RESPONSE OF GROUNDNUT (A*rachis hypogaea* L.) TO RHIZOBIA INOCULATION, NITROGEN AND PHOSPHORUS FERTILIZERS ON AN ALFISOL IN THE NORTHERN GUINEA SAVANNA OF NIGERIA

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OCTOBER, 2017

DECLARATION

I declare that the work in this dissertation entitled 'RESPONSE OF GROUNDNUT (A*rachis hypogaea* L.) TO RHIZOBIA INOCULATION, NITROGEN AND PHOSPHORUS FERTILIZERS ON AN ALFISOL IN THE NORTHERN GUINEA SAVANNA OF NIGERIA'has been carried out by me in the Department of Soil Science. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any University.

Martha Timothy Bulus

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Name of student	**Signature**	**Date**

CERTIFICATION

This dissertation entitled 'RESPONSE OF GROUNDNUT (A*rachis hypogaea* L.) TO RHIZOBIA INOCULATION, NITROGEN AND PHOSPHORUS FERTILIZERS ON AN ALFISOL IN THE NORTHERN GUINEA SAVANNA OF NIGERIA' by Martha Timothy BULUS meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello university, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to my Lord and savior JESUS CHRIST who saved me and gave me hope not only in this life but also in the life to come.

ACKNOWLEDGEMENT

All glory and honour be unto God Almighty for giving me life, privilege and the strength to start and accomplish this task. I am what I am today only by His grace. Thank you Lord.

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I pray that the Good Lord will reward you all.

ABSTRACT

Groundnut (*Arachis hypogeaa L.*) productivity in Nigeria has remained low over the years due to the inherent low fertility status of the soils of the savanna, a region where it is mostly grown by small holder farmers with limited imputs. Groundnut can fix atmospheric nitrogen through symbiotic association with native rhizobia but unfortunately, the amount of N_2 fixed is usually not enough due to the presence of ineffective or low numbers of native rhizobia. This study was designed to investigate the comparative response of groundnut to rhizobium inoculation and nitrogen fertilizer with or without phosphorus fertilizer on an Alfisol in the Northern Guinea Savannah of Nigeria. Soil samples were collected from a P-deficient plot on the Institute for Agricultural research/ Faculty of Agriculture (IAR/FOA) experimental farms located in Samaru Zaria and used to conduct two screen house trials using the groundnut genotype "SAMNUT 24" as the test crop. The first experiment consisted of six (6) inoculants (four indigenous rhizobial strains namely: SNN 343, KBU 26, SBG 234, SAMFIX 703 one commercial inoculant (HISTICK) and a reference strain (NC 92) alone, the inoculants each combined with 20 kg N ha^{-1} mineral nitrogen as starter dose, and mineral nitrogen alone at 0, 20, or 40 kg N ha^{-1}, adding up to fifteen treatments all termed as nitrogen (N) sources. The second factor was phosphorus at three rates (0, 30 or 60 kg $_2O_5$ ha^{-1}) applied as single super phosphate. The N sources (SNN 343, KBU 26, SBG 234, SAMFIX 703, NC 92, HISTICK, SNN 343 +20N, KBU 26 +20N, SBG 234 +20N, SAMFIX 703 +20N, NC 92 +20N, HISTICK +20 N, 0 kg N ha^{-1}, 20 kg N ha^{-1} and 40 kg N ha^{-1}) and P rates (0 kg P_2O_5 ha^{-1}, 30 kg P_2O_5 ha^{-1} and 60 kg P_2O_5 $ha^{-}1$) were factorially combined (fifteen x three) to give a total of forty five (45) treatments and were laid down in a Randomized Complete Block Design replicated three times. The second trial was set up to assess the residual benefit of rhizobium inoculation on following crops. Soils previously inoculated with SNN 343, KBU 26, SBG 234, SAMFIX 703, NC 92 and HISTICK and a control, were combined factorially with three levels of P (0, 30 or 60 kg P_2O_5 ha^{-1}) to give a total of twenty one (21) treatments. The RCBD was also used with three replications. A non- nodulating groundnut variety (ICGL 5) was included as a reference crop to estimate the amount of biological nitrogen fixed. In the first trial, the use of mineral nitrogen either alone or in combination with rhizobium inoculants suppressed nodulation. Addition of 20 kg mineral nitrogen per hectare to rhizobium inoculation as starter dose reduced nodule number by an average of 12.2 % but increased groundnut shoot dry weight by an average of 9.5 % and 9.8 % compared to inoculation alone and control respectively. The use of this starter dose of mineral nitrogen also increased the amount N_2 fixed by 58.4 % compared to inoculation alone and by 51.7 % compared to the control. A similar trend was also observed for % Ndfa. The application of P at 60 kg P_2O_5 ha^{-1} gave rise to significant increases in all the parameters measured. However, P uptake efficiency and P agronomic efficiency decreased when P rate increased from 30 kg P_2O_5/ha to 60 kg P_2O_5 ha^{-1}. Inoculation alone increased phosphorus uptake efficiency by 29.3 % while addition of starter nitrogen at 20 kg per hectare gave an increase of 35.4 % compared to the control. In the second trial, the residual effects of inoculation failed to produce a significant increase in most of the parameters measured. On average, shoot dry matter declined by 46.3 %, nodule number by 45 %, nodule dry weight by 53 % across all treatments when plants depended on the residual effect of the previous inoculation. Similarly the amount of nitrogen fixed decreased by an average of 34 % across all treatments except KBU 26 which indicates lack of persistence of the rhizobium strains and the

need for inoculation with each sowing. KBU 26 amongst all the inoculants increased the amount of nitrogen fixed and % Ndfa by 18.1 % and 52.8 % respectively compared to the first trial indicating that the strains persisted in the soil. The result of this study shows the importance of the use of rhizobium inoculants coupled with a starter dose of mineral nitrogen at 20 kg N/ha to enhance BNF. It also shows that the application of P at 60 kg P_2O_5 ha^{-1} greatly enhanced yield components and BNF indicating the beneficial role of phosphorus fertilizer on the growth and productivity of groundnut even though P supplied at 30 kg P_2O_5 ha^{-1} was more efficiently used by the crop than at 60 kg P_2O_5 ha^{-1}. The residual effect of KBU 26 amongst all the inoculants increased the amount of nitrogen fixed and % Ndfa indicating that the strains persisted in the soil. This potential when fully assessed and harnessed may constitute an evident advantage over the use of inorganic nitrogen fertilizer which has to be applied frequently for consistent high yields.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Groundnuts (*Arachis hypogaea* L) also known as peanuts, is a leguminous crop, a member of the genus *Arachis* in the family Leguminosae. It is widely grown in the tropics and subtropics due to its nutritional and economic importance. Groundnut is the most widely grown major legume worldwide cultivated in 118 countries and occupies more than 22.6 million ha that produce about 36.4 million MT, with average yield of about 1600 kg ha^{-1} (Abate *et al*., 2012). Groundnut seeds (kernels) contain 40 -50 % fat, 20 - 50 % protein and 10 - 20 % carbohydrates (ICRISAT, 2003). Groundnut seeds are nutritional source of vitamin E, niacin, calcium, phosphorus, magnesium, zinc, iron, ribloflavin, thiamine and potassium (Kumar and Shankar, 2013). In most of the developing countries it provides high-quality cooking oil and is an important source of protein for both human and animal diet and also provides much needed foreign exchange by exporting the kernels and cake. The uses of groundnut plant therefore, make it an excellent cash crop for domestic markets as well as for foreign trade in several developing and developed countries (FAO, 2002).

In Africa, groundnut is grown mainly in Nigeria, Sudan, Senegal, Chad, Ghana, Congo, and Niger. Average productivity is 1720 kg ha^{-1} in Nigeria which is poor when compared to the USA and other developed countries where it is close to 3500 kg ha^{-1} (Vara-Prasad *et al*., 2009). The crop is grown mostly by smallholder farmers under rainfed conditions with limited inputs (Samson, 2012).

Majority of the soils of the Guinea savanna of Nigeria are inherently low in fertility especially organic matter, phosphorus and nitrogen (Odunze and Kureh, 2009; Oluwasemire and Alabi, 2004). To address this challenge, farmers in the northern Guinea savanna use

strategies such as application of organic and inorganic fertilizers (Samson, 2012). The use of inorganic fertilizers is effective but costly (Obisesan *et al*., 2013); this therefore, leads to application at sub- optimal level that is, below the recommended rate (Olawale *et al*., 2009). On the other hand, organic fertilizers, such as crop residues and animal manures are bulky. They contain relatively low concentration of nutrients and handling them is laborious. ICRISAT (1995) recommended an application of 10 -12 t ha^{-1} of chicken manure or 20 t ha^{-1} of well decomposed farm yard- manure for groundnut production. Wamba *et al*. (2012) gave the nutrient content of poultry manure as 21.76 N g kg^{-1}, 8.74 P g kg^{-1} and 11.22 K g $kg^{-1.}$ This however depends on the handling conditions. Much of the N in manure may be lost to the air if they are allowed to dry out or stored for a long time.

Unlike cereals, legumes are able to supplement their nitrogen (N) demand and contribute to soil N through various processes. Legumes fix atmospheric nitrogen (N_2) in the soil through its symbiotic relationship with N- fixing bacteria. Legumes also add nitrogen to the soil through falling leaf litter and, to a lesser extent by decaying roots and root nodules below-ground, and thus they have great potential for restoring degraded soils. The organic matter produced by legumes is generally rich in nitrogen and of good quality, meaning that it decomposes quickly and is a good source of nitrogen for other plants (Giller, 2010). The ability of legumes to fix N_2 allows farmers to grow them with minimal inputs of N fertilizer (Lupwayi *et al*., 2011). Therefore, farmers usually intercrop non-legumes with leguminous crops including groundnut having considered legumes to stabilize crop yields and also serves as a source of income and protein for their families. In the northern savanna of Nigeria, the bulk of groundnut is produced by small scale farmers using traditional system of mixed cropping with maize, millet and sorghum. (Samson, 2012).

Nitrogen is considered as the most limiting plant nutrient for crop production in West Africa (Sangakkara *et al*., 2003). Despite its abundance in the atmosphere, plants are unable to use it

directly because it is present in an inert form (N_2) and the nitrogen in the soil is lost through microbial dinitrification, soil erosion, leaching, chemical volatilization, removal of nitrogen containing crop residues from the field. As earlier mentioned, groundnuts like other legumes play a primary role of fixing atmospheric N through their symbiotic relationship with *Rhizobium* spp., usually associated with the host's root system. This contributes nitrogenous compounds to the soil, either directly, by nodule excretion, or indirectly, by decomposition of root nodules and tissues (Giller, 2003). Biological Nitrogen Fixation (BNF) is an inexpensive, renewable resource option for smallholder farmers, permitting them to redirect limited farm investment toward other pressing household needs.

One of the ways to increase biological nitrogen fixation is by inoculating legume seeds with rhizobium inoculants. Research has also shown that legumes grow best if there is some mineral N available as nodules form and a small amount of starter nitrogen (10 to 30 kg ha^{-1}) at planting may increase total BNF over the crop's lifetime (Woomer, 2010). This starter dose of nitrogen is necessary due to the lag period between rhizobium colonization and the onset of nodule functioning. The starter dose however, increases yield only on soils that are extremely deficient in nitrogen and where crop yield potential is high (Kucey, 1989; Woomer, 2010).

Phosphorus (P), in addition to its role in crop nutrition is an essential element for rhizobium bacteria to convert atmospheric N into ammonium (NH_4^+) and ammonia (NH_3) which is usable by plants. Phosphorus influences nodule development through its basic functions in plants as an energy source. Furthermore, phosphorus plays a major role in many plant processes such as storage and transfer of energy, stimulation of root growth, flowering, fruiting and seed formation, nodule development and N_2 fixation (McLaren and Cameron, 1996).

One of the frequently asked questions with respect to rhizobial inoculation is the frequency at which legume crops should be inoculated (Drew *et al*., 2014). The answer to this question is connected to the ability of the rhizobial strain to persist in the soil. Rhizobia, once introduced to the soil, will be affected by the same factors that affect native rhizobia: vegetation, soil moisture, pH, and temperature. Where soil conditions are favourable, rhizobia are able to survive in the soil for many years, even in the absence of their legume host (Drew *et al*., 2014). Therefore the inoculation of legume seeds with reasonably large population of effective and persistent rhizobial strains constitutes an evident advantage over inorganic nitrogen fertilizer which has to be applied frequently for consistent high yields (Ojo and Fagede, 2002).

1.2 Statement of Research Problem

The soils of the dry savannas of Nigeria where groundnut cultivation is most popular are generally sandy, poor in terms of nutrient content arising from low organic carbon, low nitrogen and phosphorus content (Ajeigbe *et al*., 2015). Farmers use strategies such as application of organic and inorganic fertilizers to address this challenge. These strategies have their own limitations which include the costly nature of inorganic fertilizers leading to application at sub-optimal levels (Obisesan *et al*., 2013; Olawale *et al*., 2009), while the organic fertilizers are not available in the right quantity and quality to meet the crop's requirement. Rhizobial inoculants have been used to address the problems of soil fertility and inadequate fertilizer application in grain legume production and have been found to be a cheaper and usually more effective agronomic practice for ensuring adequate N nutrition of legumes, compared with the application of N fertilizer (Paynel *et al*., 2008). However, some results obtained from inoculation studies in comparison with the use of nitrogen fertilizers reveal that biomass production and yields were increased significantly by mineral N application over inoculation (Ahmed *et al*., 2014; Abubakar, 2015). The successful use of

rhizobial inoculants as a substitute for nitrogen fertilizers for groundnut production depends on the ability to screen and select rhizobia strains that will produce higher yields than the use of nitrogen fertilizers.

Differences have been observed in the response of groundnut to rhizobial inoculation and Phosphorus fertilizers and this may be linked to the findings of Cassman *et al*. (1981) who reported that rhizobium strains differ markedly in external P requirements for growth which seem to affect their effectiveness. Assessing the performance of different strains of rhizobia under different phosphorus levels is important in determining the strains of P- efficient rhizobia best suited for the P-deficient Alfisols of the northern Guinea savanna of Nigeria.

1.3 Justification

Woomer (2010) reported that nitrogen fixation in groundnut is about 150 kg N ha^{-1} which offers strong residual benefits to following crops. However results obtained in Nigeria with respect to the amount of nitrogen fixed by groundnut gave much lower range of values. Yakubu *et al*. (2010) reported that groundnut inoculated with rhizobia fixed 27.19 kg N ha^{-1}. There is a need for research to screen bacterial strains and traits that are useful and necessary for different environmental conditions and plants so that optimal bacterial strains that will improve nitrogen fixation and yields of groundnuts can be selected.

Biodiversity and economic potential of African rhizobia is largely unexplored, yet potential exists for native rhizobia to outperform exotic commercial strains. A study conducted by Yusuf *et al*. (2011) in the northern Guinea savanna of Nigeria showed that the commercial rhizobial inoculants used gave no increase in groundnut yield. The rhizobium strains in the commercial inoculants used were not only ineffective but were inferior to the indigenous soil population. Therefore, it is vital to assess the effectiveness and competitiveness of indigenous strains against commercial strains in order to identify potentially useful inoculants strains. There is also a need for further assessment of existing commercial inoculants to assist in the

selection of rhizobia with specific symbiotic and competitive attributes suited to a range of soil environments.

Addition of starter dose of N at planting according to Woomer (2010) has the potential to increase total BNF over the crop's lifetime and this may be useful especially on the soils of the northern Guinea savannah of Nigeria (Alfisols) which are highly deficient in Nitrogen.

Several researches carried out confirmed the importance of phosphorus for groundnut production (Kamara *et al*., 2011, Abdul-latif, 2013, Nwokwu, 2011, Amba *et al*., 2013). However, the recommended rates vary between 20 - 45 Kg P ha^{-1} depending on locations and soil types, hence the need to determine the adequate rate of phosphorus that will improve groundnut yields and in the case of inoculation, to determine the effectiveness of different rhizobial strains under different P levels in the northern Guinea savanna of Nigeria.

1.4 Aim and Objectives

The aim of this study is to improve symbiotic biological nitrogen fixation in groundnut on an Alfisol in the northern Guinea savanna of Nigeria through the use of rhizobial inoculants, nitrogen and phosphorus fertilizers. The specific objectives are:

1. To determine the effectiveness of groundnut rhizobia vis-a-vis nitrogen fertilizer and the effect of phosphorus fertilizer on the yield components and biological nitrogen fixation in groundnut.

2. To evaluate the effect of rhizobial inoculation vis-à-vis nitrogen fertilizer and the effect of phosphorus on phosphorus use efficiency in groundnut.

3. To evaluate the residual benefit of introduced rhizobia and phosphorus fertilizer on biological nitrogen fixation and yield components of groundnut.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of Groundnuts

Groundnut (*Arachis hypogaea* L.) is from the genus *Arachis* which belongs to family leguminosae, subfamily Papilionaceae, tribe Aeschynomeneae, subtribe Stylosanthinae. This genus is morphologically well defined and distinguished from other genera by having a peg and geocarpic reproductive growth. The genus *Arachis* has more than 70 wild species, of which only *Arachis hypogaea* L. is domesticated and commonly cultivated (Vara-Prasad *et al*., 2009). It is grown widely in the world due to its nutritional and economic importance. In 2011, total world production was estimated at 38.6 million tons with average yields of 1.8 tons per hectare (FAOSTAT, 2011). Developing countries account for 97 % of the world's groundnut area and 94 % of the total production.

Groundnut is an important legume used for oilseed, food and animal feed all over the world. For people in many developing countries, groundnuts are the principal source of digestible protein (25 to 34 %) (FAO, 2002). Groundnut is used to make oils and it is second largest source of vegetable oils next to soybeans. The oil can be used for cooking, as a base for confectioneries and to make peanut butter which is used as spread for bread or biscuits, in cookies, sandwiches, candies and frostings or icings (Savage and Keenan, 1994). Groundnut cake and haulms (straw stems) are used as livestock feed especially in dry season in the semi-arid tropics. Leaves and stems are particularly palatable to livestock because foliage remains green through seed ripening (Woomer, 2010). Groundnut is a high value crop that can be marketed with little processing; however, it is extremely versatile and can be used in a wide range of products. It is a good source of calcium, iron and vitamins (FAO, 2002).

