Legume fix + cattle manure and Alosca + cattle manure had MRRs of 127 and 354%, respectively. (Figure 4.7).

In the Guinea savanna, MRR results for soybean followed the same trend as observed in the Sudan savanna (Figure 4.8). Legume fix and Alosca treatments had MRRs of 917% and 361%, respectively. The 50 kg N ha^{-1} and cattle manure gave MRRs of 114% and 235%, respectively. Combination of inoculant and cattle manure generally had MRRs lower than sole inoculants. Legume fix + cattle manure and Alosca + cattle manure produced MRRs of 175% and 80%, respectively.

In the Sudan savanna (Figure 4.9), MRR result for groundnut showed that all the treatments gave negative values; an indication that farmers cannot recover their investment when they use inoculants to produce groundnut. The least MRR (-640%) was observed under 50 kg N ha^{-1} fertilizer treatment. On the other hand, in the Guinea savanna, a similar trend was observed in which all the treatments had negative values, indicating no economic advantage in shifting to new treatments.

Table 4.34: Partial cost-benefit analysis of soybean in the Sudan savanna (2015 cropping season)

	Treatments						
Parameters	**LF**	**AL**	**N**	**C M**	**LF + CM**	**AL + CM**	**C**
Grain yield (kg ha^{-1})	1366	1099	873	891	1145	1436	843
10% less than actual yield at farmer's level (kg ha^{-1})	137	110	87	89	115	144	84
Adjusted yield (kg ha^{-1})	1229	989	786	802	1031	1292	759
Gross income (soybean price: \$40/50kg bag) **	984	791	629	642	824	1034	607
N fertilizer (kg N ha^{-1})	0	0	30	0	0	0	0
P fertilizer (kg P ha^{-1})	45	45	45	45	45	45	45
K fertilizer (kg K ha^{-1})	35	35	35	35	35	35	35
CM (kg ha^{-1})	0	0	0	4000	4000	4000	0
Inoculant (g ha^{-1})	250	250	0	0	250	250	0
Cost of urea (\$ 60/50kg bag) *	0	0	36	0	0	0	0
Cost of TSP (\$ 40/50kg bag) *	36	36	36	36	36	36	36
Cost of MP (\$ 32/50kg bag) *	22.4	22.4	22.4	22.4	22.4	22.4	22.4
Fertilizer application cost (\$ 2/man/day)	14	14	14	14	14	14	14
Cost of cattle manure (\$ 0.8/50kg bag) *	0	0	0	64	64	64	0
Cost of Alosca (\$ 12/100g pack) *	0	30	0	0	0	30	0
Cost of Legume fix \$ 32/250g pack) *	32	0	0	0	32	0	0
Variable cost (\$ ha^{-1})	104	102	108	136	168	166	72
Net benefit (\$ ha^{-1})	879	689	520	505	656	868	535

*Market price of inoculants and fertilizer in Kano (US$). ** Market price in Dawanau grains market Kano (US$), LF = Legume fix, AL= Alosca, N = 50 kg N ha^{-1}, CM = cattle manure, LF + CM = Legume fix + cattle manure, AL + CM = Alosca + cattle manure, C= Control

Table 4.35: Partial cost-benefit analysis of soybean in the Guinea savanna (2015 cropping season)

	Treatments						
Parameters	**LF**	**AL**	**N**	**CM**	**LF + CM**	**AL + CM**	**C**
Grain yield (kg ha^{-1})	1342	1082	997	1188	1257	1125	890
10% less than actual yield at farmer's level (kg ha^{-1})	134	108	100	119	126	113	89
Adjusted yield (kg ha^{-1})	1208	974	897	1069	1131	1013	801
Gross income (soybean price: \$40/50kg bag) **	966	779	718	855	905	810	641
N fertilizer (kg N ha^{-1})	0	0	30	0	0	0	0
P fertilizer (kg P ha^{-1})	45	45	45	45	45	45	45
K fertilizer (kg K ha^{-1})	35	35	35	35	35	35	35
CM (kg ha^{-1})	0	0	0	4000	4000	4000	0
Inoculant (g ha^{-1})	250	250	0	0	250	250	0
Cost of urea (\$ 60/50kg bag) *	0	0	36	0	0	0	0
Cost of TSP (\$ 40/50kg bag) *	36	36	36	36	36	36	36
Cost of MP (\$ 32/50kg bag) *	22.4	22.4	22.4	22.4	22.4	22.4	22.4
Fertilizer application cost (\$ 2/man/day)	14	14	14	14	14	14	14
Cost of cattle manure (\$ 0.8/50kg bag) *	0	0	0	64	64	64	0
Cost of Alosca (\$ 12/100g pack) *	0	30	0	0	0	30	0
Cost of Legume fix \$ 32/250g pack) *	32	0	0	0	32	0	0
Variable cost (\$ ha^{-1})	104	102	108	136	168	166	72
Net benefit (\$ ha^{-1})	862	677	609	719	737	644	568

*Market price of inoculants and fertilizer in Kano (US$). ** Market price in Dawanau grains market Kano (US$), LF = Legume fix, AL= Alosca, N = 50 kg N ha^{-1}, CM = cattle manure, LF + CM = Legume fix + cattle manure, AL + CM = Alosca + cattle manure, C= Control

Table 4.36: Partial cost-benefit analysis of groundnut in the Sudan savanna (2015 cropping season)

	Treatments						
Parameters	**HS**	**BF**	**N**	**CM**	**HS + CM**	**BF + CM**	**C**
Grain yield (kg ha^{-1})	452	506	335	339	372	445	551
10% less than actual yield at farmer's level (kg ha^{-1})	45	51	34	34	37	45	55
Adjusted yield (kg ha^{-1})	407	455	302	305	335	401	496
Gross income (groundnut price: $ 50/50kg bag) **	407	455	302	305	335	401	496
N fertilizer (kg N ha^{-1})	0	0	30	0	0	0	0
P fertilizer (kg P ha^{-1})	45	45	45	45	45	45	45
K fertilizer (kg K ha^{-1})	35	35	35	35	35	35	35
CM (kg ha^{-1})	0	0	0	4000	4000	4000	0
Inoculant (g ha^{-1})	250	250	0	0	250	250	0
Cost of urea ($ 60/50kg bag) *	0	0	36	0	0	0	0
Cost of TSP ($ 40/50kg bag) *	36	36	36	36	36	36	36
Cost of MP ($ 32/50kg bag) *	22.4	22.4	22.4	22.4	22.4	22.4	22.4
Fertilizer application cost ($ 2/man/day)	14	14	14	14	14	14	14
Cost of cattle manure ($ 0.8/50kg bag) *	0	0	0	64	64	64	0
Cost of Histick ($ 12/100g pack) *	30	0	0	0	30	0	0
Cost of Biofix ($ 16/250g pack) *	0	16	0	0	0	16	0
Variable cost ($ ha^{-1})	102	88	108	136	166	152	72
Net benefit ($ ha^{-1})	304	367	193	169	168	248	424

*Market price of inoculants and fertilizer in Kano. ** Market price in Dawanau grains market Kano (US$). ** Market price in Dawanau grains market Kano (US$). HS = Histick, BF = Biofix, N= 50 kg N ha^{-1}, CM = cattle manure, HS + CM= Histick + cattle manure, BF + CM = Biofix + cattle manure, C= Control

