UNIVERSITY FOR DEVELOPMENT STUDIES

EFFECTS OF VARIETY, INOCULANT AND PHOSPHORUS ON NODULATION AND YIELD OF GROUNDNUT (*Arachis hypogaea L.*) IN THE GUINEA SAVANNAH ZONE OF GHANA

BY

RICHARD NAABE YARO

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JULY, 2019

DECLARATION

I, Richard Naabe Yaro, hereby declare that, except for references to other people's work, which have been duly acknowledged, this thesis is the result of my own research work carried out in the department of Agronomy faculty of Agriculture, under the supervision of Dr. Mahama Rufai Ahmed and Dr. Joseph Xorse Kugbe. I further declare that, this thesis is entirely my own work and that no part of it has been presented for another degree elsewhere either in whole or in part for the award of any degree in this University or elsewhere.

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ABSTRACT

The experiment was conducted at Gurumanchaenyili and Zangbalun Fandu in the Tolon and Kumbungu districts, respectively in the Northern region of Ghana to assess the effect of variety, phosphorus fertilizer and inoculant on the nodulation and yield of groundnut, to solve the problem of low productivity in the production of the crop, in 2017 cropping season. Three farmers' fields were used and each farm represented a replicate. The experiment was a 2×3×3 factorial study laid out in randomized complete block design. Data collected included: soil chemical properties (soil pH, organic carbon, available P, exchangeable K, and total N), crop growth parameters (canopy spread, haulm weight, biomass, plant height, nodule number per plant at flowering, effective nodules, ineffective nodules, number of branches N,P,K content in plants) and yield parameters (pod number, pod weight and harvest index). The findings showed that the interaction of variety, phosphorus fertilizer and inoculant increased ($p < 0.05$) pod yield of groundnut (4200 kg/ha) over the control. Haulm weight, canopy spread at 8 WAP, plant height at 4 and 8 WAP were significantly affected by variety and inoculant interaction. Plant height at 4 WAP and pod weight was significantly increased by phosphorus fertilizer and inoculant interaction. Phosphorus fertilizer and inoculant significantly affected biomass, effective nodules and harvest index. For efficient pod yield and total biomass production, phosphorus fertilizer should be combined with rhizobium inoculant at a rate of 60 kg/ha P_2O_5 + 6 g/kg inoculant by farmers in the Northern zone of Ghana.

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DEDICATION

To Victoria Yaro my mother, my late father Mr. Emmanuel N. Yaro, Mrs. Rosemond Yaro my wife and children Richmond Yaro and Richael Yaro, Mr. Fred Yaro, Godwin Yaro and Coleman Yaro for their commitment and sacrifice towards my education.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Groundnut *(Arachis hypogaea* L.) originated from South America, where the crop thrives in tropical and sub-tropical climates (Arya *et al*., 2016; Prasad *et al*., 2009). Groundnut is a leguminous crop grown mainly for its edible seeds and classified as both a grain legume and an oil crop (ICRISAT, CARDA, IITA, 2017).

Groundnut grows well in sandy loam soil with a pH of 5.5 - 6.2, an annual rainfall of 200 – 400 mm and temperature of 24^{o}C-30^{o}C with required for germination and 24^{o}C for flowering and seed setting (Tweneboah, 2000; Hamakareem *et al*., 2016).

World production of groundnuts in the 2014/2015 season was estimated at nearly 27 million tons on shelled basis (INC Global Statistical Review, 2015). Sub – Saharan Africa contributes 26% of the world's groundnuts production (Angelucci and Bazzucchi, 2013). In West Africa, Ghana is among the major producers of groundnut in the sub region and in terms of area under cultivation in Ghana, groundnut is the most important grain legume (Oteng – Frimpong *et al.*, 2017). Groundnut produced in Ghana come from the three Northern regions (Tsigbey *et al*., 2001) where it is both cultivated on commercial and subsistence ventures by many farmers (Dapaah, 2014).