Groundnut is utilized in various forms including roasted, boiled, raw, ground or paste. In Nigeria, a large number of food products are prepared from groundnut such as boiled nut, roasted nut, 'Kunun gyeda'(a porridge prepared from groundnut popular among Hausa speaking people of Nigeria), groundnut soup, the by-product obtained after oil is extracted from groundnut paste is processed into a cake popularly called "kuli kuli'(Abdulrahman *et al*., 2014).

As a legume, groundnut improves soil fertility by fixing nitrogen and thereby increasing productivity of the semiarid cereal cropping systems. In many countries including Nigeria, it is used as a component of crop rotation and such rotations with cereals give higher grain yields and better nitrogen uptake by the following plants than continuous cereal cultivation (Yusuf *et al*., 2009).

2.2 Groundnut Production in Nigeria

Groundnut in Nigeria, as in other major producing areas, is largely a smallholder crop, grown under rainfall conditions in semi-arid areas. Nigeria is the largest groundnut producing country in West Africa, accounting for 51 % of production in the region. The country contributes 10 % of total global production and 39 % that of Africa (Ajeigbe *et al*., 2015). Groundnut is grown in the majority of the states of Nigeria. Kano and Niger states account for about 19.6 % and 10.7 %, respectively of the total area for groundnut, followed by Kaduna 9.4 %, Benue, Zamfara, Taraba, Bauchi, Borno, Katsina and Nasarawa. These top 10 producing states account for nearly 80 % of the total area of groundnut for Nigeria (ICRISAT, 2011).

Traditional commercial groundnut producing areas encompass the Sahel, Sudan and derived savanna, northern Guinea and most parts of the southern Guinea vegetation zone (Ajeigbe *et al*., 2015). The Guinea Savanna zone (divided into the southern Guinea savanna and northern

Guinea savanna) is located in the middle of the country, extends southwards to southern Nigeria and pushes northward beyond Zaria. It covers an area that has 100 – 150 cm of annual rainfall and where the wet season lasts for 6 - 8 months (Aregheore, 2011). The soils are classified under Alfisols, Inceptisols, Entisols and Ultisols (Vanlauwe *et al.*, 2002).

The soils of the dry savannas where groundnut cultivation is most popular are mostly Alfisols and are generally sandy, poor both in terms of nutrient content and water-holding capacity, and prone to erosion by wind and water. With the increase in demand for agricultural products arising from the ever increasing human population, farmers are forced to crop the same piece of land year after year, without allowing for any fallow period that would encourage soil restoration (Ajeigbe *et al.*, 2015). According to Odunze *et al.*, (1996), the subhumid zone Alfisols of Nigeria are fragile, dominated by low-activity clays, and have inherently moderate to low fertility status. Following intensive use of the soils for crop and livestock production, the soils are being degraded by soil erosion, drought, complete removal of both crop and residues, improper management practices, and overgrazing. Most soils of the northern guinea savanna (Alfisols) have been reported to be slightly acidic with low values of organic carbon (< 5 g kg^{-1} soil), total N (<1 g kg^{-1} soil) and available phosphorus (<10 mg/kg soil) (Oyinlola *et al.*, 2010; Yakubu *et al.*, 2010).

2.3 Nitrogen and Crop Production

In view of the low levels of available nitrogen in many soils, and the high nitrogen (N) requirements of crops, N is the most important nutrient in agriculture. N is the most abundant mineral nutrient in plants, constituting about 2 – 4 % of plant dry matter. It is a part of the chlorophyll (the green pigment in leaves) and is an essential constituent of all proteins. It is responsible for the dark green colour of stem and leaves, vigorous growth, branching/tillering, leaf production, size enlargement, and yield formation (FAO, 2006). N is also required by all living organisms for the synthesis of proteins, nucleic acids and other

nitrogen-containing compounds (Burdass, 2002). Crops and pastures require N in relatively large amounts and because of this, it can easily become deficient. N deficiency in plants results in marked reduction in growth rate. Plants deficient in N usually have short and spindly appearance, poor tillering, and small leaf area. In a case of severe deficiency, leaves turn brown and die. As a result, crop yield and protein content are reduced (FAO, 2006).

Nitrate and ammonium are the major sources of nitrogen for plants. Under normal, aerated conditions in soils, nitrate is the main source of nitrogen. Nitrate is readily mobile in plants and can be stored in vacuoles, but for nitrate to be used in the synthesis of proteins and other organic compounds in plants, it must be reduced to ammonium (Barker and Bryson, 2007). The earth's atmosphere contains almost 80 % nitrogen gas. It cannot be used in this form by most living organisms until it has been fixed, that is reduced (combined with hydrogen), to ammonia. Nitrogen can be fixed in three ways:

(1) Atmospheric fixation - this occurs spontaneously due to lightning; a small amount only is fixed this way.

(2). Industrial fixation - the Haber process, which is very energy inefficient, is used to make nitrogen fertilizers.

(3). Biological fixation - nitrogen-fixing bacteria fix 60% of nitrogen gas. (Burdass 2002).

2.4 Nitrogen Fertilizers

Mineral fertilizers supplies about 50 % of the total N required for global food production. Worldwide mineral fertilizer nutrient use is expected to increase from 165 million tonnes in 2009/2010, to 175 million tonnes in 2015 and to 199 million tonnes in 2030 (FAO, 2005). All N in fertilizers originates from the nitrogen gas (N_2) in the atmosphere, which contains 79 % N by volume, synthesized by the Haber-Bosch reaction which combines the very stable

molecule of atmospheric N_2 with hydrogen, e.g. from natural gas, under a pressure of 200 atmospheres at 550 °C (FAO, 2006).

Of all the inputs, N fertilizer additions have had the single largest effect on crop yields and also have contributed most to environmental concerns, discussions and problems. Added N that is not absorbed by the crop or immobilized by the soil can be lost from the soil by various means, these include: leaching of nitrate to groundwater thereby contaminating the ground water, loss through soil erosion and surface runoff, and volatilization of ammonia into the atmosphere and as nitrous oxide (NO) to the atmosphere resulting from denitrification of nitrate by soil organisms. Further, cumulative application of ammonia-based fertilizers often leads to soil acidification, which in turn requires redress through the application of lime or dolomite to forestall soil degradation (Gruhn *et al.*, 1995). The quantity of nitrogen needed for agriculture is projected to increase in the next decades, which could lead to greater environmental pollution. Lesser dependence on fertilizer N and more attention to practices that favor biological nitrogen fixation (BNF) in farming systems will benefit both agriculture and the environment (Olivares *et al*., 2013).

2.5 Biological Nitrogen Fixation

Biological nitrogen fixation (BNF) is the process whereby atmospheric nitrogen (N=N) is reduced to ammonia in the presence of nitrogenase. Nitrogenase is a biological catalyst found naturally only in certain microorganisms such as the symbiotic *Rhizobium* and *Frankia,* or the free-living *Azospirillum* and *Azotobacter.* Biological nitrogen fixation is brought about by free-living soil microorganisms and by symbiotic associations of microorganisms with higher plants such as the legume-rhizobium symbiosis (Molungoy, 1995). The reduction of nitrogen gas to ammonia is energy intensive. It requires 16 molecules of ATP and a complex set of enzymes to break the nitrogen bonds so that it can combine with hydrogen (Burdass, 2002). Its reduction can be written as:

$N_2 + 8H_2 + 16\ ATP \longrightarrow 2NH_3 + 2H_2 + 16ADP + 16Pi$ …….. (2.1)

(Cheng, 2008).

2.5.1 Symbiotic nitrogen fixation

In agricultural settings, perhaps 80 % of biologically fixed N_2 comes from symbiosis involving leguminous plants and α-proteobacteria, order Rhizobiales, family Rhizobiaceae, including species of *Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium* and *Mesorhizobium* (Willems and Collins, 1993; Gaje, 2004). The process of symbiotic nitrogen fixation involves a N_2-fixing prokaryote and a photosynthetic host. The bacteria obtain food (Carbon C, which is fixed from atmospheric CO_2 during photosynthesis) from the host plant and the host plant benefits from the nitrogen fixed. This relationship is called a symbiosis, with both partners deriving benefits from the association. The bacteria respond to and invade the roots of the host plant, and the host responds by forming a nodule that surrounds the bacteria, and in which nitrogen is fixed (Foth, 1990, Gaje, 2004)

Legumes are the most widely recognized N_2-fixing symbioses and the most important N_2-fixing agents in agricultural systems because of their importance as a food source (Gaje, 2004; Paul, 2007). Many non-legume plant species have root nodules and also fix nitrogen symbiotically. This means that symbiotic fixation of nitrogen is important in natural ecosystems by both legume and non-leguminous plants. Red alder (alnus) is an example of a non-legume capable of symbiotic nitrogen fixation (Foth, 1990).

2.5.2 Mechanisms of symbiotic nitrogen fixation in legumes

Symbiotic Nitrogen fixation in legumes is an interactive process involving the eukaryotic host legume and the prokaryotic rhizobium. The bacterium rhizobium is one of the most studied symbiotic nitrogen fixing bacteria because it nodulates legumes which are environmentally significant in soil N fertility management of cultivated lands (Prevost and Antoun, 2007).

Nodulation of legumes by rhizobia involves a complex process of biochemical recognition, infection, nodule formation, N transformation and senescence. First, rhizobia multiply near the host roots, the two exchange biological signals and rhizobia attach to the root (Burdass, 2002). The molecular dialogue between the two symbionts, the legume and Rhizobium, starts when flavonoids exuded by the legume root interact with the bacterial protein NodD i.e the Rhizobia produced lipo-chitin oligosaccharide signal molecules, called LCOs or Nod factors (Gaje, 2004). Different legumes secrete different types of flavonoids, and only certain rhizobia will respond to the specific flavonoid signals by producing rhizobia specific LCOs, thereby establishing a checkpoint for defining the specificity of the relationship between the legume host and the infecting rhizobia (Wang *et al*., 2012). This flavonoid-NodD interaction provokes the transcriptional activation of rhizobial nodulation genes. Expression of nodulation genes ultimately results in the production and secretion of Nod factors which in turn induce hair curling and nodule meristem initiation in the plant root (Gage, 2004). The root infection process is under the genetic control of both rhizobial and plant genes, and a high degree of genetic compatibility between partners is essential for the development of nodules containing highly effective rhizobia (Drew *et al.*, 2014).

The infection thread grows through the root hair cells and penetrates other root cells nearby often with branching of the thread. The bacteria multiply within the expanding network of tubes, continuing to produce nod factors which stimulate the root cells to proliferate, eventually forming a root nodule. Each root nodule is packed with thousands of living Rhizobium bacteria, most of which are in the misshapen form known as bacteroids (Burdass, 2002). As the nodule forms, the host plant provides energy in the form of sugars from photosynthesis (Woomer, 2010).

For nitrogen fixation to occur, two unique compounds are produced in the nodules: Nitrogenase and Legheamoglobin. Nitrogenase is produced by the rhizobia. It is the enzyme that facilitates the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3), i.e. N_2 fixation. The enzyme requires molybdenum (Mo) to function optimally, which is why this microelement is often added as a fertilizer when legumes are sown. Leghaemoglobin is produced by the plant. This compound provides the characteristic pink/red colour of healthy nodules, and is essential for nitrogen fixation to occur (Drew *et al*., 2014). Like human haemoglobin, leghaemoglobin fixes O_2 which helps in realizing the low oxygen tension needed for nitrogenase to function well since it is an oxygen sensitive enzyme (Mulongoy, 1995).

Nitrogen fixation requires a source of electrons. Sources of electrons for the nitrogenase activity vary with the organism. They are all small proteins and highly reductive molecules such as flavodoxin, ferredoxin, nicotinamide, or ademine dinucleotide (phosphate) (Shamseldin, 2013).

2.5.3 Factors that affect biological nitrogen fixation

Virtually all agriculturally important legumes can fix nitrogen in symbiosis with their rhizobial counterparts. However, the contribution of BNF to legume production may have declined over the years, owing to a number of factors, such as the population of the indigenous/ native rhizobia, availability of N fertilizers (particularly for farmers in developed countries), edaphic and environmental constraints (temperature, water availability, soil pH, etc.), the use of legume varieties with limited ability to fix N_2 and diseases of legumes may affect nodulation and/or N_2 fixation, obscuring the beneficial effects which are being sought (Peoples *et al*., 1989, Graham *et al*., 2004).

2.5.3.1 Population of the indigenous/ native rhizobia.

The nature of soil rhizobial populations may affect the N_2 fixation potential of legumes. Native rhizobial populations can be defined in terms of number and effectiveness for a particular host, and these characteristics determine whether inoculation will enhance N_2 fixation. First, the number of available invasive rhizobia may be insufficient to nodulate the host adequately. Second, the average effectiveness of the population may be inadequate to support the host's fixed N_2 requirements. When one or both conditions are present, we might reasonably expect that successful inoculation with an effective rhizobium strain would enhance N_2 fixation (Singleton and Tavares, 1986). Therefore, the presence of background rhizobia is not always sufficient in itself, to ensure optimal N_2-fixing capacity in the host legume as the effectiveness of strains to fix N within naturalised populations can vary considerably, (Slattery and Pearce, 2002).

The most probable number (MPN) plant-infection technique has been used for many years for the enumeration of rhizobia in soils. The MPN procedure relies upon the pattern of positive or negative nodulation responses of host plants inoculated with consecutive series of dilutions of sample (soil, inoculants) containing rhizobia (Prevost and Autoun, 2007). Nodulation indicates that infective rhizobia were present in the inoculum; no nodulation indicates they were absent. The sum of nodulated growth units or the pattern of nodulated and non nodulated units is then used to derive a population estimate, the MPN (Woomer *et al*., 1988). The MPN relies on the assumption that one viable rhizobial cell can give rise to a nodule but it is implicit that this cell must multiply after the inoculation event to produce sufficient nod- factor to begin the nodulation process. For the method to give valid results, all replicates of the last dilution should be negative (Howieson and Dilworth, 2016).

2.5.3.2 Nitrogen availability

Because BNF is energetically expensive, bacteria do not usually fix N_2 in the presence of reactive N sources (Paul, 2007). In a soil of higher mineral-N content, the legume may compensate for poor N_2 fixation by scavenging N from the soil. Although production in this situation may not be impaired, the net result of cropping with a legume deficient in nodulation is an exploitation of N reserves. Soil N fertility is lost and it represents a wasteful use of a legume in a cropping sequence (Peoples *et al*., 1989). Nonetheless, legumes grow best if there is some mineral N available as nodules form and a small amount of starter nitrogen (10 to 30 kg ha^{-1}) at planting may increase total BNF over the crop's life time. The starter dose however, Increases yield only on soils that are extremely deficient in nitrogen and where crop yield potential is high (Woomer, 2010).

2.5.3.3 Environmental constraints

Environmental constraints to BNF include soil acidity and Aluminium (Al) toxicity, particularly in highly weathered tropical soils, temperature, moisture, and several chemical components of the soil such as phosphorus, calcium, Iron and molybdenum content (FAO, 1984, Woomer, 2010).

Every bacterium has its own optimum conditions, under which it grows at its best. For most rhizobia, the optimum temperature range for growth is 28 – 31 °C, and many are unable to grow at 37 °C (Zahran, 1999). Not only do the bacteria themselves have an optimum temperature range, but the processes within them do as well. High soil temperature adversely affects the growth and survival of rhizobia in soils and their symbiotic association with legumes, and prevents nodulation. Soil and root temperatures in tropical and subtropical regions are often in the range of 35 ° to 40 ° C and are detrimental to nodule formation and nitrogen fixation (Vara-Prasad *et al*., 2009). Groundnut-*Bradyrhizobium* symbiosis is

completely inhibited by a soil temperature of 40 ° C. The effects are due not only to the failure of nodulation but also to the inability of nodules to function even if they were formed. Hot temperatures adversely affect the process of infection more than the process of nodule growth. Many aspects of the Rhizobium-legume symbiosis are affected by hot root temperatures, including: growth and survival of rhizobia, formation of root hairs; binding of rhizobial cells to the surface of root hairs; formation of infection threads; structure and development of root nodules; leghemoglobin content of nodules; activity of the nitrogenase enzyme; and the nitrogen concentration and dry matter production of nodulated plants (Vara-Prasad *et al*., 2009). Temperatures of 30° and 35 °C significantly reduce the nitrogenase activity of groundnut root nodules as compared to those at a temperature of 25 °C (Vara-Prasad *et al*., 2009)

Desiccation is also detrimental to the survival of rhizobia. Rhizobial numbers can decline by the end of a dry summer. Soils that experience long dry summers and are subject to higher temperatures may have fewer rhizobia, particularly where clay content is low or other soil stresses are present (Drew *et al*., 2014). Soil moisture deficit has a marked effect on N_2 fixation as nodules initiation and growth are more sensitive to moisture than roots in general. Nodules and N_2 fixation response to water factor depends on the stage of plant development. Water stress during growth has a direct effect on the development of nodules than in other stages and the possibility of recovery is almost impossible (Niste *et al*., 2013). Many legumes are very sensitive to excess water. Nodule development and function are usually more affected than the infection itself, and some effects such as decreased nitrogenase activity may be even more intense than in the case of water deficit. Reduced to zero, the contribution of O_2 to the nodule appears to be the main problem of the effect of waterlogging (Andres *et al*., 2012).

Agricultural soils are either alkaline or acidic. This may have effect on rhizobial growth, survival and subsequent formation of nitrogen-fixing symbiosis with a legume host. The correct soil pH is crucial for the survival of *Rhizobium* spp, and in adverse soil pH environment strains of rhizobia differs in their ability to infect the host plant (Brockwell *et al*., 1995). Rhizobia can be more sensitive to acidic condition than their legume host. Indeed, it is in many cases the inability of the rhizobia to persist and survive under acidic conditions that reduces the effectiveness of the symbiosis. Many agricultural fields are alkaline with an average pH above 7.0 to 8.5. A major problem in alkaline soils is reduced nutrient availability. Alkalinity stress can also retard rhizobium from growing and subsequent establishment of a viable nitrogen-fixing symbiosis with a legume host. Therefore, it makes good sense agriculturally to select rhizobial isolates that are tolerant of alkaline conditions as well as capable of nodulating legumes (Abd-Alla *et al*., 2013).

Salinity stress is an important factor limiting the productivity of leguminous crops. Soil salinity reduces survival and growth of rhizobia in the soil and inhibits rhizobia-legume symbiosis, resulting in lower productivity of legumes. Rhizobia are known to be more salt tolerant than their respective plant partners. Maximal limit of tolerance to salinity is superior in rhizobia as compared to their host plant which frequently constitute the limiting factor in saline soils (Abd-Alla *et al*., 1998).