Table 4.37: Partial cost-benefit

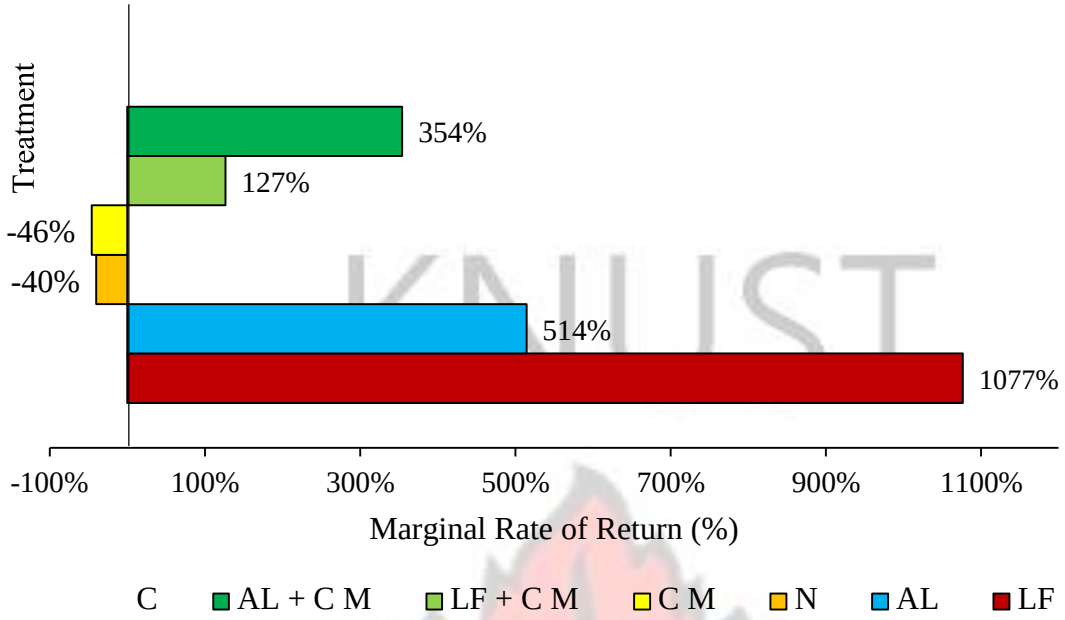
analysis of groundnut in the Guinea savanna (2015 cropping season)

	Treatments						
Parameters	**HS**	**BF**	**N**	**CM**	**HS + CM**	**BF + CM**	**C**
Grain yield (kg ha^{-1})	634	589	554	558	580	591	671
10% less than actual yield at farmer's level (kg ha^{-1})	63	59	55	56	58	59	67
Adjusted yield (kg ha^{-1})	571	530	499	502	522	532	604
Gross income (groundnut price: $ 50/50kg bag) **	571	530	499	502	522	532	604
N fertilizer (kg N ha^{-1})	0	0	30	0	0	0	0
P fertilizer (kg P ha^{-1})	45	45	45	45	45	45	45
K fertilizer (kg K ha^{-1})	35	35	35	35	35	35	35
CM (kg ha^{-1})	0	0	0	4000	4000	4000	0
Inoculant (g ha^{-1})	250	250	0	0	250	250	0
Cost of urea ($ 60/50kg bag) *	0	0	36	0	0	0	0
Cost of TSP ($ 40/50kg bag) *	36	36	36	36	36	36	36
Cost of MP ($ 32/50kg bag) *	22.4	22.4	22.4	22.4	22.4	22.4	22.4
Fertilizer application cost ($ 2/man/day)	14	14	14	14	14	14	14
Cost of cattle manure ($ 0.8/50kg bag) *	0	0	0	64	64	64	0
Cost of Histick ($ 12/100g pack) *	30	0	0	0	30	0	0
Cost of Biofix ($ 16/250g pack) *	0	16	0	0	0	16	0
Variable cost ($ ha^{-1})	102	88	108	136	166	152	72

Table 4.38: Partial cost-benefit

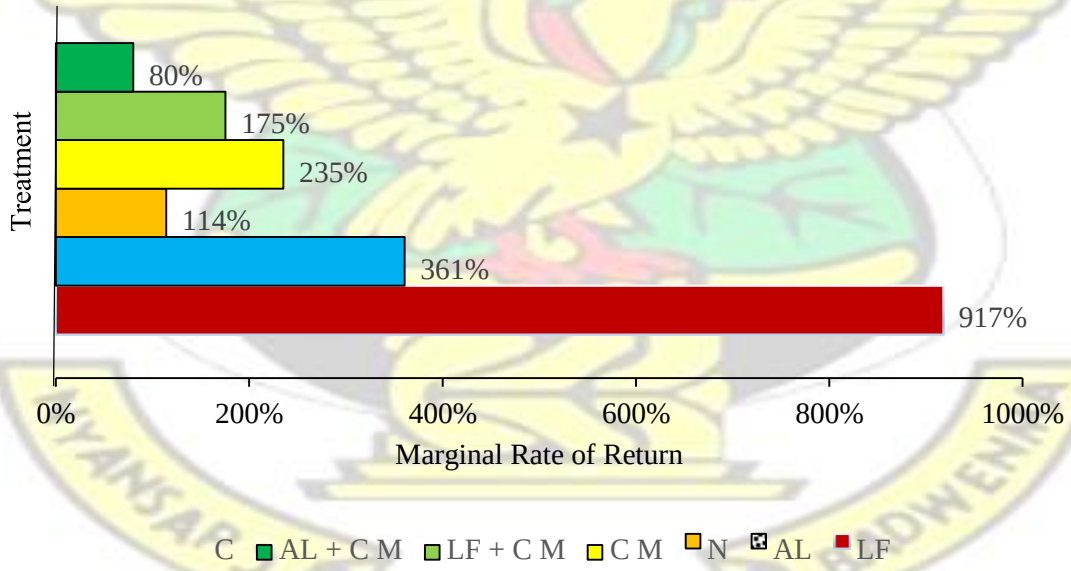
Net benefit ($ ha^{-1})	468	442	390	366	356	380	532

*****Market price of inoculants and fertilizer in Kano. ** Market price in Dawanau grains market Kano (US$). ** Market price in Dawanau grains market Kano (US$). HS = Histick, BF = Biofix, N= 50 kg N ha^{-1}, CM = cattle manure, HS + CM= Histick + cattle manure, BF + CM = Biofix + cattle manure, C= Control



C = control Al = Alosca CM = cattle manure LF = Legume fix N = nitrogen (50 kg N ha^{-1})

Figure 4.7: Marginal rates of return for different soybean treatments in the Sudan savanna



C = control Al = Alosca CM = cattle manure LF = Legume fix N = nitrogen (50 kg N ha^{-1})