Groundnut is among the legume crops that have the ability to fix atmospheric nitrogen (N_2) by forming symbiotic associations with rhizobia in their root nodules (Starker *et al.,* 2006; Kaba *et al*., 2014). The bacteroid within the root nodules converts atmospheric nitrogen (N_2) which cannot be

utilized by plants into ammonia (NH_3) which is easily utilized by plants (Mus *et al*., 2016). The conversion of nitrogen is mediated naturally by rhizobia bacteria (Sorensen and Sessitisch, 2007). To enhance this relationship, seeds of legumes or the soil are inoculated with effective bacteria. Inoculation is the process of applying a recommended type of bacteria (rhizobia) to the seed or soil before planting (Robert and Idowu, 2015; Janouskova *et al*., 2017). Inoculants used on legumes are always species specific and so the right type of inoculant must be used for the right crop (Burdass, 2002).

Inoculation ensures that enough of the right type of bacteria is present in the soil for a successful legume-bacteria symbiosis (Benizri *et al*., 2001). Burdass (2002) asserts that pre-inoculating the seeds provide more protection for the bacteria which helps it to survive in the soil for about three (3) weeks. According to Li *et al*. (2009), inoculation of legumes facilitate nodulation and nitrogen fixation which leads to increases in yield of the crop.

Inoculation is done especially if the host plant has not been cultivated on that field for about 3-5 years (Janouskova *et al*., 2017). It is also recommended when dealing with a high value crop for which one want to ensure successful growth (Janouskova *et al*., 2017).

Direct application of inoculants to seed is done by moistening the seeds with a sticker solution (20% sugar solution) and uniformly mixing it before drying the seeds in a shade within 24 hours before planting. The inoculant can also be applied to the soil directly before planting using a calibrated standard planter to ensure constant flow of inoculant (Bashan *et al*., 2014).

Benefits of using inoculants include increased legume grain yield, increased biomass production, increased soil nitrogen and friendliness to the environment (Sajid *et al*., 2011).

Increase use of inoculants directly reduces the use of chemical fertilizers and increase agronomic efficiency by reducing cost of production and environmental pollution (Souza *et al*., 2015).

The efficiency of legumes to form nodules and fix nitrogen is affected by factors such as soil moisture, temperature and nutrients, especially phosphorus (Waswa, 2013; and Aziz *et al*., 2016). Among the soil nutrients, P and N are the most limiting elements, however, P is more limiting than N in tropical legume production systems (Weih, 2016). The application of phosphorus fertilizer has been reported to have increased the number of filled pods and the yield of seeds (Vinh, 2003). Nadia (2012) asserts that the application of P improves the action of rhizobia, increase the number of branches and pod per plant and the efficiency of plants to photosynthesis. The application of rhizobium inoculation and phosphorus have been noted to increase significantly nodulation and N fixation in legumes which, result in total yield increase (Bhuiyan *et al*., 2008).

1.2 Problem statement and justification

The World population is expected to reach about 8 billion by 2025 (United Nations, 2006) and Africa and Asia will be the major contributing continents to this increase in population. This calls for increase in the cultivation of crops such as groundnut so as to meet the consumption demand of the increasing population. Although groundnut is a legume that is widely produced in Ghana especially in the Northern Regions, its cultivation has not yielded its full potential (Frimpong *et al*., 2017). World average yield of groundnut in 2010, was about 1.49 metric tones per hectare (FAOSTAT, 2010) and the average yield for groundnut in Ghana was 1.5 metric tones per hectare whiles the achievable yield was 2.5

metric tones per hectare (FAO, 2011). Among the reasons for the low yield of groundnut among farmers in Africa has been poor soil fertility and low nutrient availability in the soil (Raimi *et al*., 2017). This low yields result in wide spread poverty among African farmers (Al-Falih, 2002). Raimi *et al*. (2017) asserts that the lack of inputs such as fertilizer and labour to smallholder farmers increase the poverty level of farmers in Africa.

The introduction of synthetic fertilizer has become a major source of plant nutrients that farmers now rely on worldwide. In the 1960s to 1980s, chemical fertilizer was subsidized in Asia and Sub – Saharan Africa to promote its use and this led to increased crop yield (Dawson *et al*., 2011). However, in Africa farmers are not able to afford these inorganic fertilizers because of the high prices and in some cases the fertilizers are not available for farmers to purchase, especially in the rural areas where smallholder farmers depend on crops for their livelihood (FAO, 2012).

The formation of nodules in legumes help plants to use fertilizer efficiently (Robert and Idowu, 2015) leading to an increase in yield. The best yields are achieved when nutrients come from a mix of mineral fertilizers and natural sources such as manure and nitrogen - fixing crops and trees (FAO, 2011).