2.5.3.4 Nutritional constraints

In symbiotic nitrogen fixation, soil nutrient status has a tremendous influence on the symbiosis as well as independent growth and survival of both partners. In some cases, nutrient stresses are indirectly caused by changes in soil matric potential or acidity, which limits the nutrient bioavailability, rather than the lack of presence of nutrients per se (Mohammadi *et al*., 2012).

Iron is required for several key enzymes of the nitrogenase complex as well as for the electron carrier ferredoxin and for some hydrogenases. A particular high iron requirement exists in legumes for the heme component of hemoglobin. Therefore, in legumes iron is required in a greater amount for nodule formation than for host plant growth (Wang *et al*., 2012). In most legumes including groundnut, early nodule development after nodule initiation is most sensitive to Fe-deficiency. In groundnut, Fe-deficiency decreases the number of excisable nodules, nodule mass, number of bacteroids and concentrations of leghemoglobin, nitrogenase activity and nitrogen fixing ability. Fe-deficiency does not limit the growth of rhizosphere populations of peanut *Bradyrhizobium*, and there is no effect on root infection processes or nodule initiation. This suggests that nodule development processes are more sensitive to Fe-deficiency than nodule initiation processes. (Vara-Prasad *et al*., 2009).

Molybdenum is a micronutrient used by plants that form root nodules with nitrogen-fixing bacteria, though plants that do not form nodules also use trace amounts of it in a protein involved with nitrogen metabolism and uptake. Its relevance to N_2 fixation is clear, given that the Mo in 'FeMoCo' cofactor is at the heart of the nitrogen reduction process - at least for most nitrogenases (Allen *et al*., 1999). The symbiotic bacterial enzyme nitrogenase is comprised of two subunits one of which is the MoFe protein directly involved in the reduction of N_2 to NH_3. Supply of molybdenum and Fe to bacteroids is therefore an important process and most likely a key regulatory component in the maintenance of nitrogen fixation in legumes (Kaiser *et al*., 2005). Molybdenum deficiency-induced nitrogen deficiency in legumes relying on N_2 fixation is widespread, particularly in acid mineral soils of the humid and subhumid tropics. There are reports that foliar applications of Mo to grain legumes in field conditions increased levels of N_2 fixation and nodule mass, resulting in higher overall N content and seed yield (Yanni, 1992). It was also reported that a B. japonicum strain deficient

in molybdenum transport showed impaired nitrogen fixation activity when inoculated to soybean roots (Delgado *et al.*, 2006).

2.5.3.5 Biological constraints.

Biotic constraints to yield include insect pests, diseases and weeds (Vara-Prasad *et al.*, 2009). Other limiting biotic factors could be: excessive defoliation of host plant and crop competition. Defoliation (e.g., pruning and lopping) decreases the photosynthetic ability of legumes. It impairs N_2 fixation and can lead to nodule decay. Intercropping legumes with non-leguminous crops can result in competition for water and nutrients. This competition can affect N_2 fixation negatively. However, it has been shown that when mineral N is depleted in the root zone of the legume component by the non-leguminous intercrops, N_2 fixation of legumes may be promoted. (Mulongoy, 1995)

2.5.4 Methods for estimating biological nitrogen fixation

Many attempts have been made to quantify BNF in both natural and agricultural ecosystems, and the values vary widely. The demand for accurate determinations of global inputs of biologically fixed N is strong and will continue to be fuelled by the need to understand and effectively manage the global N cycle. Herridge *et al*. (2008) gave some basic methodologies available to quantify biological N_2 fixation.

2.5.4.1 Acetylene reduction Assay

The acetylene reduction assay is a useful diagnostic tool for the detection of nitrogenase activity and has been widely used in all areas of N_2 fixation research because of its high sensitivity and simplicity (Peoples *et al*., 1989). The enzyme nitrogenase, universally responsible for biological N_2 fixation, is also capable of reducing acetylene (C_2H_2) to ethylene (C_2H_4). Therefore, the C_2H_2 reduction assay is a sensitive measure of nitrogenase

activity at a point in time and can be very useful for detecting N_2 fixation activity of, for example, bacterial cultures or plant residues that may be harbouring N_2-fixing bacteria (Herridge *et al*., 2008). The acetylene reduction assay (ARA) is carried out on detached nodules, detopped roots, or whole plants in a closed vessel containing 10% acetylene. A gas chromatograph is used to determine the amount of ethylene formed. Data are usually expressed as nanomoles or micromoles of ethylene produced per hour per plant or per weight unit of nodules (Mulongoy, 1995).

However, in enclosing the particular agent in a gas-tight vessel to evaluate ethylene (C_2H_4) evolution, physical disturbance of the N_2-fixing species is almost inevitable and this results in a decline in activity (Minchin *et al*., 1986). Also, the method has never been applied as a routine field assay owing to practical difficulties (Herridge *et al*., 2008).

2.5.4.2 Xylem sap analysis

Xylem sap carries N-containing compounds from the roots to the shoots of field-grown legumes originating from (i) nodules as assimilation-products of N_2 fixation, and (ii) soil mineral N taken up by the roots. If there are well established differences in xylem N-solute composition between fully symbiotic plants and non-nodulated plants which are totally dependent upon soil N, it should be possible to devise an assay system based on analysis of xylem sap to assess the extent to which plants rely on N_2 fixation or soil mineral N (Peoples *et al*., 1989). Many legumes of tropical origin e.g. Soybean (Glycine max), Common bean (Phaseolus vulgaris) transport the bulk of their fixed N from nodules in the form of the ureides, allantoin and allantoic acid. In these legumes, the ratio of ureide N to total N in xylem sap or stem segments is highly correlated with %Ndfa (Herridge *et al*., 2008). In some other legumes which have been studied e.g groundnuts, nodule products are exported predominantly as the amides, asparagine and glutamine (Peoples *et al*., 1989).

2.5.4.3 N difference method

The N-difference method is a relatively simple procedure. The principal assumption is that both legume and control contain the same quantity of soil-derived N in their shoots. For this to be valid the two plant types should explore the same soil-rooting volume, have the same ability to extract N, and accumulate soil N over the same period of time (this is particularly important if several estimates of N_2 fixation are desired during a growing season). The distribution of N between tops and roots of the two plant types should also be similar. A non-N_2-fixing control plant may be:

(i) A non-legume

(ii) An uninoculated legume of the same species (requires soil to be devoid of effective *Rhizobium* spp.).

(iii) A non-nodulating legume genotype.

This method can be used with considerable success, especially if the N_2- fixing plants derive large amounts of N from N_2 fixation (Herridge *et al*., 2008).

2.5.4.4 ^{15}N Isotope methods

In general, the "isotope dilution" approach to measuring BNF under soil conditions involves the addition of a small amount of ^{15}N-enriched inorganic N to the soil to increase the $^{15}N/^{14}N$ ratio of available soil N. In principle, the $^{15}N/^{14}N$ ratio of organisms that assimilate soil N will be the same as the $^{15}N/^{14}N$ ratio of the available soil N. In the case of an organism that is fixing atmospheric N_2 and assimilating soil N, its $^{15}N/^{14}N$ ratio will be lowered proportional to the amount of N_2 being fixed; i.e., the ^{15}N content of the biomass will be "diluted" as a result of BNF (Paul, 2007). The percentage of N derived from the atmosphere (% Ndfa) is represented by the following equation:

$$\% \text{ Ndfa} = 1 - \frac{\text{atom \% } ^{15}\text{N excess in N -fixing plant}}{\text{atom \% } ^{15}\text{ N excess in non fixing plant}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (2.2)$$

2.5.5 Importance of biological nitrogen fixation in agriculture

Biological nitrogen fixation is a phenomenon occurring in all known ecosystems and it is undoubtedly of greater agricultural importance. It is a major source of fixed N for plant life (Al-Falih, 2002). Estimates of global terrestrial BNF range from 100 to 290 million tonnes of N/year. Of this total, 40–48 million tonnes is estimated to be biologically fixed in agricultural crops and fields (FAO 2006). Biological nitrogen fixation is highly important for the nutrition of leguminous plants in natural and agricultural systems (Cheng, 2008), and being an important nitrogen input in the nitrogen cycle, could lessen pollution problems by lowering the demand for chemical fertilizers (Al-Falih, 2002).

Biological nitrogen fixation is an inexpensive, renewable resource option for smallholder farmers, permitting them to redirect limited farm investment toward other pressing household needs (Woomer, 2010). Since nitrogen is commonly the most limiting plant nutrient in arable farming in the tropics and also the most expensive element as a mineral fertilizer, biological nitrogen fixation (BNF) holds great promise for smallholder farmers in sub-Saharan Africa (Mulongoy,1995)

Currently, new methods designed to increase nitrogen use efficiency are being intensely studied, especially through the recognition of biochemical and molecular pathways of absorption and assimilation in plants. Agro ecological methods, such as BNF, are proposed to allow the sustainable use of this nutrient without production loss (Herridge *et al*., 2008).

2.6 Rhizobial Inoculants and Inoculation

Bacterial inoculant is a formulation that contains one or more beneficial bacterial strains or species in an easy-to-use and economical carrier material. Inoculants are the “vehicle” to

transport living bacteria from the factory to living plants to produce the desired effects on plant growth (Bashan, 1998).

In many soils, the nodule bacteria are not adequate in either number or effectiveness. Many African soils contain large populations of compatible but less effective rhizobia capable of inducing nodulation without providing much benefit to the legume host (Woomer, 2010). These situations call for provision of external source of rhizobia to enable effective nodulation and N_2 fixation, known as inoculation. Three such situations were identified, that legumes generally need inoculation; (1) where compatible rhizobia are absent (2) where the population of compatible rhizobia is small and (3) where the indigenous rhizobia are ineffective or less effective in N_2-fixation with the intended legume than selected inoculant strains (Date, 2000; Vanlauwe and Giller, 2006). Under these conditions, it is necessary to inoculate legume seed with elite strains of rhizobium bacteria.

Rhizobial strains selected for the production of inoculants must be able to form highly effective nodules with the host plant for which they are recommended, and under a wide range of crop conditions. In addition, if the soil where the crop will be established contains rhizobia strains able to form nodules with the legume crop, the inoculants strain must be competitive with indigenous rhizobia for nodule formation. The quality of the inoculant and its survival during the process of inoculation is critical in this competition (Date, 2000; Drew et al., 2014). Other important characteristic to take into account during the process of strain selection of rhizobia and other beneficial microorganisms is the capacity to exert the beneficial effect under a wide range of field conditions. These bacterial strains should be able to grow in industrial culture media, rising high cell densities, and to survive throughout the process of manufacturing and storage of the inoculants. Persistence in the soil in the absence of the host plant, strain genetic stability, compatibility with agrochemicals and survival under

a wide range of soil physical or chemical constraints are other desirable characteristics (Herridge *et al*., 2002; Obaton *et al*., 2002; O'Hara *et al*., 2002).

There are several different commercial inoculant formulations available to farmers to allow flexibility of application:

(i) Peat inoculants: which are prepared by introducing rhizobial broths into gamma-irradiated (sterilised) finely milled peat,

(ii) Granular pellets or chips containing rhizobia made from either peat or clay,

(iii) Freeze-dried powder: where a rhizobial broth culture is concentrated as a powder in a glass vials after all the water has been removed,

(iv) Liquid inoculants which are suspensions of rhizobia in a protective liquid formulation (GRDC, 2013).

The use of rhizobial inoculants for improvement in N-fixation and productivity of grain legumes, though established in developed countries over a long time, is still in the developing stage in most parts of sub-Saharan African countries like Nigeria (Abdullahi *et al*., 2013).

2.7 Persistence of Introduced Rhizobia in the Soil

If farmers inoculate one legume crop, will they have to inoculate again the next time they plant the same legume? In other words, will introduced rhizobia persist, or continue to live in the soil until the next crop? These are some of the frequently asked questions with respect to rhizobial inoculation (Drew *et al*., 2014).

Rhizobia live freely in the soil, and even in the absence of a legume host plant, a rhizobial community can persist for several years (Downie, 2010). Once rhizobia are introduced to the soil, they will be affected by the same factors that affect native rhizobia: vegetation, soil moisture, pH, and temperature. Where soil conditions are favourable, rhizobia are able to survive in the soil for many years, even in the absence of their legume host. In this state, the

rhizobia are known as saprophytes (microorganisms that live on dead or decaying organic matter). They can also live in or near the rhizospheres of non-leguminous plants and utilize their root exudates (Drew *et al*., 2014).

Ojo and Fagede, (2002) in a study to evaluate the persistence of *Rhizobium* inoculants originating from *Leucaena leucocephala* fallowed plots in southwest Nigeria reported that at the time of introduction of the inoculants, the number of homologous rhizobia able to nodulate *L. leucocephala* were 360 cells/g of soil on the experimental field while after the ten-year fallow period their population had risen to 8.5 x 10^4 cells/g of soil. This they attributed to nodule senescence which encourages rhizobia release and thus increase the population of the appropriate rhizobium symbiont in the rhizosphere. They therefore concluded that the inoculation of legume seeds with reasonably large population of effective and persistent rhizobial strains constituted an evident advantage over inorganic nitrogen fertilizer which has to be applied frequently for consistent high yields. Similarly, Wigley *et al*. (2015), in a study to determine the changes in rhizobia population over time in inoculated and uninoculated lucerne plants using a commercial inoculants, reported that the commercial inoculant was dominant in the nodules of lucerne plants grown from peat and coated seed three years after sowing.

2.8 Role of Phosphorus in Plant Nutrition and BNF

After nitrogen, phosphorus (P) is the second most limiting nutrient in African soils. Phosphorus is vital to plant growth and is found in every living plant cell. It is involved in several key plant functions, including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from one generation to the next (Armstrong, 1999). It is also essential for growth, cell division, root lengthening, seed and fruit development, and early ripening. It is a part of several compounds including oils and amino acids. The P compounds adenosine diphosphate

(ADP) and adenosine triphosphate (ATP) act as energy carriers within the plants. Phosphorus plays an indispensable role as a universal fuel for all biochemical activity in living cells. High-energy adenosine triphosphate (ATP) bonds release energy for work, when converted to adenosine diphosphate (ADP) (Foth, 1990).

In legumes, phosphorus is essentially required for healthy growth with efficient root system and profuse nodulation which in turn can affect the N_2-fixation potential (Kwari, 2005). Phosphorus influences nodule development through its basic functions in plants as an energy source (Woomer, 2010). Rhizobial activities and N_2 fixation without suitable fertilization by phosphorus (P) is depressed as it promotes early root development and the formation of lateral, fibrous and healthy roots (Badar *et al*., 2015). P is absorbed as the orthophosphate ion (either as $H_2PO_4^-$ or HPO_4^{2-}) depending on soil pH. As the soil pH increases, the relative proportion of $H_2PO_4^-$ decreases and that of HPO_4^{2-} increases (Foth, 1990).

2.9 Response of Groundnut to Rhizobial Inoculation

Over time, various responses have been obtained with respect to inoculation of groundnuts with rhizobia. The major drawback to inoculation technology is the wide variability in yield responses in time and space for a given rhizobium -legume symbiosis. Responses can vary from no response, and sometimes negative responses, to positive yield increases. Response to inoculation with a strain of *Rhizobium* vary with sites, legume cultivars, and the form of inoculant. Changes in climate, such as Africa's long droughts in recent years, and management factors including cropping systems and inoculant handling will also introduce variability in response to inoculation (Mulongoy, 1995).

Studies conducted by Mohamed and Abdalla (2013) indicated that rhizobial inoculation significantly increased nodulation, nodule dry weight, root and shoot dry weight, seed yield and nitrogen contents in the shoots. Similarly, Ashraf *et al*. (2006) observed a significant increase in nodule number per plant, nodule dry weight, shoot and root N content, 100 seed

weight and number of pods per plant over uninoculated plants. Yakubu *et al*. (2010) observed a similar trend in the sudano- sahelian zone of north eastern Nigeria where a positive response to rhizobial inoculation was observed in groundnut; N content increased by 32 % and amount of fixed N by 39 % over the control.

Inoculation trials were also conducted on Eutric Cambisols (EC) and Rhodic Nitisols (RN) soils in a greenhouse study. Soybean (TGx 1448-2E), cowpea (IT90K-277-2) and groundnut (SAMNUT 21) were used as test crops along with rhizobial inoculants (MAR 1495, TSBF Mixture, Legumefix, HiStick and IRj 2180A) to determine their response to soil type and ability to form symbiotic relationship with the crops. Rhizobia strains MAR 1495 and TSBF mixture showed similar ability to improve the productivity of soybean and groundnut and thus recommended for use as common inoculants for the two crops (Aliyu *et al*., 2013).

A contrary result however, was obtained by Yusuf *et al*. (2011) who conducted a field trial in 2010 to evaluate the effects of three P sources and three rhizobial inoculants on the yield and yield components of groundnut in the northern Guinea savanna of Nigeria using rhizobial inoculants Biofix, Vault and mixture of the two. There was no significant difference in nodulation and shoot dry matter yield between inoculated and uninoculated plants. Similarly, there were no significant differences in pod and haulm yields among the rhizobial inoculants. Instead, the uninoculated plants produced significantly higher pod (20 %) and haulm (28 %) yields than the average yield of the inoculated plants. This, they attributed to the ineffectiveness of the inoculants strains.

2.10 Response of Groundnut to Phosphorus Fertilizer

Groundnut response to phosphorus fertilizer application depends on the crop, environment and management factors. Different rates of phosphorus application have been recommended to increase the growth, grain yield and yield components of groundnut. Kamara *et al*. (2011)

evaluated four groundnut varieties for their response to P fertilization in two Nigerian agro-ecological zones (sudan and northern Guinea savanna) during 2005 and 2006. The treatments included 0, 20, and 40 kg P ha^{-1} and groundnut varieties ('Samnut 22', 'local Wadabura', 'Samnut 21', and 'Samnut 23'). The results showed significant response of grain yield and yield components to P application confirming the importance of P for groundnut production in the Nigerian savannas. Pod yield increased linearly with increasing P rates in both years. Mean pod yield was higher by 49.3 % at 20 kg and by 57.8 % at 40 kg P ha^{-1} compared with unfertilized plots with 'Samnut 23' having more grain yield than other varieties at both locations in 2005.