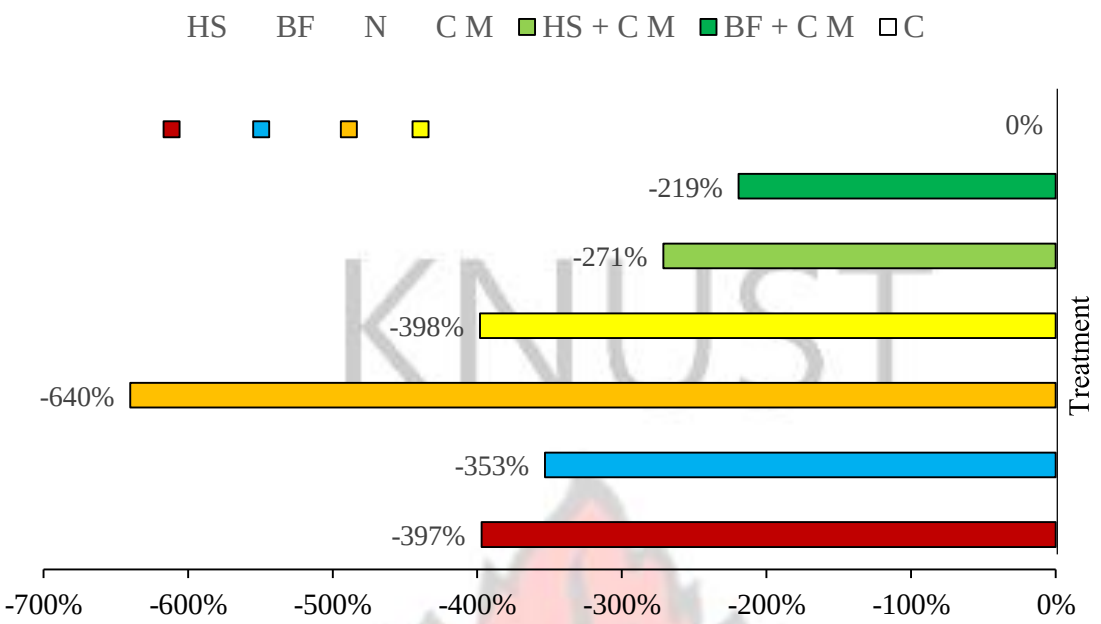
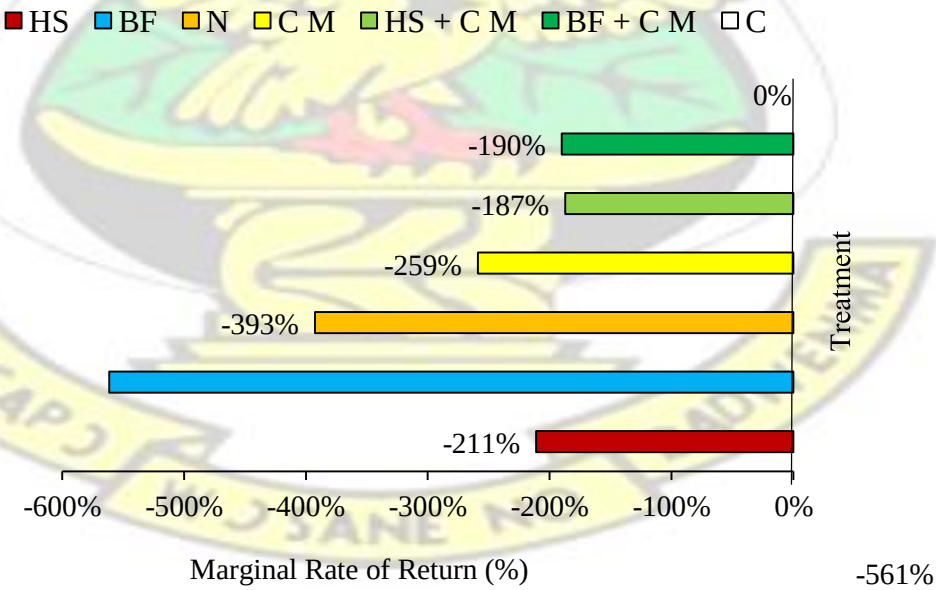


Figure 4.8: Marginal rates of return for different soybean treatments in the Guinea savanna

Marginal Rate of Return (%)

C = control HS = Histick CM = cattle manure BF = Biofix N = nitrogen (50 kg N ha^{-1})

Figure 4.9: Marginal rate of return for different groundnut treatments in the Sudan savanna



Marginal Rate of Return (%)

-561%

C = control HS = Histick CM = cattle manure BF = Biofix N = nitrogen (50 kg N ha^{-1})

Figure 4.10: Marginal rates of return for different groundnut treatments in the Guinea savanna

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physical and chemical properties of soils of the study locations

The results of the initial soil properties (Table 4.1) showed that the soil texture was sandy loam in both the Sudan and Guinea savanna experimental fields. However, soil in the Sudan savanna was moderately acidic while that of the Guinea savanna was slightly acidic according to the ratings of Landon (2014). Soil organic carbon was low (< 1%) in both study locations (Landon, 2014). The low organic carbon can be attributed to the continuous cultivation of the area coupled with limited addition of organic amendments as typical of most savanna soils (Ogundare *et al*. 2012). The available N and P contents of the soil in the two study locations are considered low relative to critical levels (N < 0.10% and P < 10 mg kg $^{-1}$) (Landon, 2014). The K values of soil in the Sudan savanna were relatively lower than in the Guinea savanna and this could be a result of the parent material being rich in K. Parent material have direct relationship with nutrient content of soils (Sudhir *et al*., 2014). In general, it can be said that the soils in the two study locations have low inherent fertility (Singh *et al*., 2011). The indigenous rhizobia population (IRP) was found to be low in both study locations; 1.02 x 10^1 cells g^{-1} of soil and 2.20 x 10^1 cells g^{-1} of soil in the Sudan and Guinea savanna, respectively. There is therefore a high tendency for the soils to respond to inoculation. According to Sanginga *et al*. (1996a), Houngnandan *et al*. (2000) and Zoundji *et al*. (2015), response of soil to inoculation is likely to occur when the population of indigenous rhizobia is between 5 to 10 cells g^{-1} soil.

5.2 Quality assessment of commercially produced microbial inoculants

Result of the MPN experiment (Table 4.33) revealed that, the inoculants contained enough number of rhizobia that could be used to nodulate host legume under field conditions. The

findings further showed that, the inoculants had pH range (threshold 6.00) ideal for the survival of rhizobia strains and the moisture content (threshold 32- 35%) was also enough to support rhizobia present in the peat carrier. Rhizobia cells are temperature and pH sensitive and as such care must be taken during handling and storage especially in African countries where most smallholder farmers lack storage facilities (Boonkerd, 1991). Boonkerd (1991) highlighted the importance of temperature in the survival of rhizobia cells as high number of cells in peat are present under 10 ^{o}C than at 30^{o}C.

The success of any commercial microbial inoculant is dependent on the number of the viable and effective rhizobia capable of infecting nodulation at the point of use (Catroux *et al*., 2001). It is always paramount to investigate the quality of commercial rhizobia rather than to depend on the claim of manufacturers to avoid use of low quality products. There is also need to intensify effort in identifying effective strains that can perform better than the native strains even under adverse environmental conditions (Aliyu *et al*., 2013). However, it is imperative to note that, no matter the quality of inoculants, it can only be successful and yield positive results when the native rhizobia are ineffective and low in population.