The application of fertilizer together with inoculants by farmers is imperative to increase the productivity of groundnut farmers in the savannah zone.

1.3 Research questions

i. What is the effect of inoculant on groundnut varieties?
ii. What is the effect of inorganic phosphorus fertilizer on groundnut varieties?

iii. What is the interactive effect of inoculants and phosphorus fertilization on groundnut varieties?

iv. What is the effect of inoculants and inorganic phosphorus fertilizer on nitrogen, phosphorus and potassium content, soil properties and biological nitrogen abilities of groundnut varieties?

1.4 Objectives of Study

The main objective in this study is to determine the productivity of inoculants and phosphorus application in groundnut production in the Tolon and Kumbungu Districts.

The specific objectives are**:**

i. To assess the effect of inoculant on groundnut varieties

ii. To ascertain the effect of inorganic phosphorus fertilizer on groundnut varieties

iii. To ascertain the interactive effects of inoculants and phosphorus fertilization on groundnut varieties

iv. To evaluate the effect of inoculants and inorganic phosphorus fertilizer on nitrogen, phosphorus and potassium contents, soil properties and on biological nitrogen abilities of groundnut varieties

1.5 Hypotheses

i. Inoculant increases number of nodules per plant, effective nodules at flowering, number of branches per plant, phosphorus and potassium content in plant material as well as harvest index.

ii. Inorganic phosphorus fertilizer increases haulm weight, biomass, plant height and nodule number per plant

iii. The interactive effects of inoculants and inorganic phosphorus fertilizer increased nodule number per plant, haulm weight, biomass, plant height, number of branches, yield and harvest index.

iv. Inoculant and inorganic phosphorus fertilizer increased nitrogen, phosphorus and potassium contents, soil properties and biological nitrogen abilities of groundnut varieties

CHAPTER TWO

LITERATURE REVIEW

2.0 Botany and morphology of groundnut

Groundnut (*Arachis hypogaea*) is an annual herbaceous plant which belongs to the family Leguminnosae and sub-family Papilionoideae (Waele and Swanevelder, 2001). Like all other legumes, groundnut develops a symbiotic nitrogen fixing relationship with bacteria in the nodules of their root (Badar *et al*., 2015). The bacteria live in the root nodules and depend on the nutrients of the plant and in turn convert atmospheric nitrogen (N_2) into ammonia (NH_3) which the plant benefits from (Sharma *et al*., 2011; Biswas and Gresshoff, 2014). The seeds of groundnut consist of three parts namely the embryo, food storage and the seed covering (Weitbrecht *et al*., 2011). The embryo of a groundnut seed is a diploid (2N) which is unusually advanced in morphological development (Prasad *et al*., 2011). The embryo consists of the epicotyl, hypocotyl, radicle, and two cotyledons. The tip of the epicotyl is the plumule which develops in to the shoot of the plant. The hypocotyl is the zone between the shoot and the root and it is white and easily distinguishable especially during the early stages of growth. As the plant matures, the hypocotyl becomes indistinguishable from the root of the plant. The radicle is the embryonic root which further develop in to a tape root system with many lateral branches (Weitbrecht *et al*., 2011). The root of groundnut can grow to a depth of 5 to 35 cm. The cotyledons are the seed leaves which develop from the plumule. The seed covering of groundnut consist of the seed coat which ranges in colour from dark purple to whitish and is partially impermeable to water. The seed coat prevents the entry of parasites and excessive loss of water. All species of Arachis are geocarpic that is where the gynoecium after fertilization develops in to the

pegs. The pegs function as the root of the plant to some extent by absorbing nutrients from the soil and growing to a depth of 10 cm or more. The tips of the pegs develop in to a mature pod underground which contains the seeds. The pods are neither round nor spherical but may be oblong in shape (Maduako and Hamman, 2014) and are normally 3 to 7 mm long with two to three seeds in a pod covered by a thin netted spongy shell (Kumar, 2013) (Kumar, 2013).