Similarly, field experiments were conducted by Nwokwu (2011) to evaluate the effect of four levels of phosphorus (0, 20, 40 and 60 kg P ha^{-1}) and three plant spacing (20 x15 cm, 30 x15 cm and 40 x15 cm) on the vegetative and yield parameters of groundnut (*Arachis hypogeae* L). The results showed that vegetative parameters such as plant height, number of branches and number of leaves increased significantly from 0 kg P ha^{-1} (Control) to 60 kg P ha^{-1} while yield parameters such as harvest index, 100 seed weight, pod weight per plots, pod weight per plant and number of mature pods were highest at 20 kg. However, the phosphorus rates increased yield significantly above 0 kg P ha^{-1}.

An experiment on the optimum planting density and phosphorus requirement of a local groundnut (*Arachis hypogaea* L.) cultivar, 'Graffi' was carried out by Shiyam, (2011) on an Ultisol in the humid area of southeastern Nigeria, during the growing seasons of 2007 and 2008. Five phosphorous rates 0, 20, 30, 40 and 50 kg P_2O_5 ha^{-1}) were applied to five plant densities of groundnut (47 ,619, 57, 128, 71, 428, 95, 238 and 142, 857 plants ha^{-1}). The results showed that phosphorus rates influenced the number of filled pods/plant and seed yield/ha. It was concluded that the plant density of 95,238 plants/ha with application of 40 kg

P_2O_5 ha^{-1} would enhance optimum productivity of groundnut in the humid areas of southeastern Nigeria.

2.11 Phosphorus Efficiency Concepts

Nutrient use efficiency is a critically important concept in the evaluation of crop production systems. The objective of nutrient use is to increase the overall performance of cropping systems by providing economically optimum nourishment to the crop while minimizing nutrient losses from the field.

The efficient use of fertilizer phosphorus (P) is very important in crop production. Three main reasons for this are outlined by Syers *et al*. (2008). First, phosphate rock, from which P fertilizers are manufactured, is a finite, non- renewable resource, and it must be used efficiently in order to maximize its life span. Second, there is a need to maintain and improve the P status of many soils for the growth of crops for food, fibre and bioenergy and Third, the transfer of soil P (derived from fertilizers and organic manures) is a major cause of P - induced eutrophication in surface waters.

Efficiency concepts in plant mineral nutrition have been defined based on the process by which plants acquire, transport, store and use the nutrient in order to produce dry matter or grain, at low or high nutrient supply (Ciarelli et al., 1998). It therefore means that, plants with high nutrient use efficiency should be able to tolerate lower nutrient availabilities: thus, they should be effective competitors in diverse communities where nutrients are in short supply.

Phosphorus efficiency can be divided into P acquisition (uptake) efficiency (PAE) and P utilization efficiency (PUE). PAE refers to the ability of plants to take up P from soils, whereas PUE is the ability to produce biomass or yield using the acquired P ((Good et al., 2004). Therefore, enhancement of P efficiency in plants can be achieved through improving P acquisition and/or utilization.

There are a number of agronomic indices and methods for measuring the efficiency of plant nutrient use in agriculture. In summary, the methods and indices, based on those of Cassman et al. (1998), are: direct method; difference method; partial factor productivity index; physiological efficiency index; and balance method. Syers *et al*. (2008) reported that of all the methods for calculating the recovery and efficiency of fertilizer P, the "balance method" is preferred because it takes residual P in the soil into account. This method, expresses total P uptake by the crop as a percentage of the P applied i.e. total P in the crop divided by the P applied, expressed as a percentage.

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

Two screen house trials were conducted between May and October, 2015 at the screen house of the Department of Soil Science (11^0 9‟ N, 7^0 36‟ E), Faculty of Agriculture / Institute for Agricultural Research (FOA/ IAR), Ahmadu Bello University, Samaru Zaria.

3.2 Soil Sampling and Preparation

Soil samples were taken from a P deficient field, on the Institute for Agricultural Research (IAR) farm located in Samaru (Latitude 11° 11’ 0’’ N, and Longitude 7° 36’ 52’’ E). Rainfall in Samaru is monomodal which starts in May or June to September or October. The mean rainfall for the research year (2015) was 1096 mm and the mean temperature was 26 ^oC (IAR, 2017). The soil was classified to be leached tropical ferruginous, Typic Haplustalf in Soil Taxonomy, Acrisol in the FAO system or Alfisol in the USDA system (Jones and Wild, 1975; Uyovbisere *et al*., 2000). Bulk soil samples were taken at the depths of 0 –15 cm from the field, bulked and thoroughly mixed to form composite samples. The soil was sieved using a sieve of 4.00 mm size and 4 kg of soil was weighed into plastic pots for the screen house trials. A subsample of the soil was sieved with 2 mm and 0.5 mm sieve size for analysis of some physical and chemical properties. Surface soil at 5 cm depth (a depth where soil microbiological activities is prevalent) was taken for the determination of the number of viable rhizobia in the soil using the most probable number (MPN) plant- infection technique.

3.3. Physical and Chemical Analysis of the Soil

The following analyses were conducted on the soil samples in the Department of Soil Science laboratories at FOA/IAR Samaru. Particle size distribution was determined by the hydrometer method, as described by Gee and Bauder (1986), using distilled water and calgon (sodium

hexametaphosphate) as dispersing agents. The soil texture was determined using the USDA soil textural triangle. Soil pH was measured in both water and 0.01 M $CaCl_2$ solution using a 1:2.5 soil to solution ratio with a glass electrode pH meter (Hendershot *et al.*, 1993). Organic carbon was determined by the Walkey-Black (chromic acid) method as described by Nelson and Sommers (1982). Total nitrogen was determined by micro-Kjeldahl digestion method (Bremner and Mulvaney (1982). Available phosphorus was determined by the Bray 1 method as outlined by Dipak and Abhijit (2005). Exchangeable bases for the soil were extracted with 1N ammonium acetate at pH 7.0 buffer (Chapman, 1965). Exchangeable K and Na was determined using flame photometer while exchangeable Ca and Mg was determined using Atomic absorption spectrophotometer (AAS) (Jackson, 1958). Exchangeable acidity was determined by titration method as described by McLean, (1982).

3.4 Determination of Rhizobia Population in the Experimental Soil

In order to estimate the total viable rhizobia in the experimental soils, the most probable number (MPN) plant infection method was used. The host plant used was groundnut (SAMNUT 24) which was cultured in growth pouches (Somesegaran and Hoben, 1985). The seeds were first sterilized by immersing them in 70 % ethanol for 10 seconds after which it was drained. The seeds were then submerged in 3 % sodium hypochlorite for three minutes and then drained. This was followed by rinsing the seed six times with sterile distilled water and the seeds were then pre- germinated using 1 % agar water inside an incubator at 30 ºC for 72 hours. Upon emergence of the radicle, the pre-germinated seeds were taken to the growth chamber and were transferred aseptically into growth pouches (two plants per pouch) containing 100 mL of sterilized nitrogen-free plant nutrient solution (Woomer *et al.*, 1988). The pouches were put into a racket for support. A week later, a six step five-fold dilution series of a subsample of the experimental soil was carried out replicated four times and 1 ml

of aliquot was inoculated onto the root zone of the cultured plants. The plants were watered using the nitrogen free- nutrient solution. Nodulation was assessed after six weeks.

The MPN was then calculated using the following formula:

$$X = \frac{m \times d}{v} \qquad \text{………………………………(3.1)}$$

Where:

X= Most probable number

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

d = the lowest dilution (first unit or any unit in which all replicates are nodulated)

v = volume of the aliquot applied to plants. (Woomer *et al*., 1990).

3.5. First Screen House Trial

3.5.1 Treatment and experimental design

The first screen house experiment included an inoculation trial with groundnut (SAMNUT 24) as test crop. The crop was grown in plastic pots with Four kilograms (4 kg) of soil. The treatments consisted of two factors. The first factor consisted of six (6) inoculants (four indigenous rhizobium strains namely: SNN 343, KBU 26, SBG 234, SAMFIX 703, a reference strain (NC 92) and a commercial inoculant (HISTICK) alone, the inoculants each combined with 20 kg N ha^{-1} mineral nitrogen as starter dose, and mineral nitrogen alone at 0, 20, or 40 kg N ha^{-1}, adding up to fifteen treatments all termed as nitrogen (N) sources. The second factor was phosphorus at three rates (0, 30 or 60 kg P_2O_5 ha^{-1}) applied as single super phosphate. The N sources (SNN 343, KBU 26, SBG 234, SAMFIX 703, NC 92, HISTICK, SNN 343 +20N, KBU 26 +20N, SBG 234 +20N, SAMFIX 703 +20N, NC 92 +20N, HISTICK +20N, 0 kg N ha^{-1}, 20 kg N ha^{-1} and 40 kg N ha^{-1}) and P rates (0 kg P_2O_5 ha^{-1}, 30 kg P_2O_5 ha^{-1} and 60 kg P_2O_5 $ha^{-}1$) were factorially combined (fifteen x three) to give a total of

forty five (45) treatments and were laid down in a Randomized Complete Block Design replicated three times. In order to estimate the amount of biological nitrogen fixed by SAMNUT 24, a non- nodulating groundnut variety (ICGL 5) was included as a reference crop (minus rhizobium, minus mineral N). The rhizobial inoculants used and their sources are listed in Table 3.1 below:

Table 3.1 Inoculants used and their sources

Inoculant	Source	Formulation
SAMFIX 703	Samaru, Nigeria	Liquid
SNN 343	Shanono, Nigeria	Peat based
KBU 26	Kubau, Nigeria	Peat based
SBG 234	Sabongari, Nigeria	Peat based
NC 92	Murduch University, Australia	Peat based
HISTICK	BASF, Germany	Peat based

3.5.2 Trial management

Four kilograms (4 kg) of soil was weighed into plastic pots perforated at the bottom to ensure adequate drainage. The pots were saturated with water and allowed to stand for twenty four hours to achieve equilibrium before sowing. Five seeds of groundnuts (SAMNUT 24) were sowed in each pot on the 27 May, 2015 and were thinned down to one fourteen days after sowing. The peat based inoculants were coated to the seeds using gum Arabic as a sticking agent. The Slurry method outlined by Woomer *et al*. (2010) was used. The seeds were transferred into bowls and the inoculant was then poured over the seeds in the bowl after sprinkling the gum Arabic mixture over the seeds. The seeds and inoculants in the bowl were mixed carefully until seeds were coated with black film of inoculants and allowed to dry for a few minutes under a shade after which they were planted in the pots. The liquid inoculant

was prepared in a yeast mannitol broth (YMB) and 5 mL was applied to the root zones of each of the plants fourteen days after sowing. Potassium at 20 kg K_2O ha^{-1} was applied one week after sowing as muriate of potash, as well as three levels of phosphorus (0, 30 or 60 kg P_2O_5 ha^{-1}) applied as single super phosphate and three levels of nitrogen (0, 20, or 40 kg Nha^{-1}) applied as urea. The fertilizers were placed in holes about 5 cm away from the plants and covered with the soil. Due to the unusually hot nature of the screen house environment which gives rise to high evapotranspiration, plants were irrigated once a day at the beginning and twice a day as the plants grow to flowering. In order to prevent any external source of nitrogen, deionized water was used to irrigate the plants throughout the period of the experiment. The crop was grown for seven weeks before nodulation assessment and harvesting. Harvesting was carefully done using sterile polythene bags for each pot to avoid contamination. The soils were carefully returned to the pots and preserved for the second trial.

3.5.3 Inoculation

3.5.3.1 Rhizobial counts in the inoculants used

In order to ascertain the viable bacterial concentration of the inoculants, colony forming units (CFU) were determined using the pour plate method. Seven steps ten-fold serial dilution of each strain using 1 mL of liquid inoculants and 1 g of peat inoculants was carried out. (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}). Using the dilution levels of 10^{-5}, 10^{-6} and 10^{-7}, 1 mL of each strain was transferred aseptically into a sterile plate which was quickly followed by a pouring of already prepared nutrient agar medium (the constituents are listed in Appendix 1) amended with congo red dye and maintained in a water bath at 50 ^{o}C was poured, and swirled gently. The plates were incubated at 25 ^{o}C for three to five days. The plates with 30-300 colonies were selected per dilution and counted on a digital colony counter and the

number of colony units per ml (CFU/ ml) of the liquid inoculant and the number of colony units per gram (CFU/g) of the peat based inoculants were determined using;

$$N = \frac{n}{C \times V} \quad \text{……..} \quad \text{……..……(3.2)}$$

Where: N = number of cells per gram

n = number of isolated colonies counted on the plate

C = Dilution

V = Volume spread (Somasegaran and Hobben, 1985).

3.5.3.2 Preparation of the liquid inoculants

In order to have sufficient quantity of liquid inoculant for the screen house work, the strain which was stored in small quantity in vials was multiplied. From the stock culture of the strain, fresh broth culture of the strain was prepared using a sterile yeast mannitol broth (YMB). The pH of the medium was adjusted to 6.8 $\pm$ 0.02**.** One hundred milliliter (100 mL) of the broth was transferred to a 250 ml Erlenmeyer flask and autoclaved at 121 ^{o}C for 15 minutes. It was allowed to cool to room temperature (25 ^{o}C). From the original inocula, a loop was taken and used to inoculate the sterile YMB and put in a shaker incubator for seven days to get actively growing rhizobia.

3.6 Second Screen House Trial

3.6.1 Treatment and experimental design

A second trial was conducted to assess the residual benefit of rhizobial inoculation on following legumes. Groundnut (SAMNUT 24) was sown in pots containing soil inoculated in the first trial with KBU 26, SAMFIX 703, SBG 234, SNN 343, NC 92 and HISTICK. A control treatment (minus rhizobium, minus mineral N) was also included giving a total of

seven treatments. Phosphorus was applied at three rates (0, 30 and 60 kg P_2O_5 ha^{-1}). The inoculants were factorially combined with three levels of phosphorus giving a total of twenty one (21) treatments arranged in a randomized complete Block design replicated three times. A non- nodulating groundnut variety (ICGL 5) was also included as a reference crop (minus rhizobium, minus mineral N) in order to estimate the amount of biological nitrogen fixed by SAMNUT 24.

3.6.2 Trial management

Four kilograms of soil was weighed into plastic pots perforated at the bottom to ensure adequate drainage. The soil was saturated with water and allowed to stand for twenty four hours to achieve equilibrium before sowing. Five seeds of groundnuts (SAMNUT 24) were sowed in each pot on the 25 of August, 2015 and were thinned down to one fourteen days after sowing. Potassium at 20 kg K_2O ha^{-1} was applied one week after sowing as muriate of potash as well as three levels of phosphorus (0, 30 or 60 kg P_2O_5 ha^{-1}) applied as single super phosphate. The fertilizers were deposited in holes 5 cm away from the plants and covered with the soil. Plants were irrigated once a day at the beginning and twice a day as the plants grow to flowering. In order to prevent any external source of nitrogen, deionized water was used to irrigate the plants throughout the period of the experiment. The crop was grown for seven weeks before harvesting and data collection.

3.7 Data Collection

3.7.1 Determination of nodule number, nodule fresh/dry Weight

At seven weeks after sowing, plants were carefully removed from the pots and placed on 1 mm sieve size to avoid loss of nodules during cleaning. The soil was then gently washed off the roots in a bucket containing water. The nodules were carefully removed from the roots,

counted and weighed. The nodules were then transferred to a paper bag and placed in an oven and allowed to dry to a constant weight at 65 °C, and the dry weight was then taken.

3.7.2 Determination of root and shoot dry weight

The plant biomass was determined. After harvesting, the plants were cut above the ground to separate the shoot from the roots. The shoots and the roots were oven dried in the oven at 65 °C to a constant weight. The dry weights of the shoots and roots were recorded and later ground and sieved with 0.5 mm sieve for tissue analysis.

3.8 Plant Analysis

3.8.1 Determination of plant total nitrogen and total phosphorus

After harvesting, the plant samples were separated into shoots and roots and were washed with water to remove adhering soils. The samples were placed in envelopes and oven dried at 65 ^{o}C to a constant weight and ground to pass through a 0.5 mm mesh using a Panasonic mixer grinder. Total N concentration was determined using the micro Kjedhal method (Bremmer and Mulvaney, 1982), while total P was determined using the yellow molybdate method (Anderson and Ingram, 1993).

3.9 Assessment of Biologically Fixed Nitrogen

The amount of biological nitrogen fixed from each treatment was assessed using the Total Nitrogen Difference (TND) method. The total amount of nitrogen in SAMNUT 24 and ICGL 5 was determined and the amount of N fixed was calculated using the equations reported by Murray *et al*. (2008) :

$$\text{Total N in plants} = \frac{\text{Dry matter weight} \times \%\ \text{N in plants}}{100} \quad \ldots\ldots\ldots\ldots\ldots (3.3)$$

$$\text{N fixed} = \text{Total N in SAMNUT 24} - \text{Total N in ICGL 5} \quad \ldots\ldots\ldots\ldots\ldots (3.4)$$

$$NDFA = \frac{\text{Total N in SAMNUT 24} - \text{Total N in ICGL 5}}{\text{Total N in SAMNUT 24}} \times 100 \quad \ldots\ldots\ldots\ldots (3.5)$$

Where, NDFA means nitrogen derived from atmosphere.

3.10 Calculation of Phosphorus Use Efficiency

Phosphorus uptake efficiency and phosphorus agronomic efficiency were calculated using the following formula:

$$\text{Phosphorus uptake efficiency (PUE)} = \frac{\text{Total P in the plant}}{\text{P supplied}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.6)$$

$$\text{Phosphorus agronomic efficiency (PAE)} = \frac{\text{Dry matter weight}}{\text{P supplied}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (3.7)$$

3.11 Statistical Analysis

Data collected was analyzed using SAS 9.2 software (SAS, 2008). Data was subjected to Analysis of Variance (ANOVA). Means were separated using the Duncan's Multiple range Test (DMRT) at 5 % level of probability where the F ratios were found to be significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Physical and Chemical Properties and Rhizobial Count of the Experimental Soil

The results for the physical and chemical properties and rhizobial count of the experimental soils are shown in Table 4.1. Results of the particle size distribution of the soils show that the soil is sandy loam in texture. The pH of the soil in water was slightly acidic. The organic carbon content, the available phosphorus and the total nitrogen contents of the soil were low. The exchangeable bases (Mg^{2+}, Na^{+} and K^{+}) were moderate, while Ca^{2+} was low. Exchangeable cation exchange capacity (ECEC) of the soil was low. The rhizobial counts of the indigenous groundnut rhizobia using the plant most probable number plant infection method indicates a population of 2.0×10^{1} rhizobial cells g^{-1} of soil.