5.3 Study 1. Influence of microbial inoculants on growth and yield of soybean and groundnut

5.3.1 Nodule number, number of effective nodules and nodule dry weight

Nodulation is the major criterion for assessing biological nitrogen fixation by *Bradyrhizobium* and therefore an important index for fixation potential (Singleton and Tavares, 1986; Ampomah *et al*., 2008; Workalemahu, 2009; Argaw, 2014;). Legume fix gave relatively higher nodule number compared to Alosca. The efficacy of Legume fix over

Alosca is associated with the relatively higher number of strains it contained (Table 4.33). Several researchers have reported significant increases in nodule number due to inoculation with appropriate *Bradyrhizobium* (Martins *et al.*, 2003; Osunde *et al*., 2003; Kumaga and Ofori, 2004). Yakubu *et al*. (2010a) while working with cowpea in north eastern Nigeria, observed that inoculation with *Rhizobium* increased nodule number of cowpea by more than 30% compared to the control.

In this current study, nodule dry weight under all treatments in the Sudan and Guinea savannas was higher than the control. This result is in agreement with that of Santos *et al*. (2011) and Solomon *et al*. (2012) who observed that rhizobia inoculation significantly increased nodule dry weight. The cropping history of the two experimental sites suggest that soybean had not been grown, hence it was expected that inoculation will work well. In their study, Asei *et al*. (2015) opined that compatible rhizobia population was low in soil where soybean had not been grown for long and hence inoculation with superior strains of rhizobia is necessary if good nodulation and subsequent yield boost is to be achieved. Other researchers have reported that, soybean grown in Africa was believed to be capable of forming effective nodules with indigenous *Bradyrhizobium* species in some soils where soybean has been grown over a long period of time (Maingi *et al*., 2006; Yoseph *et al*., 2017). The relatively higher number of nodules under Legume fix and Alosca treatments compared to the control is in line with the claims of Okereke *et a*l. (2004), Tahir *et al*. (2009) and Bekere and Hailemariam (2012a) who reported higher number of nodules relative to the control following inoculation. This further confirms the hypothesis of this study that inoculation will enhance nodulation. Several workers also reported successful nodulation in legumes following inoculation with compatible rhizobia (Kishinevsky *et al*., 1987; Elsheikh and Wood, 1995; Sanginga e*t al*., 1996b; Burdass, 2002; Gwata *et al*.,

2003; Singh and Usha, 2003; Abaidoo *et al*., 2007; Ndusha *et al*., 2017; Kumar *et al*., 2018).

Plots amended with nitrogen at 50 kg N ha^{-1} gave low percentage effective nodules. This corroborates with the findings of Weisany *et al*. (2013) who reported that application of mineral nitrogen above the recommended rate (40 – 60 kg N ha^{-1}) suppressed nodulation in bean plants. This is as a result of the fact that when there is abundant quantity of mineral nitrogen is in the soil, the legume will use it instead of establishing relationship with compatible rhizobium. The high number of nodules under cattle manure treatment may be due to improvement in organic carbon content of the soil under this treatment. Manure addition has been reported to result in the multiplication of rhizobia due to the high organic C content which acts as energy source for bacteria leading to the provision of a favourable growing environment (Zengeni *et al*., 2006). Better responsiveness of soil microbial biomass under organic matter amendments in smallholder farm was also reported by Ngullie and Srivastava (2009).

Generally, the nodule number of soybean in the Guinea savanna was lower than that of Sudan savanna. This is attributable to variability in soil and climate in the two study locations as well as differences in the indigenous rhizobia population. Ngullie and Srivastava (2009) opined that favourable soil condition and microclimate environment enhance performance and persistence of microbes in an area. Nodule dry weight followed the same trend as nodule number. Research in smallholder farms in Mozambique showed that cowpea inoculation influenced nodule dry weight significantly compared to control treatments (Kyei-Boahen *et al*., 2017).

Results for nodule number, number of effective nodules and nodule dry weight of groundnut showed that, there were significant differences among the treatments (Table

4.5). However, it was observed that among the inoculants, Biofix performed better than Histick in terms of nodule number and number of effective nodules in both the Sudan and Guinea savannas. This was possibly due to higher number of rhizobia cells of Biofix and higher effectiveness as compared to Histick. Appreciable nodule number and nodule dry weight were observed under inoculants plots + cattle manure amended plots for soybean and groundnut fields in both agro-ecologies. This is attributed to improved soil structure and enhanced moisture retention capacity as a result of cattle manure addition. Another reason could be due to micronutrients contribution from the cattle manure (Table 4.2). Results of the study revealed that soils of the study area in both locations had low nutrient content especially phosphorus resulting in obstructed nodulation of the host plant and ultimately yield decline in groundnut sole inoculated plots (Wasike *et al*., 2009). According to Kabir *et al*. (2013) and Kyei-Boahen *et al*. (2017), deficiency of phosphorus in soil inhibits proper nodulation in legumes. The presence of specific rhizobia population in a soil that was well adopted to soil condition and poorly effective in their nitrogen fixation can limit nodule occupancy by the introduced strains resulting in inoculation failure (Rodriguez-Navarro *et al*., 2011).

5.3.2 Shoot biomass N, P and K uptake at 50% flowering

In this study, it was observed that inoculation enhanced P uptake especially under soybean relative to the control. This corroborates with the findings of Fituma *et al*. (2018) that inoculation of soybean with *Bradyrhizobium* significantly enhanced plant N concentration and P uptake compared to the control treatment in calcareous soils of Ethiopia. Furthermore, Laditi *et al*. (2012) while working on some soils of Nigeria confirmed that inoculation enhanced shoot N uptake in soybean. The fact that the inoculants performed better than the control in terms of uptake of nutrients suggests enhanced nitrogen fixation capacity of the introduced strains. Phosphorus and K uptake in soybean was also affected

by inoculation contrary to the finding of Aliyu *et al*. (2013) who observed that N uptake, dry matter yield and BNF of the control treatment performed better than inoculated treatments.

It was observed that shoot N, P and K uptake of soybean were better in cattle manure plus inoculant amended plots than sole inoculant and 50 kg N ha^{-1} treatments (Tables 4.6, 4.8 and 4.10). This could be due to complementary effect of inoculant and cattle manure application. Several workers also found significant increases in uptake of major nutrients when cattle manure was used with inoculants or mineral fertilizer than sole N fertilizer or inoculant (Asawalam and Onwudike, 2011; Abdullahi *et al*., 2014; Chinthapalli *et al.*, 2015). Shoot nitrogen uptake of inoculated plots were not significantly different when compared to control treatments in both soybean and groundnut fields in the two study locations. The same trend was also observed for P and K uptake in the Sudan savanna groundnut field where treatments were not significantly different from the control. This is due to the fact that the native rhizobia strain was more effective than the introduced strain or the introduced strain was unable to adopt to the new environment to impact any meaningful difference (Bala *et al*., 2003; Okogun and Sanginga, 2003; Samuel *et al*., 2012; Thuita *et al*., 2012). Biofix was relatively better than Histick inoculant in terms of nutrient uptake. This could be attributed to higher number of strains in Biofix which could be more effective than Histick (Table 4.33). Various researchers related high performance of inoculants with effectiveness and higher number of introduced strains (Osunde *et al*., 2003; Mehrpouyan *et al*., 2012; Watanabe *et al*., 2013).

5.3.3 Grain yield and harvest index (soybean)

Inoculant treatments significantly influenced grain yield in both the Sudan and Guinea savanna agro-ecological zones (Table 4.11). This is due to nitrogen contribution as a result

of symbiotic association between legumes and rhizobium. This was confirmed by the findings of many researchers who reported that inoculation of legumes with rhizobia resulted in improved grain yield, quality and profitability (Singleton and Tavares, 1986; Albareda *et al*., 2009; Solomon *et al*., 2012; Argaw, 2014; Kyei-Boahen *et al*., 2017). Alosca + cattle manure treatment produced the highest grain yield followed by Alosca alone. The highest harvest index was also produced under inoculated treatments. This finding was expected because soybean is a newly introduced crop in the study locations. Therefore, the inoculant strains established a healthy symbiotic relationship with the crop to fix the required nitrogen and consequently increased grain yield. The result tallies with the findings of many workers who established that inoculation influenced yield especially in an area where a new legume is introduced (Martins *et al*., 2003; Unkovich *et al*., 2008; Osunde. *et al*., 2003; Kolapo, 2011; Meghvansi, Prasad and Mahna, 2010; Osman, 2011; Binang *et al*., 2013; Mutuma *et al*., 2014; *Asei et al*., 2015; Rahim *et al*., 2017). Zimmer *et al*. (2016) reported that it is necessary to inoculate when a new crop is introduced to an area to achieve the potential yield. This was also reported by Thuita *et al*. (2012) that commercial rhizobium inoculants can supply effective strains for use in areas where soybean is being introduced as a new crop. The low grain yield obtained under 50 kg N ha^1 is due to effect of high nitrogen which inhibits nodulation. Research by Tahir *et al*. (2009) showed that application of 25 kg N in combination with TSP fertilizer resulted in increased soybean grain yield.

5.3.4 Grain yield and harvest index (groundnut)

The low performance of inoculants under groundnut (Tables 4.14 and 4.15) could be attributed to the groundnut cropping history of the experimental site over a long period of time. This could have resulted in the buildup of the native rhizobia which nodulate

groundnut. Inoculation may not perform excellently in the soil when the native rhizobia strains are effective (Kwakye and Dennis, 1988). The native rhizobia have competitive advantage over introduced strains. This probably explains the low response to inoculation. The long-term adaptation to edaphic and agro-climatic conditions by the indigenous rhizobia could account for the low response of the exotic strains contained in Histick and Biofix inoculants. This result agrees with the findings of Romdhane *et al*. (2007) who reported that native rhizobia are well adapted to native bean variety and compete more effectively in root colonization with the introduced strains.

Research has shown that, soil nutrient deficiencies especially calcium and phosphorus are the most limiting factors in groundnut production (Tarawali, 2014). Though, phosphorus level was less than 10 mg kg^{-1} in the study locations, the level was higher in the Sudan Savanna (8.05 mg kg^{-1}) than in the Guinea Savanna (4.03 mg kg^{-1}). Phosphorus was found to be essential nutrient for nodule development and nitrogen fixation. It was established that nitrogenase activity required high ATP (Yakubu *et al*., 2010a; Safapour *et al*., 2011; Bekere and Hailemariam 2012a; Kamara *et al*., 2012). Kamara *et al*. (2007) reported that, groundnut production in north eastern Nigeria savanna zone was inhibited by inadequate soil phosphorus. This could be the reason for the low performance of groundnut in the two agro-ecological zones under the present study. The low yield under 50 kg N ha^{-1} as compared to the control may be due to the laxity of rhizobium in fixing atmospheric nitrogen when nitrogen is not limiting.

The interaction effect of *Bradyrhizobium* and cattle manure may explain the good yield performance under the combined applications in both soybean and groundnut plots. The mechanism behind yield increment when inoculants are combined with organic manure is linked to the availability of carbon contained in organic manure which enhance survival

and persistence of rhizobia (Zengeni *et al*., 2006). Very high yield increment was achieved under soybean when compared with the control in both the Sudan and Guinea savannas. The significant yield increment can contribute to increase livelihood of smallholder poor farmers in the study zones.

5.3.5 Pearson correlation of some yield parameters

In the soybean field in the Sudan savanna, positive correlations were observed between shoot dry weight and nodule dry weight. This, somewhat confirms the dependence of shoot biomass accumulation and nitrogen fixation on nodulation. Root dry weight was also found to correlate with grain yield (Table 4.18). A clear indication of successful nodulation is a sign of good seed yield (Kyei-Boahen *et al*., 2017). This possibly accounted for the positive correlation between nodule dry weight and 100 seed weight. These results further support earlier claims by Kawaka *et al*. (2014) and Koskey et al. (2017) that, there is a direct relation between plant growth, nodulation and nitrogen accumulation in legume plants.

At the groundnut field in the Sudan savanna, significant ($P < 0.05$) positive correlations were also observed between shoot dry weight and nodule dry weight, shoot dry weight and grain yield and nodule dry weight and grain yield (Table 4.18). Thies *et al*. (1992) also documented a positive correlation in nodule occupancy against yield parameters while observing the effects of soybean inoculation and the influence of native rhizobia strains.

On the contrary, in the Guinea savanna, a significant negative correlation was observed between shoot dry weight and 100 seed weight and 100 seed weight and grain yield for soybean (Table 4.19). This is contrary to the earlier finding reported by Kyei-Boahen *et al*. (2017) which indicated a positive relationship between shoot dry weight and nodulation and nodulation and yield. At the groundnut field in the Guinea savanna, results showed that

only root dry weight vs 100 seed weight showed significant ($P < 0.05$) positive correlation (0.447**) with the growth and yield parameters measured. All the other parameters did not show any significant correlation (Table 4.20). This is in contrast to the findings of Bekere *et al*. (2012a) who reported significant positive correlation between nodule dry weight and growth parameters while studying the influence of *Bradyrhizobium* inoculation on the growth and nodulation of soybean.

Generally, in soybean field, Legume fix performed better than Alosca in terms of nodulation capacity. This is attributed to the effectiveness of the *Bradyrhizobia* strains in Legume fix compared to Alosca. This is a common occurrence in inoculation with different strains (Waswa *et al*., 2014; Ulzen *et al*., 2016; Osei *et al*., 2017). On the contrary, inoculation did not enhance the growth and yield performance of groundnut, probably due to the presence of native rhizobia that occupied and became well adapted to the soil of the study area. It has been reported that native rhizobia population above 50 cells g^{-1} soil prevent inoculation response (Fening and Danso, 2002; Chianu, Nkonya, Mairura, Chianu and Akinnifesi, 2011; Kanonge-Mafaune *et al*., 2018). However, in this study, the

population of rhizobia from the study sites was less than 50 cells g^{-1} soil but it still obviated significant response in the case of groundnut inoculation in most of the parameters observed.