4.2 Rhizobial Counts in the Inoculants Used

The results obtained for the rhizobial counts in the inoculants used **(**colony forming units of cells per gram of the rhizobial inoculants) is given in Table 4.2. The result shows that all the inoculants used had lower number of cells g^{-1} than the recommended number of viable cells expected in high quality inoculants.

Table 4.1 Physical and Chemical Properties and Rhizobial Count of the Experimental Soil

Property	Unit	Test value
pH (H_20) 1:2.5		6.40
pH (0.01M $CaCl_2$) 1:2.5		5.40
Organic carbon	g kg^{-1}	7.30
Total N	g kg^{-1}	0.70
Available P	mg kg^{-1}	2.25
Total Phosphorus	mg kg^{-1}	818.19
Exchangable cations	cmol(+)/kg	
Ca		1.51
Mg		0.90
Na		0.28
K		0.21
$Al^{3+,}$ H^{+}	cmol(+)/kg	0.30
ECEC	cmol(+)/kg	3.20
Sand	g kg^{-1}	640
Silt	g kg^{-1}	200
Clay	g kg^{-1}	160
Textural class		Sandy loam
Rhizobial count (MPN)	cells g^{-1} soil	2.0×10^{1}

MPN- Most probable number

Table 4.2. Rhizobial Counts in the Inoculants Used.

Inoculant	Count	Formulation
HISTICK	3.1×10^7 cells g^{-1}	Peat
NC 92	7.7×10^6 cells g^{-1}	Peat
SNN 343	4.9×10^7 cells g^{-1}	Peat
KBU 26	7.2×10^6 cells g^{-1}	Peat
SBG 234	4.9×10^6 cells g^{-1}	Peat
SAMFIX 703	3.1×10^6 cells ml^{-1}	Liquid

4. 3. Effect of Nitrogen Sources and Phosphorus Fertilizer on Shoot and Root Dry Weights of Groundnut

The results of shoot and root dry weight is presented in Table 4.3. The application of Nitrogen (N) sources and phosphorus (P) fertilizer had a significant effect ($P < 0.05$) on both shoot and root dry weight of plants. The result of shoot dry weight shows that only inoculation with SNN 343 + 20N produced better shoot dry weight than the control. Generally, the use of inoculants in combination with 20 kg N ha^{-1} increased shoot dry weight by 9.5 % over the use of inoculants alone, and by 9.8 % when compared with the control even though the values were statistically similar to the control.

The results of the root dry weight reveals SBG 234 + 20N to be the best treatment but not statistically different from the control. All the other treatments except KBU 26 + 20N were just as good as the control. KBU 26 +20N produced the smallest root biomass.

Phosphorus application at 30 kg P_2O_5 ha^{-1} and at 60 kg P_2O_5 ha^{-1} significantly ($P < 0.05$) dry matter yields in the groundnut plants. Shoot dry weight was significantly higher at 60 kg P_2O_5 ha^{-1} than at 30 kg P_2O_5 ha^{-1}. The least was observed with the control (0 kg P_2O_5 ha^{-1}) which was significantly lower than 30 kg P_2O_5 ha^{-1}. Application of 60 kg P_2O_5 ha^{-1} increased shoot dry weight of plants by 54 % while application of 30 kg P_2O_5 ha^{-1} gave an increase of 41 % when compared with the control (0 kg P_2O_5 ha^{-1}). A similar trend was observed in the root dry weight of plants where application of phosphorus increased root dry weight. Though there was no significant difference between the two rates applied, both gave an increase of 24 % over the control. There was no significant interaction ($P > 0.05$) between N sources and P fertilizer on shoot dry weight of plants, but a significant interaction was observed in the root dry weight (Fig. 4.1). Most of the no P treatments produced values statistically similar to most of the interaction of N sources with 30 kg P_2O_5 ha^{-1} and / or 60 kg P_2O_5 ha^{-1} except the interaction of the control with P both at 30 and 60 kg P_2O_5 ha^{-1} and

Table 4.3. Effect of Nitrogen Sources and Phosphorus Fertilizer on Shoot and Root Dry Weights of Groundnut

Treatment	Dry weight (g plant^{-1})	
	Shoot	Root
Nitrogen sources		
0 kg N ha^{-1}	6.42bcd	0.52ab
20 kg N ha^{-1}	6.90b	0.48abc
40 kg N ha^{-1}.	6.98ab	0.44bc
NC 92	6.93ab	0.49ab
SBG 234	6.57bcd	0.51ab
KBU 26	6.37bcd	0.42bc
SNN 343	6.03d	0.46bc
SAMFIX 703	6.37bcd	0.42bc
HISTICK	6.12cd	0.48abc
NC 92+20N	7.12ab	0.49ab
SBG 234+20N	7.01ab	0.57a
KBU 26+20N	6.89b	0.39e
SNN 343+20N	7.63a	0.50ab
SAMFIX 703+20N	6.69bcd	0.52ab
HISTICK+20N	6.85bc	0.56ab
SE±	0.033	0.221
P rates (kg P_2O_5 ha^{-1})		
0	5.10c	0.42b
30	7.21b	0.51a
60	7.87a	0.52a
SE±	0.010	0.093
Interaction		
N*P		
SE±	0.391	0.053
Significance	NS	*

Means with the same letter(s) within a treatment group are not significantly different ($P \leq 0.05$) using DMRT (Duncan Multiple Range Test). NS= Not significant at 5% level of probability, *= significant at 5% probability

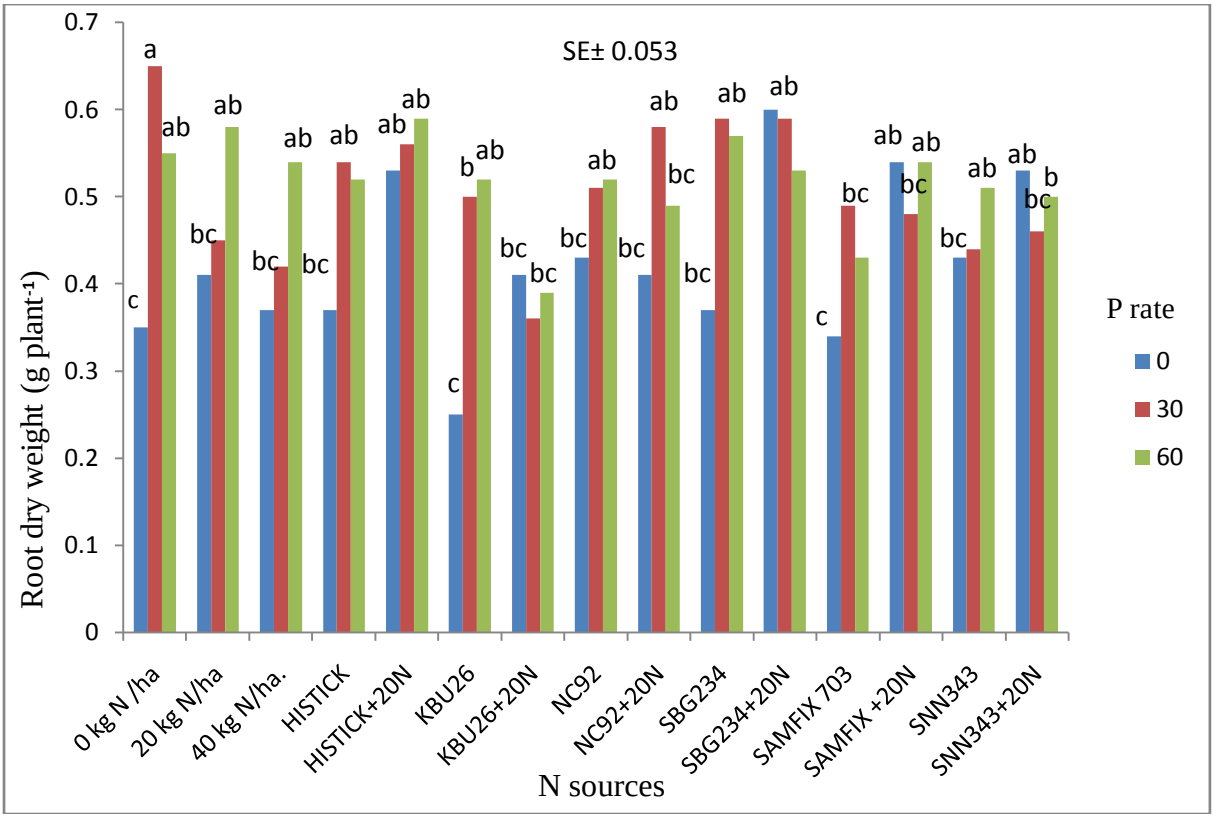


Fig 4.1. Interaction of N sources and P fertilizer on root dry weight of groundnut

KBU 26 with P at 30 and 60 kg P_2O_5 ha^{-1} that produced statistically higher root dry weight than the absolute control.

4.4. Effect of Nitrogen Sources and Phosphorus Fertilizer on Nodulation of Groundnut

4.4.1 Nodule number

Application of N sources had a highly significant effect ($P < 0.01$) on nodule number (Table 4.4). All the treatments produced statistically similar number of nodules than the control except 20 kg N ha^{-1} and SBG 234 + 20N that produced fewer numbers of nodules than the control. Generally, the use of inoculants alone did not increase nodulation while the use of inoculants in combination with mineral fertilizer at 20 kg N ha^{-1} decreased nodulation by an average of 12.2 %.

Phosphorus application significantly ($P < 0.01$) increased number of nodules. The result shows that nodule number increased with increase in phosphorus rates. Application of 30 kg P_2O_5 ha^{-1} gave an increase of 36 % while application of 60 kg P_2O_5 ha^{-1} further increased nodule number of plants by 52 % over the control (0 kg P_2O_5 ha^{-1}). A significant interaction was also observed between N sources and P fertilizer on nodule number (Fig 4.2). The combination of HISTICK and 60 kg P_2O_5 ha^{-1} produced statistically higher nodule number than the absolute control (0 kg N ha^{-1} and 0 kg P_2O_5 ha^{-1}) and most of the no P treatments. Generally, the interactions of N sources with 30 kg P_2O_5 ha^{-1} and 60 kg P_2O_5 ha^{-1} produced similar number of nodules than the no P treatments except the interaction of the control with 30 kg P_2O_5 ha^{-1}, HISTICK with 30 kg P_2O_5 ha^{-1} , NC 92 + 20N with 60 kg P_2O_5 ha^{-1}, SBG 234 with 60 kg P_2O_5 ha^{-1} and SNN 343 with 60 kg P_2O_5 ha^{-1}.

4.4.2 Nodule fresh weight

The result of nodule fresh weight shows that application of N sources had a significant effect ($P < 0.01$) on nodule fresh weight. Inoculation with SBG 234 and NC 92 + 20N produced

Table 4.4. Effect of Nitrogen Sources and Phosphorus Fertilizer on Nodulation of Groundnut

Treatment	Nodule no Plant^{-1}	Nodule fresh wt (mg plant^{-1})	Nodule dry wt (mg plant^{-1})
Nitrogen source			
0 kg N ha^{-1}	133a-d	180cde	70c-f
20 kg N ha^{-1}	97e	160de	70c-f
40 kg N ha^{-1}.	115b-e	130e	60f
NC 92	127a-d	210bc	90abc
SBG 234	143ab	290a	90abc
KBU 26	114cde	200bcd	70c-f
SNN 343	136a-d	210bcd	90abc
SAMFIX 703	132a-d	180cde	80b-f
HISTICK	156a	230bc	110a
NC 92+20N	144ab	260ab	90abc
SBG 234+20N	96e	190cde	60def
KBU 26+20N	122b-e	220bc	100ab
SNN 343+20N	110de	190cde	60edf
SAMFIX 703+20N	108de	190cd	60edf
HISTICK+20N	124b-e	220bc	70c-f
SE±	8.970	17.000	6.000
P rates (kg P_2O_5 ha^{-1})			
0	96c	130c	40c
30	130b	220b	90b
60	146a	270a	110a
SE±	4.011	7.000	2.000
Interaction			
N*P			
SE±	15.533	32.000	10.000
Significance	**	*	**

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). NS= Not significant at 5% level of probability, *= significant at 5% probability, ** significant at 1 % probability

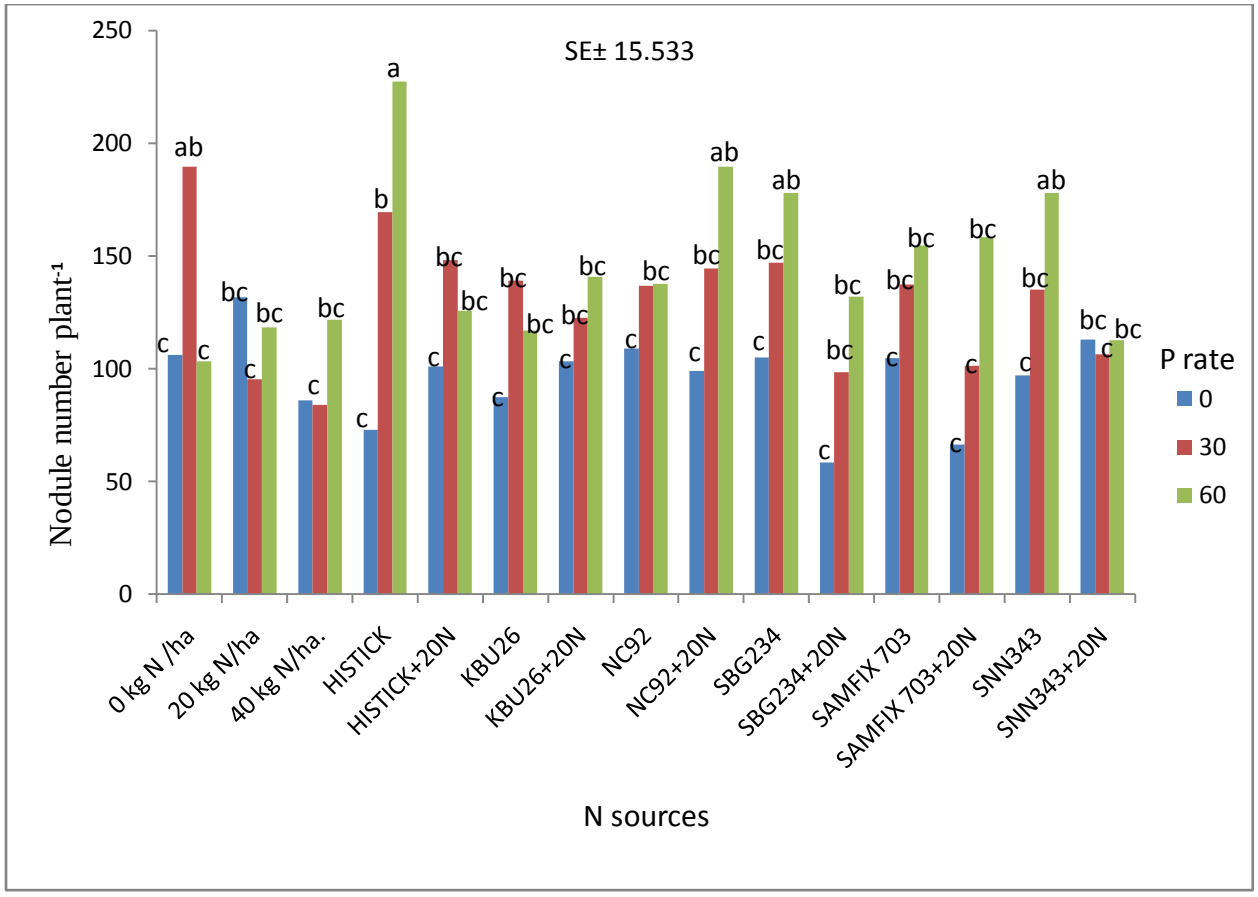


Fig: 4.2. Interaction of N sources and P fertilizer on nodule number of groundnut

higher nodule fresh weight than the control. All the treatments were statistically similar to the control.

Similarly, phosphorus application significantly ($P < 0.01$) enhanced the nodule fresh weight of plants. The enhancement was dependant on the rates of phosphorus applied. When compared with the control (0 kg P_2O_5 ha^{-1}), the application of 60 kg P_2O_5 ha^{-1} increased the nodule fresh weight of plants from 130 - 270 mg $plant^{-1}$ which was better than the application of 30 kg P_2O_5 ha^{-1} which gave an increase from 130 -.220 mg $plant^{-1}$. The N sources and P fertilizer interaction was significant in respect of nodule fresh weight (Fig. 4.3). Differences between the no P and P treatments were only observed with HISTICK, NC 92, NC 92 + 20N, SBG 234 and SNN 343 where interaction with 30 kg P_2O_5 ha^{-1} and 60 kg P_2O_5 ha^{-1} produced statistically higher nodule fresh weight than with 0 kg P_2O_5 ha^{-1}. All the other interactions were similar to the control.

4.4.3 Nodule dry weight

Nodule dry weight was significantly influenced ($P < 0.01$) by application of N sources where inoculation with HISTICK and KBU 26 + 20N gave nodule dry weights better than the control. All the other treatments were similar to the control. Generally, the use of inoculants increased nodule dry weight by an average of 26 % over the control.