5.4 Grain uptake of major plant nutrients

5.4.1 Grain N uptake for soybean and groundnut

In the Sudan savanna, Grain N uptake increases of 58, 57, 21, 14 and 13% over the control were observed for Legume fix, Legume fix + cattle manure, Alosca + cattle manure, Alosca and cattle manure, respectively (Figure 4.1). The same trend was also observed in the

soybean field in the Guinea savanna. Satpute *et al*. (2018) while working on soybean in vertisol soils under rainfed condition reported significant N uptake when soybean was inoculated with biofertilizer. However, a higher N uptake (57.98 kg ha^{-1}) was achieved when combined application of biofertilizer and green manure was used (Satpute *et al*., 2018). Legume fix + cattle manure and Alosca + cattle manure were also higher also higher in N uptake than the control treatment in both study locations. Inoculation with effective strains of rhizobia and P supplementation was an effective way of enhancing the growth of soybean (Tairo and Ndakidemi, 2014). Rhizobia inoculation and combined treatment of rhizobia inoculant and cattle manure enhanced N uptake more than the mineral nitrogen or cattle manure alone. This may be due to the attributes of manure in enhancing soil carbon content and soil moisture. Reports of other researchers show significant increment in uptake of major nutrients when inoculants were used in combination with cattle manure or other organic manure than sole application of inoculants or mineral nitrogen fertilizer alone (Asawalam and Onwudike, 2011; Abdullahi *et al*., 2014; Chinthapalli *et al*., 2015). Another reason could be the high-quality organic manure used during the field experiment (Table 4.2). Organic manure with lignin and phelophenol contents of 10 – 15% and 3 – 4%, respectively have been reported to be of good quality (Vanlauwe *et al*., 2002; Verkaik *et al*., 2006 and Rezig *et al*. 2014). In this study, lignin and polyphenol contents of 10.73% and 2.97%, respectively (Table 4.2) were observed giving an indication that the manure used was of good quality. The low performance in Guinea savanna may be due to the presence of native competitive strains of rhizobia. One of the reasons behind failure of successful inoculation with efficient rhizobia is the competition for nodule occupancy postured by native rhizobia (Simon et al., 2014).

5.4.2 Grain P uptake of soybean and groundnut

Legume fix and Alosca inoculants enhanced P uptake relative to the control in the Sudan savanna (Figure 4.3). The Alosca + cattle manure and Legume fix + cattle manure treatments produced the highest uptake values. This may be due to the added benefit of P contents of the cattle manure. This tallied with report by Alsamowal *et al*. (2013) that *Bradyrhizobium* and AMF positively affected peanut plant growth, nutrients uptake and yield in both greenhouse and field experiments than sole inoculants.

Plant growth, nodule formation and nitrogen fixation need a lot of energy due to enzymatic activities, as such P is necessary (Safapour *et al*., 2011). The high grain P uptake obtained under Legume fix and Alosca in both agro – ecological zones was not surprising because earlier studies in Ethiopia reported that high grain P uptake was influenced by inoculation with *rhizobium* strains than uninoculated treatment (Belachew and Halemariam, 2010). Belachew and Halemariam (2010) also reported that, microbial activity during N fixation highly correlated with P uptake. It was also noted that, treatments that gave the highest grain P uptake produced higher grain yield. This confirms the finding of Sinclair and Vadez (2014) that adequate supply of P enhanced grain yield of legumes.

In the Sudan savanna, groundnut inoculation with Biofix gave the highest P uptake. In the Guinea savanna zone, groundnut responded to inoculation. Biofix gave the highest uptake followed by Histick. This corroborates the earlier finding of Belachew and Halemariam (2010) who reported increased P uptake following inoculation of peanut. The non-response of inoculation on P uptake was also contrary to the findings of Ndakidemi *et al*. (2011) who reported that *Bradyrhizobium* inoculation of leguminous plants enhanced uptake of minerals like P, K, Mg, Ca, S, Fe, Cu, Zn, Mn, and Mo. It was however observed that, location or morphological differences could result in variation in nutrient uptake in leguminous plants (Mefti *et al*., 1998). In this study, low P uptake levels on groundnut field

was observed and this could be attributed to the low soil P level and pH (Table 4.1) which could have reduced photosynthetic activities (Marschner and Dell, 1994). Phosphorus has been found to be associated with fat, carbon, hydrogen and oxygen metabolism and in photosynthesis and respiration (Mfilinge *et al*., 2014). It was reported by Hinsinger (1998) and Schachtman *et al*. (1998) that increase in pH result in decreased P uptake. An optimum pH for P uptake is between 5 and 6. The relatively low P uptake in the Guinea savanna agro-ecology compared to the Sudan savanna could be due to relatively lower soil available P in the Guinea savanna (Table 4.1). Phosphorus has been reported to be an important nutrient for all crops including legumes since it is a key constituent of ATP (Farag and Ahmed, 2014).

5.4.3 Grain K uptake of soybean and groundnut

The P and K contents of cattle manure might have accounted for relatively greater K uptake in the combined inoculant and cattle manure amended plots (Figure 4.5). This corroborates with the findings of Mfilinge et al. (2014) who outlined significant increases in N fixation, nutrient uptake and grain yield of treated plots over the control. Groundnut K uptake was generally higher than soybean. In groundnut field at the Guinea savanna, there were no significant differences in potassium uptake among the observed treatments Eutropia *et al.* (2013) reported effective rhizobia strains enhanced the growth and uptake of macronutrients in soybean.

5.4.4 Nitrogen fixation in the Sudan and Guinea savanna zones

Several researchers have conflicting findings on the response of soybean to mineral nitrogen fertilization (Salvagiotti *et al*., 2008). The genetic components of a crop and

compatibility with nitrogen fixing organisms can also influence BNF capability of a crop (Meghvansi *et al*., 2010; Singh, 2010).

Inoculating groundnut in the Sudan savanna showed some response in nitrogen fixation capacity of the legumes. This observation could be due to introduction of effective rhizobia or highly competitive and ineffective native strains (Samuel *et al*., 2012). Biofix had the highest nitrogen fixed (11.58 kg ha^{-1}) which was not significantly different from that of biofix + cattle manure and control. However, nitrogen fixation under cattle manure was the least (7.41 kg ha^{-1}) but not significantly different from 50 kg N ha^{-1}, Histick and Histick + cattle manure amended plots. This tallied with the findings of other researchers who reported that application of mineral nitrogen inhibits the biologically fixed nitrogen. Under this condition the plant will not release flavonoid (chemical substances responsible for attracting the compatible rhizobia to form association) (Heidari *et al*., 1992; Kubota *et al*., 2008; Bekere *et al*., 2012b; Ntambo *et al*., 2017). However, in the Guinea savanna, there were no significant differences among all the treatments observed (P= 0.412). This could be due to the presence of highly competitive native rhizobia which had advantage over the introduced strains as was also reported by Aliyu *et al*. (2013).

In the soybean experiment showed that the microbial inoculants and cattle manure influenced nitrogen fixation and uptake of major plant nutrients positively. Several workers reported similar findings of improved nitrogen fixation when inoculants were used in combination with organic manure (Santos *et al*., 2011; Mohammadi *et al*., 2012; Muthuri, K and Kirigiah, 2014). However, in the case of the groundnut experiment, it has been observed by many researchers that inoculants seldom significantly affect yields and general performance of crops (Prasad *et al*., 2009; Sajid *et al*., 2011; Mohamed and Abdalla, 2013; Muhammad, 2014). This is especially in areas that have long term history of groundnut production due to high competition in nodule occupancy between the newly introduced

strains and the native strains of rhizobia already present in the soil. Several workers have also opined that soil contain substantial population of highly competitive native rhizobia strains and thus chances of persistence for the introduced strains diminishes (Thies *et al.*, 1992; Abaidoo *et al*., 2007; Didagbe *et al*., 2014).