Similarly, the nodule dry weight of plants was significantly ($P < 0.01$) influenced by the application of Phosphorus. A 125 % increase was observed with application of 30 kg P_2O_5 ha^{-1} when compared with the control (0 kg P_2O_5 ha^{-1}) and as the phosphorus rate was increased to 60 kg P_2O_5 ha^{-1}, the increase was as high as 175 % showing clearly that the increase observed was dependent on the rates of phosphorus applied. A significant interaction ($P < 0.01$) was observed between N sources and P fertilizer (Fig 4.4). Inoculation with HISTICK and P rate at 60 kg P_2O_5 ha^{-1} produced nodule dry weight which was statistically

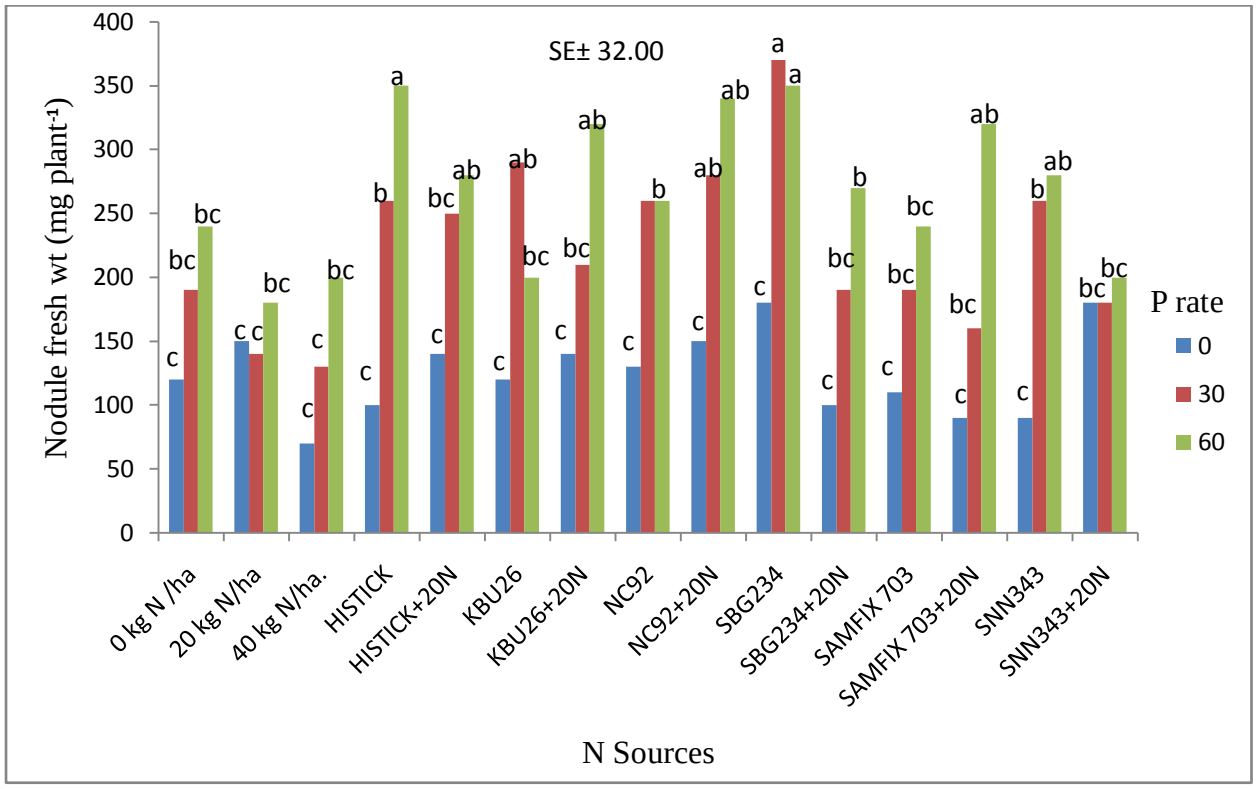


Fig. 4.3. Interaction of N sources and P fertilizer on nodule fresh weight of groundnut

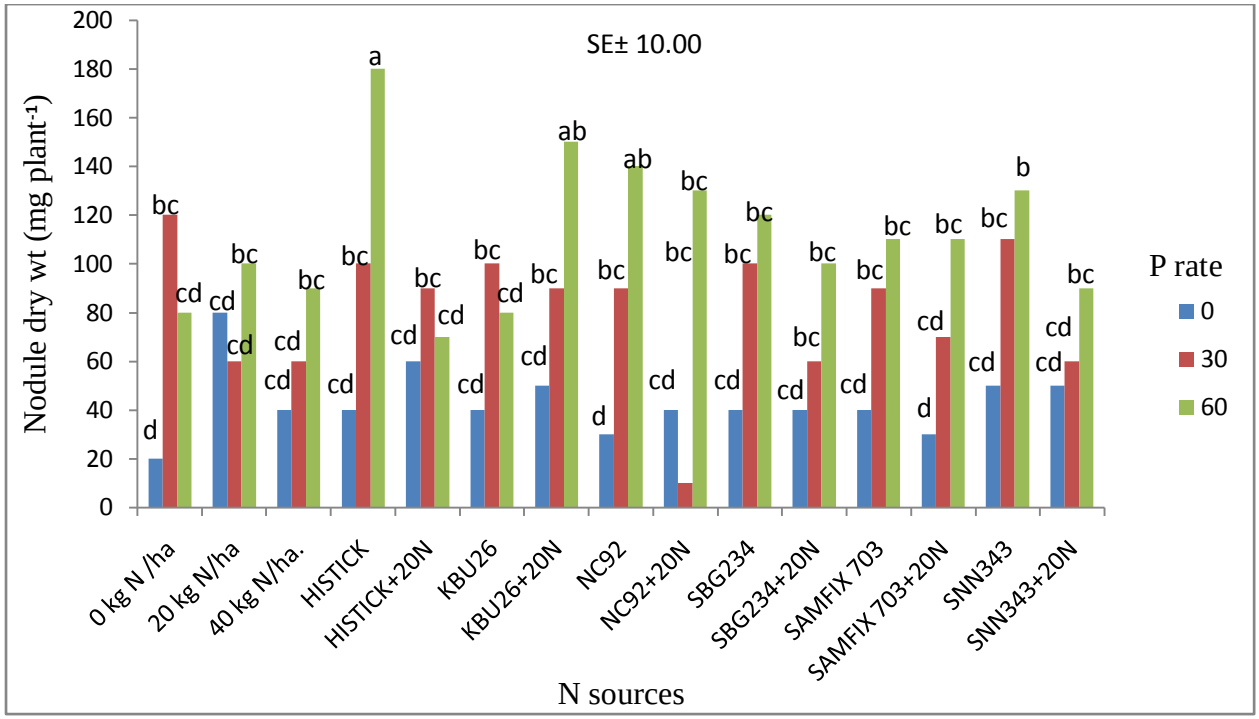


Fig.4.4. Interaction of N sources and P fertilizer on nodule dry weight of groundnut

higher than all the other treatments except KBU 26 + 20N and NC 92 + 20N at 60 kg P_2O_5 ha^{-1}. All the other N sources produced statistically similar values irrespective of the P rates.

4.5. Effect of Nitrogen Sources and Phosphorus Fertilizer on Biological Nitrogen Fixation by Groundnut

4.5.1 Amount of N_2 fixed

The amount of N_2 fixed was significantly affected ($P < 0.01$) by the application of N sources (Table 4.5). The use of inoculants alone did not increase the amount of N_2 fixed when compared with the control. Most of the treatments that received mineral fertilizer (20 kg N ha^{-1}, 40 kg N ha^{-1}, SBG 234 + 20N, KBU 26 + 20N, SNN 343 + 20N and SAMFIX 703 + 20N) however, fixed statistically higher amounts of nitrogen than the control. The addition of 20 kg N ha^{-1} to most of the indigenous inoculants (KBU 26, SAMFIX 703, SBG 234 and SNN 343) increased the amount of N_2 fixed. On average, the amount of N_2 fixed increased by 51.7 % when inoculants were combined with 20 kg N ha^{-1}.

There was a significant ($P < 0.01$) increase in the amount of N_2 fixed due to phosphorus application. Application of 60 kg P_2O_5 ha^{-1} increased the amount of N_2 fixed from 140 to 470 mg $plant^{-1}$, which was better than application of 30 kg P_2O_5 ha^{-1}. The interaction of N sources and P rates had a significant effect ($P < 0.05$) on the amount of N_2 fixed. The result presented in Fig. 4.5 shows that HISTICK, NC 92, SAMFIX 703 , SAMFIX 703 + N20 and the control responded positively to the application of P at 60 kg P_2O_5 ha^{-1} by fixing statistically higher amounts of nitrogen than application at 30 kg P_2O_5 ha^{-1} which gave similar values than the no P treatments.

Table 4.5. Effect of Nitrogen Sources and Phosphorus Fertilizer on Biological Nitrogen Fixation by Groundnut

Treatment	N fixed (mg plant^{-1})	%Ndfa
N sources		
0 kg N ha^{-1}	210def	30.6efg
20 kg N ha^{-1}	350abc	41.2bcd
40 kg N ha^{-1}.	390a	50.6a
NC 92	300a-d	40.0bcd
SBG 234	210def	34.3edf
KBU 26	110f	23.0g
SNN 343	140ef	28.1fg
SAMFIX 703	240cde	38.3cde
HISTICK	250b-e	38.9cde
NC 92+20N	320a-d	41.6a-d
SBG 234+20N	360ab	46.9abc
KBU 26+20N	330abc	44.3abc
SNN 343+20N	370ab	49.3ab
SAMFIX 703+20N	330abc	43.1a-d
HISTICK+20N	270bcd	40.0bcd
SE±	42.000	2.884
P rates (k g P_2O_5 ha^{-1})		
P0	140c	27.4b
P30	220b	29.2b
P60	470a	61.5a
SE±	16.000	1.293
Interaction		
N*P		
SE±	61.000	4.980
Significance	*	*

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). Ndfa= Nitrogen derived from atmosphere,
*= significant at 5% probability

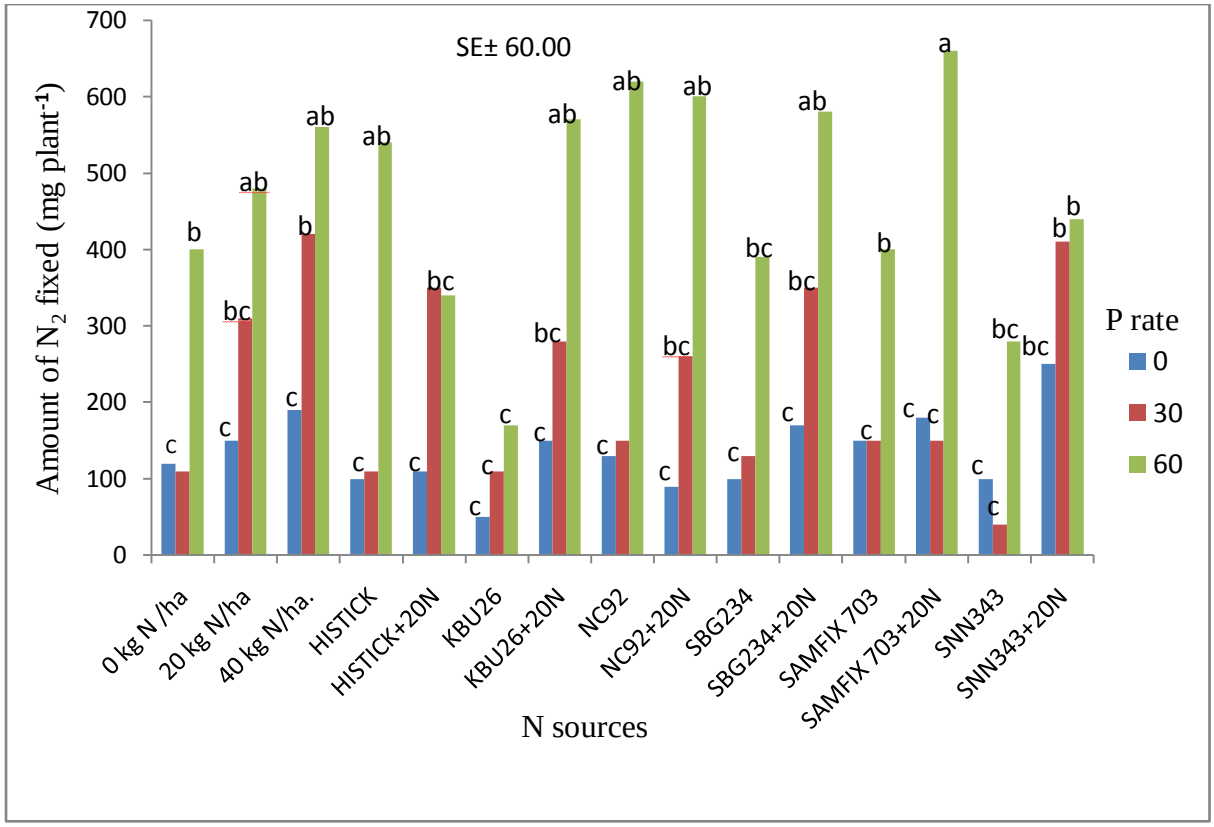


Fig. 4.5: Interaction of N sources and P fertilizer on the amount of N_2 fixed by groundnut

4.5.2 Percent nitrogen derived from atmosphere (% Ndfa)

The trend of results observed for % Ndfa was similar to the amount of N_2 fixed (Table 4.5). Generally, increase in N_2 fixation gave a corresponding increase in % Ndfa. The effect of N sources was highly significant ($P < 0.01$) on the % Ndfa of plants. The inoculation plus mineral fertilizer at 20 kg N ha^{-1} treatments derived an average of 44.2 % of their nitrogen from the atmosphere and were statistically better than the control. The use of inoculants alone on the other hand, did not significantly increase % Ndfa when compared to the control.

Similarly, application of P fertilizer had a highly significant effect ($P < 0.01$) on the % Ndfa. The trend observed was similar to that of N_2 fixed. Even though application of 30 kg P_2O_5 ha^{-1} did not produce a significant increase in % Ndfa when compared with the control, application of 60 kg P_2O_5 ha^{-1} more than doubled the % Ndfa (from 27.38 to 61.48 %). The interaction between N sources and P fertilizer was significant ($P < 0.05$) on % Ndfa (Fig. 4.6). The interaction of 60 kg P_2O_5 ha^{-1} with 20 kg N ha^{-1}, 40 kg N ha^{-1}, HISTICK, KBU 26 +20N, NC 92, NC 92 +20N, SBG 234, SAMFIX 703, and SAMFIX 703 + 20N significantly increased the % Ndfa of the plants. No significant increase however was observed in the interaction of 30 kg P_2O_5 ha^{-1} with most of the N sources when compared with the no P treatments.

4.6 Effect of Nitrogen Sources and Phosphorus Fertilizer on Phosphorus Use Efficiency of Groundnut

4.6.1 Phosphorus uptake efficiency (PUE)

The differences observed in PUE of groundnut with application of N sources were significant ($P < 0.05$) as shown in Table 4.6. Plants inoculated with SBG 234, SAMFIX 703, HISTICK, NC 92 + 20N and SBG 234 + 20N had higher and better uptake efficiency than the control. Inoculation alone increased uptake efficiency by an average of 29.30 % while the addition

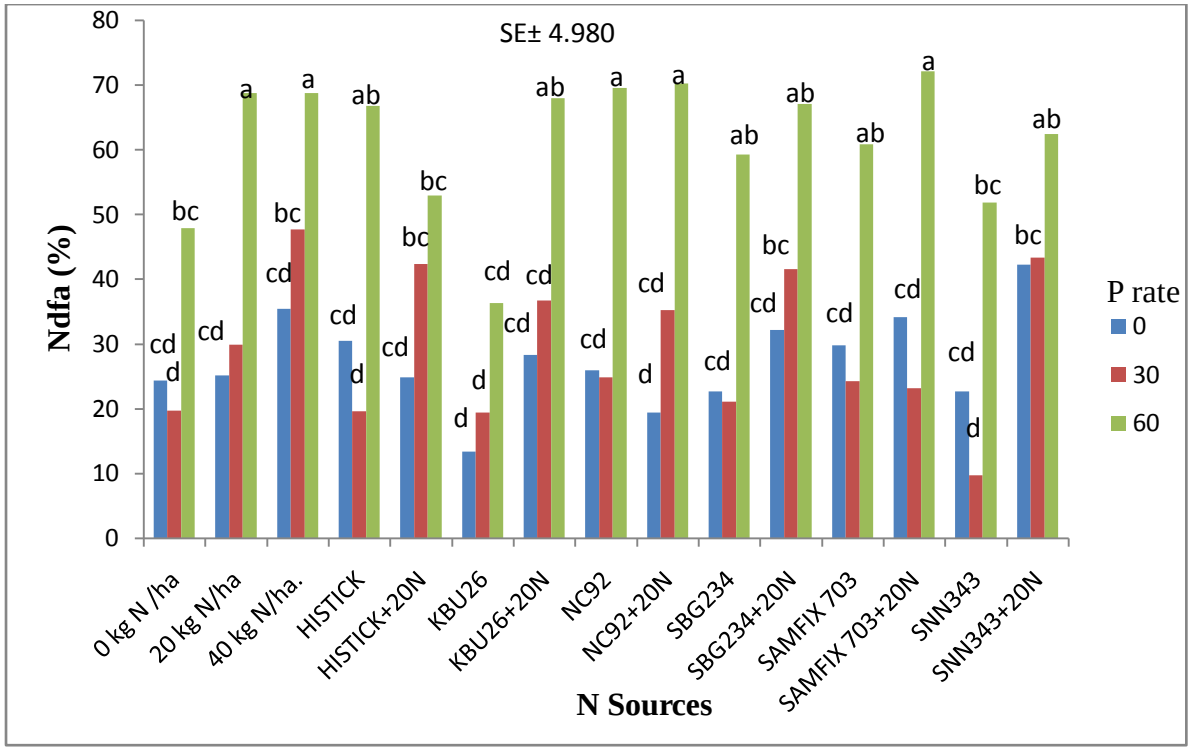


Fig.4.6. Interaction of N sources and P fertilizer on Percent nitrogen derived from atmosphere by groundnut.

of 20 kg N ha^{-1} to inoculants as starter nitrogen dose gave an average increase of 35.37 % over the control.

Remarkable differences in PUE were also observed with application of Phosphorus. The result however showed an inverse relationship between PUE and P rates. A sharp decrease in PUE was observed with increase in phosphorus application. Application of 30 kg P_2O_5 ha^{-1} gave a significantly higher value of PUE than application of 60 kg P_2O_5 ha^{-1}. Phosphorus uptake efficiency was not significantly affected ($P > 0.05$) by the interaction of N sources and P fertilizer.

4.6.2 Phosphorus agronomic efficiency (PAE)

The results of PAE followed a similar trend with that of PUE but not without some exceptions. Application of N sources significantly affected ($P < 0.05$) PAE. Only plants inoculated with NC 92, NC 92 + 20N and SNN 343 + 20N had significantly higher PAE than the control. All the other treatments were similar to the control. Generally, an average of 8.71 % increase in PAE was obtained with the use of inoculants alone while the combination of inoculants with 20 kg N ha^{-1} gave an average increase of 16.38 %.

Phosphorus fertilizer had a highly significant effect ($P < 0.01$) on phosphorus agronomic efficiency. The effect was however inverse as shown in the result. The agronomic efficiency of the plants decreased with application of 60 kg P_2O_5 ha^{-1} when compared with the application of 30 kg P_2O_5 ha^{-1}. There was no significant interaction ($P > 0.05$) between N sources and phosphorus fertilizer.