5.5 Legume residues effect on the growth and yield of maize during the 2016 cropping season

5.5.1 Grain yield and harvest index of maize under soybean residues in the Sudan and Guinea savanna zones

Results from the soybean field in the Sudan savanna revealed no significant differences among the treatments in terms of maize grain yield (P = 0.123) (Table 4.23). However, the combined treatments of cattle manure and inoculants performed better than the control and sole application of inoculants. This may be attributed to increased soil moisture content as a result of improved soil structure in maize plants that received combined application of cattle manure and inoculants. This is in line with the findings of Partey *et al*. (2014) who reported yield increase of maize in mixed than single treatments. This may also account for the highest harvest index (0.45) recorded under Legume fix + cattle manure.

5.5.2 Grain yield and harvest index of maize under groundnut residues in the Sudan and Guinea savanna zones

The results showed that, in the Sudan savanna zone, plots which previously received 50 kg N ha^{-1} had the highest maize yield (2167 kg ha^{-1}). Research has also shown that even in some situations where calculated N contribution of legumes was estimated to be negative, yield had increased (Sanginga *et al*., 2002). However, despite greater profitability achieved

through the double cash-crop sequence in groundnut-maize rotation in the long run, soil deterioration can emanate due to negative soil N balance (Okito *et al*., 2004).

It was expected that for yield of proceeding crop to be increased under inoculated plots due to N contribution, the amount of N fixed returned to the soil by legumes through their residue must have been higher than the amount of soil N that was harvested along with crop (Giller and Wilson, 1991). Yusuf *et al*. (2009b) reported that, despite the fact that most of the nitrogen harvested along with crop could lead to the negative N balance, maize succeeding soybean and cowpea gave higher yield than when maize followed maize. In this experiment, it was observed that a yield higher than the average yield (1.2 tonnes ha^{-1}) was produced in inoculated plots under both soybean and groundnut residues in the two agro-ecological zones.

5.6 Economic analysis

Net benefit and the marginal rates of return in percentage were used to quantify the economic viability of the various treatments used during the experiment.

5.6.1 Net benefit

It is important to note that farmers always make comparism between cost of changing from a production practices to the benefit that could be derived from that change. The soybean yield results in the Sudan savanna showed increase in marginal net benefit in Inoculated plots compared to control. The finding that soybean inoculation is more profitable than the control (Figures 4.7-4.8) is consistent with the findings of Masso *et al*. (2016), Ulzen *et al*. (2016) and Ulzen *et al*. (2018) who used value cost ratio (VCR) and found the economic benefit of inoculation to farmers.

Net benefit produced increases of \$ 344 ha^{-1}, \$334, \$ 154 ha^{-1} and \$ 121 ha^{-1} for Legume fix (LF), Alosca (AL) + Cattle manure (CM), AL and LF + CM, respectively. Cattle manure

alone produced the least negative value for net benefit. This result has shown that the use cattle manure alone and 50 kg N ha-^{1} fertilizer will not be profitable for soybean production. The highest profit was obtained with sole LF inoculant treatment.

In the Guinea savanna, soybean results showed marginal net benefit under all treatments including 50 kg N ha^{-1}. Legume fix had the highest marginal benefit ($ 294 ha^{-1}) while the least was obtained under 50 kg N ha^{-1} ($ 41 ha^{-1}). This agrees with finding of Asei *et al*. (2015) while using VCR method to quantify profitability of soybean production in smallholder farms in Northern Ghana and found that the VCR of mineral N was below the profitable threshold range of 3 – 4. This makes the usage of N fertilizer (50 kg N ha^{-1}) in soybean production uneconomical. Groundnut in the Sudan savanna showed a negative net benefit value in all the treatments. This indicates that neither the use of inoculant (I), nitrogen (50 kg N ha^{-1}) or cattle manure had any economic advantage over the control. Thus, it can be concluded that, the use of inoculants for groundnut production in the Sudan savanna will incur a loss to the farmers.

In the Guinea savanna, groundnut field results followed a similar trend except that the loss was generally lower compared to the Sudan savanna if inoculants were used. This was because net benefit of control was higher ($ 532). This is in conformity with the findings of Ulzen *et al*. (2016) who reported that all inoculants tested on cowpea at Nyagli in Northern Ghana were not economically viable because they had a VCR value less than the threshold (VCR $\geq$ 2). Thus, it is worth noting that yield is not always what is used to consider in the adoption of a new farming technology but farmers compare net benefit associated with yield, with the incurred cost of changing to new technology to make judgement.

5.6.2 Marginal rate of return

In the Sudan savanna, even though the highest net benefit was obtained under AL + CM in the soybean field, MRR was higher under Legume fix due to the increased cost incurred on CM under AL + CM (Appendix 3). Based on the findings of this study, the 50 kg N ha^{-1} gave a negative MRR value meaning that farmers will incur loss of 40 % if they opted for mineral N at high rate for soybean production. The I + CM treatment also gave MRR of 240% and 589% for LF + CM and AL + CM, respectively. Hirpa (2013) reported MRR of 1386 % per unit production cost on inoculated soybean in Ethiopia. Furthermore, Abera and Feyisa (2008) reported high MRR (1003 %) when Faba bean and field pea were intercropped at 75%: 25 % seed rate proportion. Buah *et al*. (2010) also reported highest MRR when maize was treated with 90 kg ha^{-1} N in Ghana.

The soybean field in the Guinea savanna showed that marginal net benefit was higher under all treatments when compared with the control (Appendix 4). This means that farmers will obtain more profit if they used inoculants for soybean production in the study area. This is in agreement with many workers who proved the economic viability of inoculating soybean (Asei *et al*., 2015; Ronner *et al* ., 2016; Ulzen *et al*., 2016). However, in the groundnut field, MRR results showed that the use of inoculant will incur loss to farmers in both agro – ecological zones. In the Guinea savanna, loss will be higher when Histick is used to produce groundnut. In the Sudan savanna, the loss will be more severe when N fertilizer is used to produce groundnut (-640%) (Appendix 5). Similar finding was also observed in the Guinea savanna except that severity of loss was higher in Biofix than 50 kg N ha^{-1} fertilizer. Researchers in Vietnam while studying the effect of inoculating soybean and groundnut reported that farmers could clearly improve profitability by reducing N fertilizer inputs (Hiep e*t al*., 2002).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

This study has added to the existing knowledge on the use of rhizobia inoculants for improving grain legume productivity through:

i. the evaluation of the effects of microbial inoculants growth and yield of two legumes under field conditions; ii. examining the effect of microbial inoculants, cattle manure and mineral nitrogen fertilizer on BNF and uptake of major nutrients in soybean and groundnut; iii. appraising the influence of legume residual effects following inoculation, inorganic and organic manure treatments on the general performance of succeeding maize crop and iv. assessing the quality of rhizobia inoculants to inoculate legumes under field condition.