4.7 Effects of Phosphorus Fertilizer and Residual Effects of Rhizobium Inoculants on Root and Shoot Dry Weights of Groundnut

4.7.1 Root dry weight

The result of the root dry weight shows that the residual effect of rhizobial inoculants significantly affected ($P < 0.05$) root dry weight (Table 4.7). However, all the treatments were

Table 4.6. Effect of Nitrogen Sources and Phosphorus Fertilizer on Phosphorus Efficiency of Groundnut

Treatments	PUE (%)	PAE (g/g)
Nitrogen Sources		
0 kg N ha^{-1}	12.33e	89.69de
20 kg N ha^{-1}	13.17de	87.89e
40 kg N ha^{-1}.	15.50b-e	104.47a-d
NC 92	14.33dce	106.51a-c
SBG 234	17.50ab	103.90a-d
KBU 26	14.67b-e	94.40b-e
SNN 343	15.33b-e	91.03cde
SAMFIX 703	16.83a-d	92.67cde
HISTICK	17.00a-d	96.54a-e
NC 92+20N	19.83a	107.75ab
SBG 234+20N	18.33ab	102.45a-e
KBU 26+20N	15.33b-e	100.79a-e
SNN 343+20N	16.33b-e	110.04a
SAMFIX 703+20N	15.50cde	100.23a-e
HISTICK+20N	14.83b-e	101.05a-e
SE±	1.100	4.416
P rates (kg P_2O_5 ha^{-1})		
P0	NA	NA
P30	19.62a	128.62a
P60	11.96b	69.97b
SE±	0.400	1.613
Interaction		
N*P		
SE±	1.700	6.245
Significance	NS	NS

Means with the same letter(s) within a treatment group are not significantly different ($P \leq 0.05$) using DMRT (Duncan Multiple Range Test). PAE= Phosphorus agronomic efficiency, PUE= Phosphorus utilization efficiency, NS= Not significant at 5% level of probability, NA=Not applicable

Table 4.7. Effects of Phosphorus Fertilizer and Residual Effects of Rhizobial Inoculants on Root and Shoot Dry Weights of Groundnut

Treatment	Root dry wt (g/plant)	% increase or decrease	Shoot dry wt (g/plant)	% increase or decrease
N sources				
Control	0.40b	- 22.08	3.48	- 45.79
NC 92	0.45ab	- 8.16	3.48	- 49.78
SBG 234	0.55a	+ 7.84	3.60	- 45.21
KBU 26	0.54ab	+ 22.21	3.29	- 48.02
SNN 343	0.58a	+ 20.65	3.46	- 42.71
SAMFIX 703	0.51ab	+ 17.58	3.25	- 42.71
HISTICK	0.49ab	+ 2.04	3.11	- 49.18
SE±	0.040		0.210	
Prates(kg P_2O_5 ha^{-1})				
0	0.47	NA	2.76c	NA
30	0.51	NA	3.34b	NA
60	0.53	NA	4.03a	NA
SE±	0.020		0.091	
Interaction N*P				
SE±	0.081	NA	0.372	NA
Significance	NS	NA	NS	NA

Means with the same letter(s) within a treatment group are not significantly different ($P \leq 0.05$) using DMRT (Duncan Multiple Range Test). NS= Not significant at 5% level of probability, NA = Not applicable.

statistically similar to the control. Root dry weight was seen to decrease in the control and treatment NC 92 by 22.08 and 8.16 % respectively , while the residual effect SBG 234, KBU 26, SNN 343 and HISTICK increased root dry weight by 2 – 22 %.

Phosphorus rates had a highly significant effect ($P < 0.01$) on the root dry weight of plants. Root dry weight increased with increasing P rates. Application of 60 kg P_2O_5 ha^{-1} produced a significantly higher root dry weight than P at 30 kg P_2O_5 ha^{-1}. Both however, were better than the control.

4.7.2 Shoot dry weight

The shoot dry weight of the groundnut plants was not significantly affected ($P > 0.05$) by the residual effect of rhizobial inoculation. However, compared with the first trial, the residual effect of inoculation decreased shoot dry weight by an average of 46.3 % across all the treatments.

Application of phosphorus had a highly significant effect ($P < 0.01$) on shoot dry weight of plants where application of 30 kg P_2O_5 ha^{-1} and 60 kg P_2O_5 ha^{-1} significantly increased shoot dry weight of plants by 21 % and 46 % respectively when compared with the control (0 kg P_2O_5 ha^{-1}).

4.8 Effect of Phosphorus Fertilizer and Residual Effects of Rhizobial Inoculants on Nodulation of Groundnut

4.8.1 Nodule number

The result in Table 4.8 shows that application of rhizobial inoculants had a significant effect ($P < 0.05$) on nodule number. The residual effect of HISTICK again produced the highest number of nodules which was similar to the control, NC 92, SBG 234, KBU 26 and SAMFIX 703. SNN 343 produced fewer numbers of nodules than the control. When compared with the first trial, nodule number decreased by an average of 45 % across all treatments when plants depended on the residual effects of the inoculants.

Table 4.8. Effects of Phosphorus Fertilizer and Residual Effects of Rhizobial Inoculants on Nodulation of Groundnut

Treatment	Nodule No plant^{-1}	% increase or decrease	Nodule fresh wt (mg plant^{-1})	% increase or decrease	Nodule dry wt (mg plant^{-1})	% increase or decrease
N sources						
Uninoculated	85ab	- 33.59	130ab	- 27.78	50	- 28.57
NC 92	76abc	- 40.15	120ab	- 42.85	40	- 55.55
SBG 234	70bc	- 44.00	120ab	- 58.62	30	- 62.50
KBU 26	60bc	- 46.43	90b	- 52.63	40	- 42.86
SNN 343	55c	- 59.55	110ab	- 47.62	30	- 66.67
SAMFIX 703	61bc	- 53.79	90b	- 50.00	30	- 62.50
HISTICK	98a	- 37.18	150a	- 34.78	50	- 54.55
SE±	8.92	NA	20.00	NA	6.00	NA
Prates (kg P_2O_5 ha^{-1})						
0	44c	NA	60c	NA	20c	NA
30	75b	NA	110b	NA	40b	NA
60	98a	NA	170a	NA	60a	NA
SE±	3.99	NA	7.00	NA	3.00	NA
N*P						
SE±	15.26	NA	30.00	NA	10.00	NA
Significance	NS	NA	*	NA	NS	NA

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). NS= Not significant at 5% level of probability, *= significant at 5% probability, ** significant at 1 % probability. NA= Not applicable.

Application of phosphorus also had a highly significant effect ($P < 0.01$) on nodule number. Application of P at 30 Kg P_2O_5 ha^{-1} increased nodule number by 71 % while application at 60 Kg P_2O_5 ha^{-1} doubled the number of nodules produced. The interaction of the residual effect of rhizobial inoculants and P fertilizer was not significant ($P > 0.01$).

4.8.2 Nodule fresh weight

The residual effect of rhizobial inoculants had a highly significant effect ($P < 0.01$) on the nodule fresh weight of plants. All the treatments had similar nodule fresh weight to the control except KBU 26 and SAMFIX 703 that had lower weight than the control. When compared with the first trial, nodule fresh weight decreased by an average 45 % across all the treatments.

Application of phosphorus also significantly affected ($P < 0.01$) nodule fresh weight where nodule fresh weight increased linearly with increase in P rates. Application of P at 30 kg P_2O_5 ha^{-1} increased the weight from 60 – 110 mg $plant^{-1}$ while application at 60 kg P_2O_5 ha^{-1} increased nodule fresh weight from 60 – 170 mg $plant^{-1}$. The interaction of P fertilizer and the residual effect of rhizobial inoculants had a significant effect ($P < 0.05$) on nodule fresh weight. Fig 4.7 shows the result of the interaction where the absolute control (0 kg N ha^{-1} and 0 kg P_2O_5 ha^{-1}) produced nodule fresh weight similar to the interactions with 60 kg P_2O_5 ha^{-1}.

4.8.3 Nodule dry weight

The nodule dry weight of plants was not significantly affected ($P > 0.05$) by the residual effect of rhizobial inoculants. On average, a decrease of 53 % was recorded across all treatments when plants depended on the residual effect of rhizobial inoculation.

Phosphorus application on the other hand, had a highly significant effect ($P < 0.01$) on nodule dry weight. Nodule dry weight was higher by 100 % due to application of 30 kg P_2O_5 ha^{-1} by 200 % by application of 60 kg P_2O_5 ha^{-1} when compared with the no P treatment.

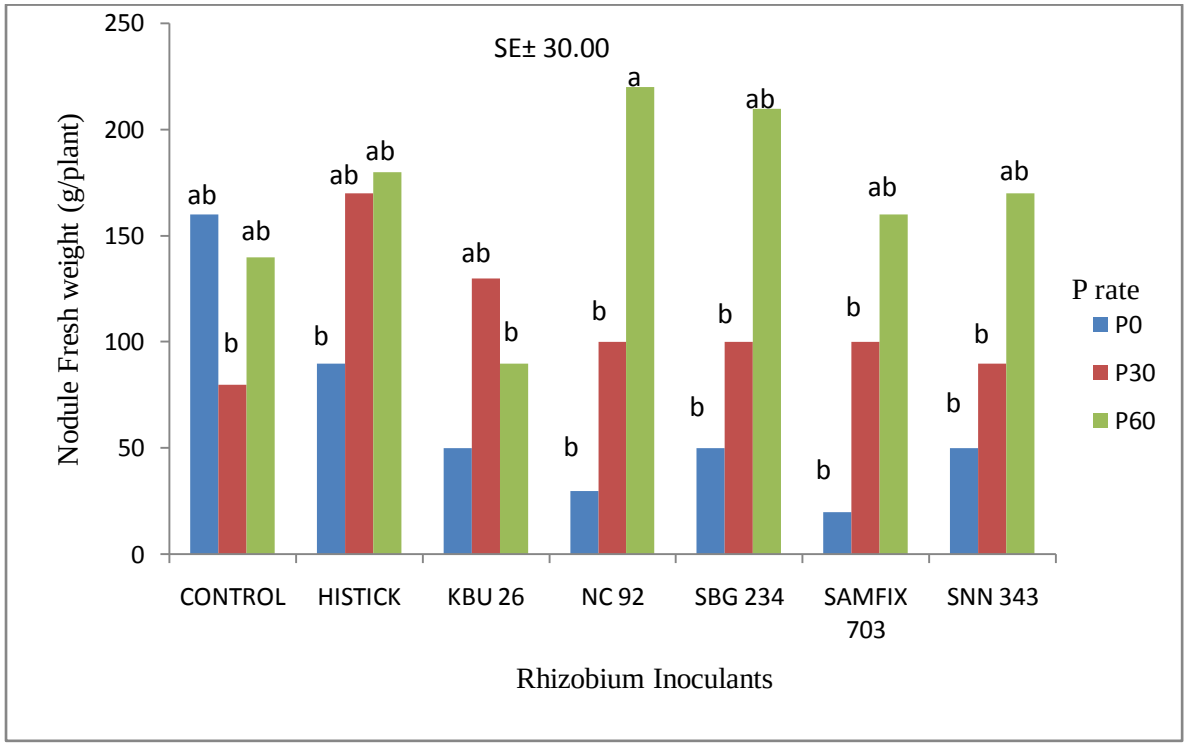


Fig 4.7. Interaction of phosphorus fertilizer and the residual effect of rhizobial inoculants on nodule fresh weight of groundnuts

4.9 Effects of Phosphorus Fertilizer and Residual Effects of Rhizobial Inoculants on Biological Nitrogen Fixation by Groundnut

4.9.1 Amount of N_2 fixed

Table 4.9 shows the result of the amount of N_2 fixed where the residual effects of rhizobial inoculants had no significant effect ($P > 0.05$) on the amount of N_2 fixed by plants. However, when compared with the first trial, the amount of N_2 fixed decreased by an average of 34 % across all treatments except KBU 26 where there was an increase of 18.1 %. Similarly, P fertilizer had no significant effect ($P > 0.05$) on the amount of N_2 fixed.

4.9.2 Percent nitrogen derived from atmosphere (% Ndfa)

A significant difference ($P < 0.05$) was observed in the % Ndfa when plants depended on the residual effect of rhizobial inoculation. All the treatments had similar values of % Ndfa as the control except SNN 343 which had lower value than the control. Comparing the result with that of the first trial, the % Ndfa of the control and treatment KBU 26 increased by 35.62 % and 52.83 % respectively, while all the other treatments recorded a decrease with treatment SNN 343 recording a decrease as high as 52 %.

Application of phosphorus significantly affected ($P < 0.05$) % Ndfa. Application of 30 kg P_2O_5 ha^{-1} significantly increased % Ndfa of plants but application of 60 kg P_2O_5 ha^{-1} gave values statistically similar to the control. The interaction P fertilizer and the residual effect of inoculation was not significant ($P > 0.05$).

Table 4.9. Effects of Phosphorus Fertilizer and Residual Effects of Rhizobial Inoculants on Biological Nitrogen Fixation by Groundnut

Treatment	N_2 fixed (mg plant^{-1})	% increase or decrease	% Ndfa	% increase or decrease
N sources				
Control	120	- 42.85	41.5a	+ 35.62
NC 92	110	- 63.33	31.5ab	- 21.25
SBG 234	190	- 9.52	40.4a	- 17.78
KBU 26	130	+ 18.18	35.0a	+ 52.83
SNN 343	70	- 50.00	12.1b	- 56.79
SAMFIX 703	200	- 16.67	32.7ab	- 14.62
HISTICK	150	- 40.00	39.2a	- 0.77
SE±	150.00	NA	7.05	NA
Prates(kg P_2O_5 ha^{-1})				
0	220	NA	12.43b	NA
30	200	NA	76.59a	NA
60	290	NA	10.77b	NA
SE±	70.00		3.15	
Interaction N*P				
SE±	260.00	NA	12.21	NA
Significance	NS		NS	

Means with the same letter(s) within a treatment group are not significantly different ($P \leq 0.05$) using DMRT (Duncan Multiple Range Test). Ndfa= percent nitrogen derived from atmosphere
NS= Not significant at 5% level of probability, NA = Not applicable

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physical, chemical and microbiological properties of the soil

5.1.1 Physical and chemical properties of the soil

The texture of the experimental soil was sandy loam which according to Raemaekers (2001) is suitable for groundnut production. The chemical parameters of the soil were rated based on the critical values for nutrients as given by Federal Ministry of Agriculture and Natural Resources (FMANR, 1990). The pH of the soil in water indicates that the soil is slightly acidic and falls within the range adequate for groundnut production (ICRISAT, 1995). The organic carbon and total nitrogen were low which is typical of savannah soils (Jones and Wild, 1975). Cultivation of legumes e.g groundnuts on such a soil can help to improve the organic carbon and nitrogen contents of the soil as well as reduce nitrogen fertilizer application to meet the needs of plants (Giller, 2010). The available phosphorus of the soil was low and therefore poses a threat to groundnut production (Weisany *et al*., 2013), hence the reason for the sharp response observed in this study with increasing application of phosphorus for most plant parameters. The values of exchangeable bases ($cmolkg^{-1}$) indicated low status of Ca^{2+}, moderate concentration of Mg^{2+}, K^{+} and Na^{+} . Effective cation exchange capacity (ECEC) was low. Generally, the soils properties were typical characteristic of Alfisols of northern Guinea savanna of Nigeria as described by Odunze (2006).

5.1.2 Rhizobial counts in the experimental soil

The rhizobial count of the soil as obtained from the most probable number showed that the population of viable rhizobia in the indigenous soil was low. The nature of soil rhizobial populations may affect the N_2 fixing potential of legumes and in soils where population of

indigenous rhizobia is less than 2 x 10^1 cells g^{-1}, inoculation is required (Singleton and Tavares, 1986). The major aim of inoculation is to increase the number of desirable strains of rhizobia in the rhizosphere. The provision of this external source of rhizobia enables effective nodulation and N_2 fixation (Date, 2000).

5.2 Rhizobial Counts in the Inoculants Used

The results obtained for the number of cells found in the inoculants used were all lower than the recommended number of viable cells expected in high quality inoculant which was estimated at 1.0 x 10^9 ml^{-1} (Woomer *et al*., 2010). The low numbers may affect the performance of the inoculants. Other factors that affect the performance of rhizobial inoculants include: the competitive ability of the inoculants strains, compatibility with agrochemicals, ability to survive under a wide range of soil physical or chemical constraints and storage conditions of the inoculants (Herridge *et al*., 2002; O' Hara *et al*., 2002).

5.3 Effect of Nitrogen Sources and Phosphorus Fertilizer on Dry Matter Yield of Groundnut

5.3.1 Root and shoot dry weight

Plant dry weight is usually well correlated to effectiveness to N_2 fixation. The use of rhizobial inoculants in this study did not significantly influence shoot and root dry weight. This could be due to the inability of the inoculants strains to outcompete the native strains. This finding is in conformity to the result obtained in a study conducted by Yusuf *et al*. (2011), where there was no significant difference in shoot dry matter yield between inoculated and uninoculated plants which they attributed to the ineffectiveness of the inoculants strains, but differs with the findings of Mohamed and Abdallah (2013) and Latif *et al*. (2014) who indicated that rhizobial inoculation significantly increased shoot and root dry weights in groundnuts.

When rhizobial inoculants were combined with mineral fertilizer as starter dose, SNN 343 + 20N produced better dry matter than the control while the others did not. This could mean that the strains of the inoculant have more competitive ability than the other inoculants. The slight increase recorded generally in the shoot dry matter due to the combination of inoculants with mineral nitrogen could be attributed to the promotive effect of mineral nitrogen on stem growth and leaf production and enlargement.

Shoot and root dry matter yield increased linearly with increase in phosphorus rates and this increase could be attributed to the beneficial effect of phosphorus on plant growth through promotion of early root development and the formation of lateral, fibrous and healthy roots (Badar *et al*., 2015). Phosphorus is also an essential nutrient for efficient growth and yield improvement of groundnut (Hemalatha *et al*., 2013). This current study agrees with the findings of Aziz *et al*., (2016) who stated that phosphorus application of 22.5 and 45 kg P_2O_5 ha^{-1} significantly enhanced biomass production in soybean. The interaction of N sources and P fertilizer on shoot dry weight reveals the ability of most of the inoculants to produce a significant increase in root dry matter weight even in the absence of phosphorus. This projects them to be useful in P-deficient soils especially that no additional cost of P will be incurred.

5.4 Effect of Nitrogen Sources and Phosphorus Fertilizer on Nodulation of Groundnut

5.4.1 Nodule number per plant

The number and weight of nodules are commonly used as the criteria of effective complementary interaction between legume and micro- symbionts (Tahir *et al*., 2009). The findings in this study as it relates to nodule number reveal that application of mineral nitrogen at 20 kg N ha^{-1} in combination with rhizobial inoculants affected the number of nodules per plant when compared with the control. Even though the difference was not significant, nodule number was found to have decreased and this could be due to the suppressing effect of

inorganic N on nodulation of legumes (Herridge *et al*.,1984). Oteino *et al*. (2007) reported that application of 20 kg N ha^{-1} depressed nodulation in soybean. Fagam *et al*. (2007) also reported a decrease in number of nodules with increased levels of nitrogen at 30, 60 and 90 kg N/ha of soybean varieties cultivated in the same study area. Sogut *et al*. (2013) observed a decrease in number of nodules of groundnuts when rhizobial inoculant was combined with 50 kg N ha^{-1} than inoculation alone. Contrary to these findings, Malik *et al*. (2006) reported that application of 30 kg N ha^{-1} as starter dose did not affect nodulation of soybean grown under phosphorus nutrition. Njobdi (1990) also reported that 20 kg N ha^{-1} of nitrogen fertilizer was the best level of obtaining yield in cowpea without comprising nodulation. However, he noted that application of nitrogen at 40 kg N ha^{-1} depressed both yield and number of nodules but significantly increased vegetative growth. Abayomi *et al*. (2008) also obtained higher yield and nodulation with the application of nitrogen at 30 kg N ha^{-1} than at 60 kg N ha^{-1}.

On the other hand, Similarity was observed in the number of nodules produced by the use of inoculants alone and the control and this is in line with the report of Aliyu *et al*. (2013) who in their inoculation study using five different strains recorded no significant difference in nodule number among the inoculated and the uninoculated control in groundnuts, while Mweetwa *et al*. (2014) observed that inoculating groundnut seeds with Biofix resulted in significantly more nodules per plant than the uninoculated control.

The significant enhancement in nodulation observed with the application of phosphorus in this study underlines the influence phosphorus has on nodule development through its basic function as an energy source. The number of nodules per plant was seen to increase with increase in phosphorus rates. This is in line with the findings of Aziz *et al*. (2016) who recorded a significant enhancement in nodulation with P application and that the degree of enhancement was dependent on the rate of P applied. In their study, application of 22.5 and 45 kg P_2O_5 ha^{-1} significantly increased number of nodules by 11.85 and 21.71 % respectively.

Amba *et al*. (2013) also claimed that application of phosphorus at 26.4 kg P ha^{-1} produced higher number of nodules than the no phosphorus treatment but observed that significantly fewer nodules were produced when the phosphorus level was raised to 39.6 kg P ha^{-1}.

5.4.2 Nodule fresh and dry weight.

It is a common expectation that higher nodule number should give rise to higher nodule fresh and dry weight. Visual observation during the experiment showed that treatments like HISTICK that produced high nodule number tend to have smaller nodules than the uninoculated treatments with fewer number of nodules. This could be due to the mechanism observed on groundnut by Singleton and Taveres (1986) that plant- controlled mechanisms appear to compensate for reduced nodulation when there are few rhizobia in the soil by increasing the average size of nodules above that of inoculated plants with more nodules and therefore maintaining a nodule dry weight similar to that of inoculated plants with more nodules.

The effect of phosphorus in enhancing nodulation is again clearly visible in the results of the nodule fresh and dry weights. This could easily be related to the low initial available P content in the experimental soil which gave rise to a high response to P fertilization. This also accounts for the high increase in nodule fresh and dry weight with increased phosphorus fertilization Irrespective of the N source applied. Contrarily, Ndlovu (2015) did not record any increase in nodule number and nodule dry weight with phosphorus fertilization at 0, 45 and 90 kg ha^{-1} and he related it to the high initial available P in the soil during the growing season.

5.5 Effect of Nitrogen Sources and Phosphorus Fertilizer on Biological Nitrogen Fixation by Groundnut

5.5.1 Amount of `N_2 fixed and % Ndfa

The use of inoculants alone did not significantly increase the amount of `N_2 fixed. This may be because the number of rhizobia introduced during this study may not have been sufficiently large to out-compete native rhizobia. The number of cells found in the inoculants used were all lower than the recommended number of viable cells expected in high quality inoculant which was estimated at 1.0×10^9 ml^{-1} (Woomer *et al*., 2010). Most of the inoculant plus mineral fertilizer treatments fixed higher amount of nitrogen and had higher % Ndfa than the control which suggests that the use of starter dose of mineral nitrogen alongside rhizobial inoculants enhanced the overall performance of the plants. This projects the importance of starter nitrogen which according to Kucey (1989), is necessary due to the lag period between rhizobium colonization and the onset of nodule functioning. The young legume plants require adequate N from external sources in order to achieve vegetative growth and establishment of the N fixing symbiosis. Generally, the indigenous inoculants competed with the commercial inoculants in nitrogen fixation and this indicates that these indigenous inoculants have comparable symbiotic effectiveness to commercial inoculants and should be tested further using other groundnut varieties to assess their potential.

When legumes depend on symbiotic nitrogen fixation and receive an inadequate supply of phosphorus, they may suffer from nitrogen deficiency as a result of poor N_2 fixation (Weisany *et al*., 2013). The sharp response to P application observed in this study with respect to N_2 fixed and % Ndfa can be attributed to the low initial available P content of the soil used and further confirms the effect of P in nitrogen fixation. This observation is in line with that of Yakubu *et al*. (2010) who observed an increase in % Ndfa from 69.33 in the control to 85.01 in cowpea that received 40 kg P ha^{-1}. Aziz *et al*. (2016) also reported an increase in the amount of N_2 fixed with phosphorus application. Phosphorus is involved in

several energy transformation and biochemical reactions including nitrogen fixation (Kwari, 2005).

5.6 Effect of Nitrogen Sources and Phosphorus Fertilizer on Phosphorus Use Efficiency of Groundnut

Efficiency concepts in plant mineral nutrition have been defined based on the process by which plants acquire, transport, store and use the nutrient in order to produce dry matter or grain, at low or high nutrient supply (Ciarelli *et al*., 1998). Nutrient acquisition efficiency and nutrient internal utilization efficiency are the two major components of plant nutrient use efficiency. These two components are related to the ability of the plant to acquire nutrient from the soil and to plants internal ability to produce yield units per unit nutrient in plant (Good *et al*., 2004).

The use of rhizobial inoculants brought about enhanced uptake of phosphorus by plants. The general enhancement observed in P uptake could be due to the activities of the rhizobia in the inoculants. Microbial activity in the rhizosphere can increase P availability by both lowering the pH and solubilizing iron –bound and aluminium – bound P, probably by complexing (or chelating) the iron (Fe) and aluminium (Al) (Syers *et al*., 2008). This therefore suggests that the use of rhizobial inoculants in growing groundnuts can increase P uptake which will inturn enhance phosphorus use efficiency of the plants. This result is in close conformity with that of Kakar *et al*. (2002) who found that inoculation increased Phosphorus Uptake Efficiency (PUE), but differed with the findings of Aziz *et al*. (2016) who reported that application of rhizobial inoculants had no significant effect on phosphorus uptake efficiency.

Phosphorus uptake efficiency was higher when P was applied at the rate of 30 kg P_2O_5 ha^{-1} than at 60 kg P_2O_5 ha^{-1}. This indicates that increasing rate of phosphorus application increases phosphorus uptake inefficiency. The utilization of nutrients decreases with increasing rate of nutrient application as stated by the law of limiting factors (Hussein, 2009).

This observation is similar to the work of Khair *et al*. (2002) who reported that P uptake efficiency decreased with increasing rate of applied P in uninoculated plots but increased in inoculated plots at NIFA. The efficiency of the applied P in this study was within the range of 12.22 - 20.30 %. This is lower than what was reported by Abdul-latif (2013) who reported an uptake efficiency of 22.22 - 33.33%.

5.7: Effects of Phosphorus Fertilizer and Residual Effects of Rhizobial Inoculants on Dry Matter Yield of Groundnut

5.7.1 Shoot and root dry matter

Previous inoculation slightly increased root dry matter over the first trial in four of the six inoculants but the shoot dry matter was decreased by 46.3 % when compared with the first trial. The inability of the residual effect of most of the inoculants to improve the shoot and root dry matter of the groundnut plants could be an indication that the introduced rhizobia did not persist in the soil. Sanginga *et al*. (1996) conducted an experiment in the moist savanna of Nigeria to determine the relationships between growth response to previous rhizobial inoculation and the indigenous rhizobial populations. They reported that previous inoculation increased shoot dry matter production by an average of 32 % over the uninoculated controls only in 94 of the 312 (30 %) legume inoculations and farmers' field combinations and that the response to previous inoculation treatments was farmers' fields dependent and inversely related to the numbers of rhizobia in the soil.

The observed increase in shoot dry weight with increase in phosphorus application could be due to active involvement of P in carbohydrate metabolism which helps in putting more vegetative growth (Patel *et al*., 1990). This finding agrees with that of Prasad *et al*. (1996) who reported a significant increase in dry matter production due to phosphorus application at 40 kg P_2O_5 ha^{-1}.

5.8 Effects of Phosphorus Fertilizer and Residual Effects of Rhizobial Inoculants on Nodulation of Groundnut

Nodulation parameters in general were seen to decrease when plants depended on the residual effect of inoculation. Nodule number decreased by 45 %, nodule fresh weight by 45 % and nodule dry weight by 53 % when compared with the first trial. Zengeni *et al*. (2006) assessed the persistence of the soyabean rhizobial inoculant strain MAR 1491 in 52 soils in Zimbabwe. The soils have been inoculated 1-4 or 6 years previously. From their study, MPN estimates of rhizobia in the soils showed that population sizes decreased with increasing time since the last inoculation. They attributed the greater rhizobial persistence in one of the soils to the higher clay (> 20 %) and organic C (> 1 %) content of the soil compared with the sandier soils and concluded that farmers can benefit from using cattle manure to enhance rhizobial survival and persistence in sandy soils, increasing the period before the next inoculation is required. Contrarily, Robert and Schmidt (1983) examined the persistence of an inoculant strain of *Rhizobium phaseoli* and its ability to compete with a resident population of *R. phaseoli* for nodule occupancy. They found out that the introduced strain *R. phaseoli* Viking 1 persisted, even in fallow soil, to produce abundant nodulation of host plants the following spring.

The ability of phosphorus application to enhance all the nodulation parameters measured underlines the influence phosphorus has on nodule development through its basic function as an energy source. P plays a significant role in early formation of roots, their proliferation and increased microbial activity in the root nodule (Hemalatha *et al*., 2013).

5.9 Effects of Phosphorus Fertilizer and Residual Effects of Rhizobial Inoculants on Biological Nitrogen Fixation by Groundnut

The amount of N_2 fixed and % Ndfa decreased in five of the six inoculants when plants depended on the residual effect of previous inoculation. This can be traced to the inability of the introduced strains to persist in the soil. However, the 18.1 % increase observed in the

amount of N_2 fixed and 53 % increase in % Ndfa arising from the residual effect of KBU 26 is outstanding. This implies that the strains of KBU 26 persisted in the soil and thus should be studied further to assess its full potential which may constitute an evident advantage over the use of inorganic nitrogen fertilizer which has to be applied frequently for consistent high yields. Wigley *et al*. (2015), in a study to determine the changes in rhizobial population over time in inoculated and uninoculated lucerne plants using a commercial inoculant, reported that the commercial inoculant was dominant in the nodules of lucerne plants grown from peat and coated seed three years after sowing.

CHAPTER SIX

6.0 SUMMARY CONCLUSION AND RECOMMENDATION

This study was designed to investigate the comparative response of groundnut to rhizobial inoculation and nitrogen fertilizer with or without phosphorus fertilizer on an Alfisol in the Northern Guinea savannah of Nigeria. Soil samples were collected from a P-deficient plot on the Institute for Agricultural Research /Faculty of Agriculture (IAR/FOA) experimental farms located in Samaru Zaria and used to conduct two screen house trials using the groundnut genotype "SAMNUT 24" as the test crop. The first experiment consisted of six (6) inoculants (four indigenous rhizobial strains namely: SNN 343, KBU 26, SBG 234, SAMFIX 703, one commercial inoculant (HISTICK) and a reference strain (NC 92) alone, each inoculant combined with 20 kg N ha^{-1} as starter dose, three levels of mineral nitrogen (0, 20, or 40 kg N ha^{-1}) all termed as N sources and three rates of phosphorus (0, 30 or 60 kg P_2O_5 ha^{-1}). The N sources (SNN 343, KBU 26, SBG 234, SAMFIX 703, NC 92, HISTICK, SNN 343 +20 N, KBU 26 +20 N, SBG 234 +20 N, SAMFIX 703 +20 N, NC 92 +20 N, HISTICK +20 N, 0 kg N ha^{-1}, 20 kg N ha^{-1} and 40 kg N ha^{-1}) and P rates (0 kg P_2O_5 ha^{-1}, 30 kg P_2O_5 ha^{-1} and 60 kg P_2O_5 ha^{-1}) were factorially combined to give a total of 45 treatments and laid down in a Randomized Complete Block Design (RCBD) replicated three times. The second trial was set up to assess the residual benefit of rhizobial inoculation on following crops. Soils previously inoculated with SNN 343, KBU 26, SBG 234, SAMFIX 703, NC 92 and HISTICK and a control, were combined with three levels of P (0, 30 or 60 kg P_2O_5 ha^{-1}) factorially to give a total of 21 treatments. The RCBD was also used with three replications.

Addition of 20 kg mineral nitrogen per hectare to rhizobial inoculation as starter dose reduced nodule number by an average of 12.2 % but increased groundnut shoot dry weight by an average of 9.5 % and 9.8 % compared to inoculation alone and control respectively. The use

of this starter dose of mineral nitrogen also increased the amount N_2 fixed by 58.4 % compared to inoculation alone and by 51.7 % compared to the control. A similar trend was also observed for % Ndfa. Generally, most of the indigenous inoculants fixed higher amount of N_2 when combined with a starter dose of mineral N than when used alone.

The application of phosphorus fertilizer significantly increased all the parameters measured but it was observed that supplying groundnut with P at 60 kg P_2O_5 ha^{-1} increased yield components and nitrogen fixation more than P at 30 kg P_2O_5 ha^{-1}. However, the interactions of N sources and P fertilizer on most of the parameters measured reveals no significant differences between the two rates indicating that the application of P fertilizer at 60 kg P_2O_5 ha^{-1} may not be economically viable.

Phosphorus uptake efficiency of the groundnut crop was enhanced by the use of rhizobial inoculants and so also phosphorus agronomic efficiency which confirms the importance of microbial activity in the rhizosphere in solubilizing bound P and making it available for plant's uptake. Inoculation alone increased phosphorus uptake efficiency by 29.3 % while addition of starter nitrogen at 20 kg per hectare to inoculants gave an increase of 35.4 % compared to the control. With respect to P application, the results show that P supplied at the rate of 30 kg P_2O_5 ha^{-1} was more efficiently used by the crop than at 60 kg P_2O_5 ha^{-1}.

The residual effects of inoculation failed to produce a significant increase in most of the parameters measured. On average, shoot dry matter declined by 46.3 %, nodule number by 45 %, nodule dry weight by 53 % across all treatments when plants depended on the residual effect of the previous inoculation. Similarly the amount of nitrogen fixed decreased by an average of 34 % across all treatments except KBU 26 which indicates lack of persistence of the rhizobium strains and the need for inoculation with each sowing. KBU 26 amongst all the inoculants increased the amount of nitrogen fixed and % Ndfa by 18.1 % and 52.8 %

respectively compared to the first trial indicating that the strains persisted in the soil. This potential when fully assessed and harnessed may constitute an evident advantage over the use of inorganic nitrogen fertilizer which has to be applied frequently for consistent high yields.

In conclusion, BNF and yield components of SAMNUT 24 was slightly increased by the combined application of rhizobial inoculants and mineral nitrogen at 20 kg N ha^{-1} and as such, a starter dose of nitrogen at 20 kg N ha^{-1} is needed alongside rhizobial inoculants to boost nitrogen fixation in groundnuts. This study also reveals the ability of the indigenous inoculants to compete favourably with commercial inoculants in nitrogen fixation. Further screening of these indigenous inoculants is needed as this will help to address the problem of the unavailability of commercial inoculants to farmers and will also enhance the local production of inoculants.

Phosphorus use efficiency of the groundnut crop was enhanced by the use of rhizobial inoculants but was seen to decrease with increase in phosphorus rates. Even though P supply at 60 kg P_2O_5 ha^{-1} significantly increased yield components and BNF, the profitability of using 60 kg P_2O_5 ha^{-1} by farmers and other consequences on the environment is in question since the P was more efficiently used by the crop at the rate of 30 kg P_2O_5 ha^{-1}.

The residual effect of KBU 26 amongst all the inoculants increased the amount of nitrogen fixed and % Ndfa indicating that the strains persisted in the soil. This potential when fully assessed and harnessed may constitute an evident advantage over the use of inorganic nitrogen fertilizer which has to be applied frequently for consistent high yields. However, further research in the field needs to be carried out in order to validate the findings of this work.

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Appendix 1

Broughton and Dilworth N-free Plant Nutrient Solution

Stock Solutions	Element	M	Form	g/l	M
1	Ca	1000	$CaCl_2 \cdot 2H_2O$	294.1	2.0
2	P	500	KH_2PO_4	136.1	1.0
3	Fe	10	Fe-citrate	6.7	0.02
	Mg	250	$MgSo_4 \cdot 7H_2O$	123.3	0.5
	K	250	K_2SO_4	87.0	0.5
	Mn	1	$MnSO_4 \cdot H_2O$	0.338	0.002
4	B	2	H_3BO_3	0.247	0.004
	Zn	0.5	$ZnSO_4 \cdot 7H_2O$	0.288	0.001
	Cu	0.2	$CuSO_4 \cdot 5H_2O$	0.100	0.0004
	Co	0.1	$CoSO_4 \cdot 7H_2O$	0.056	0.0002
	Mo	0.1	$Na_2MoO_2 \cdot 2H_2O$	0.048	0.0002

Appendix 2

Yeast-Mannitol Agar (YMA)

Constituents

Mannitol	10.0 g
K_2HPO_4	0.5 g
$MgSO_4.7H_2O$	0.2 g
NaCl	0.1 g
Yeast Extract	0.5 g
Distilled Water	1.0 liter
Agar	15 g