Among the soybean inoculants, Legume fix outperformed Alosca in grain yield. However, among groundnut inoculants, Biofix performed better than Histick in most of the growth and yield parameters measured. Combined application of inoculants with cattle manure proved in yield enhancement in both soybean and groundnut plots when compared to sole application of cattle manure in both locations.

On the legume residual effect on subsequent maize crop, results showed significant difference between inoculated plots and the control in some yield parameters (grain yield, harvest index and stover yield) in soybean and groundnut plots in both Sudan and Guinea savanna agro-ecological zones. Significant difference was observed in N, P and K uptake in soybean and groundnut in both study locations.

Economic analysis also revealed that application of inoculants was profitable under soybean but not in groundnut.

6.2 Conclusion

i. The use of Legume fix and Alosca enhanced the growth and yield of soybean positively in both the Sudan and Guinea savanna agro-ecological zones. Legume fix performed generally better than Alosca. Combination of Alosca and cattle manure produced the highest grain yield of soybean than sole Alosca. Furthermore, the use of rhizobia inoculants proved economically viable. This confirmed the hypothesis of the study that inoculation of soybean with rhizobium inoculants will enhance growth and yield of soybean. The combined application of Biofix or Histick with cattle manure gave significantly higher nodule numbers than sole Biofix and Histick inoculants on groundnut field. Groundnut yield was not significantly affected by Histick and Biofix in both study locations.

ii. Legume fix and Alosca inoculants gave the highest fixed N in soybean field in both the Sudan and Guinea savanna agro-ecological zones. However, in the groundnut field in the Sudan savanna, higher N fixation was obtained under Biofix followed by Histick. Nitrogen fixation was suppressed under 50 kg N ha^{-1} treated plots in both legumes in the Sudan and Guinea savanna agro–ecologies. Inoculation with appropriate rhizobia and combination of rhizobia and quality cattle manure enhanced nitrogen, phosphorus and potassium uptake in both study locations.

iii. Residual effect experiment during the second year showed that in the groundnut field in the Sudan savanna, the 50 kg N ha^{-1} fertilized plots produced highest yield in maize even though not significantly different from Histick, cattle manure, Biofix + cattle manure and Biofix. Furthermore, maize stover yield increased as a result of inoculation.

iv. The use of inoculants on soybean resulted in high NPV and MRR, but on groundnut, inoculant use proved otherwise (low NPV and negative MRR values).

6.3 Recommendations

The findings of this study were not exempted from some limitations due to time frame and limited number of varieties for the trial. However, it leads to these recommendations:

i. farmers should be encouraged in the study areas to use inoculants in combination with cattle manure (4 tonnes ha^{-1}) for legumes production and ii. policy makers should assist smallholder farmers to ensure adequate supply of quality inoculants at subsidized prices.

iii. further studies should be conducted in multiple locations and multi varietal trials to investigate inoculation response in legumes in the agro-ecological zones.

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APPENDICES

Appendix 1: Physico-chemical properties of experimental sites (final 2015)

Parameters (year 1)	**Sudan savanna (Bagauda location)**	**Guinea savanna (Gubi location**
pH (1:2.5 H_2O)	6.10	6.30
Organic carbon (%)	0.53	0.58
Total nitrogen (%)	0.07	0.14
Available P (mg kg^{-1})	6.13	7.35
Exchangeable cations		
Ca^{2+}	8.87	4.32
Mg^{2+}	2.56	0.64
K^{+}	0.10	0.23
Na^{+}	0.15	0.33
Total exchangeable acidity		
(Al^{3+} +H^{+}) (cmol $_{(+)}$ kg^{-1})	0.60	0.60
Sand (%)	72	74
Silt (%)	18	16
Clay (%)	10	10
Texture	Sandy loam	Sandy loam
CEC	12.40	6.80

Appendix

2: Physico-chemical properties of experimental sites (initial 2016)

Parameters (year 1)	**Sudan savanna (Bagauda location)**	**Guinea savanna (Gubi location)**
pH (1:2.5 H_2O)	5.61	5.80
Organic carbon (%)	0.41	0.47
Total nitrogen (%)	0.14	0.14
Available P (mg kg^{-1})	2.64	4.53
Exchangeable cations		
Ca^{2+}	1.64	1.40
Mg^{2+}	0.39	0.37
K^{+}	0.10	0.15
Na^{+}	0.20	0.23
Total exchangeable acidity		
(Al^{3+} +H^{+}) (cmol $_{(+)}$ kg^{-1})	0.40	0.40
CEC	3.02	2.60
Sand (%)	66	74
Silt (%)	26	18
Clay (%)	8	8
Texture	Sandy loam	Sandy loam

3: Marginal Rate of Return (MRR) soybean in the Sudan savanna

Treatments		**Variable cost ($ ha^{-1})**	**Net benefit ($ ha^{-1})**	**Marginal cost ($ ha^{-1})**	**Marginal net benefit ($ ha^{-1})**	**MRR**
Inoculants	LF AL	104	879	32	344	1077%
		102	689	30	154	514%
Fertilizer	N	108	520	36	- 14	-40%
	CM	136	505	64	- 30	-46%
Inoculant+ C M	LF CM AL	168	656	96	121	127%
	CM	166	868	94	333	354%
	C	72	535	0	0	0%

Appendix

Appendix 4: Marginal Rate of Return (MRR) soybean in the Guinea savanna

	Treatments	Variable cost ($ ha^{-1})	Net benefit ($ ha^{-1})	Marginal cost ($ ha^{-1})	Marginal net benefit ($ ha^{-1})	MRR
	LF AL	104	862	32	293	917%
Inoculants		102	677	30	108	361%
Fertilizer	N	108	609	36	41	114%
	CM	136	719	64	151	235%
Inoculant+	LF CM AL	168	737	96	168	175%
CM	CM	166	644	94	75	80%
	C	72	568	0	0	0%

5: Marginal Rate of Return (MRR) groundnut in the Sudan savanna

	Treatments	Variable cost ($ ha^{-1})	Net benefit ($ ha^{-1})	Marginal cost ($ ha^{-1})	Marginal net benefit ($ ha^{-1})	MRR
	HS	102	304	30	-119	-397%
Inoculants	BF	88	367	16	-57	-353%
Fertilizer	N	108	193	36	-230	-640%
	CM	136	169	64	-255	-398%
Inoculant+	HS CM	166	168	94	-256	-271%
CM	BF CM	152	248	80	-176	-219%
	C	72	424	0	0	0%

Appendix

Appendix 6: Marginal Rate of Return (MRR) groundnut in the Guinea savanna

	Treatments	Variable cost ($ ha^{-1})	Net benefit ($ ha^{-1})	Marginal cost ($ ha^{-1})	Marginal net benefit ($ ha^{-1})	MRR
Inoculants	HS	102	468	30	-63	-211%
	BF	88	442	16	-90	-561%
Fertilizer	N	108	390	36	-141	-393%
	CM	136	366	64	-166	-259%
Inoculant+	HS CM	166	356	94	-176	-187%
CM	BF CM	152	379	80	-152	-190%
	C	72	532	0	0	0%

7: Monthly cumulative rainfall distribution in the Sudan and Guinea savannas in 2015 cropping season