The main stem of groundnut is upright or prostrate and grows up to 30 to 50 cm tall (Kumar, 2013) or 1m in the Virginia type. Groundnuts have many lateral branches and the pattern of flowering differs based on the type of groundnut variety. Groundnut begins to produce flowers 25 to 30 days after emergence (Prasad *et al.*, 2009) Prasad *et al.*, 2009) and continue to increase in flower production until it reaches a peak at 60 to 70 days (Kaba *et al.*, 2014) after emergence during which flower production begins to decline. The flower constitutes the reproductive unit of groundnut (kaba *et al.*, 2014) and is perfect and self-pollinated. The leaf axils bear the flower on primary and secondary branches on which each node produces several flowers. However, 15 to 20 percent of the flower only produces a harvestable pod (Kaba *et al.*, 2014). Awal and Ikeda (2003) reported that, groundnut pod takes 8 weeks to reach maturity from the time of flowering and this varies from one variety to another. The flower has a showy yellow bloom with folded petals when it emerges. The petals of the flower unfold early in the morning for pollen to be shed on it for fertilization to take place which last for about 3 to 6 hours. Cultivated groundnuts have been classified into two botanical groups which are erect or bunch types and the trailing or runner types (Prasad *et al.*, 2009) .The bunch or Valencia type of groundnut is an erect plant that has pods clustered about the base of the root. According to Craufurd *et al.* (2000), the bunch or

erect type commence flowering 26 to 34 days after planting and produces fewer flowers. The plant produces fewer reproductive nodes and a short period of flower production. It is early maturing with a maximum growth height of 60 cm with yellow flowers appearing 4 – 6 weeks after planting. The peg develops from the ovary to form the pod which contains 1 – 6 seeds surrounded by a shell which is thick and fibrous. According to Waele and Swanevelder (2001), a mature cylindrically shaped pod of the groundnut consists of an exocarp, mesocarp and endocarp which are the layers which covers the seeds. The radicle after germination emerges within 24 hours.

Virginia, trailing or runner type has pod that spread along the secondary and tertiary branches and is late maturing. It produces more flowers which ranges from 18 to 142. The radicle after germination emerges within 36 – 48 hours and the branching pattern of the plant is alternate ((Prasad *et al*., 2009) Prasad *et al*., 2009).

2.1 Climate

Temperature highly influences the rate of growth of plants (Hatfield and Prueger, 2015). For every species, there is a definite range of maximum and minimum temperature which serve as a limit within which growth occurs. The optimum temperature for groundnut ranges between 28 -30^{o}C (Prasad *et al*., 2009).Germination, growth and development of groundnut is reduced rapidly and at 14 ^{o}C growth ceased.

Optimum temperature influences the net rate of photosynthesis, flower formation and the growth of the pods (Bunce, 2004).

Groundnut is a shade tolerant plant and so can be cultivated with tree crops. Plants under shade develop bigger leaves and smaller reproductive organs (Martins *et al*., 2014) which

reduces the yield of the crop under extremely shady conditions (Polthanee *et al.,* 2011) When light is very intense, groundnut as a C3 - plant achieves a level of photosynthesis which can be comparable to that of the C4 – plants (Wang *et al.,* 2012; Raines, 2011).

The optimum planting time should correspond with the raining season which helps to prevent reduction in yield of the crop. A mature groundnut plant can tolerate flooding conditions for about a week (Pucciariello *et al.,* 2014) provided the soil is well drained.

The late ripening varieties (145 days vegetation period) require 500 – 1000 mm of rainfall during the growth period for good yield whiles the early ripening varieties (up to 100 days vegetation period) need 300 – 500 mm of rainfall. A minimum of 300 mm rainfall is needed between emergence of plant and flowering to ensure a good vegetative growth (Tweneboah, 2000).

2.2 Factors influencing biological nitrogen fixation

For Arid and semiarid soils were the fixation of nitrogen is limited due to poor nodulation of legumes as a result of reduce population of rhizobia bacteria in the soil especially in the dry season (Mohammadi *et al.,* 2012). Stresses encountered by rhizobia bacteria in the soil affect their growth, initial steps of symbiosis and their ability to fix nitrogen in the soil (Monica *et al.,* 2013; Mohammadi *et al.,* 2012). Under suitable soil conditions according to Bernap, (2001) factors that influence nitrogen fixation are temperature, light, and moisture. Monica *et al.* (2013) also asserts that the factors that affect biological nitrogen fixation in the soil are salinity, drought and temperature. Factors that limit biological nitrogen fixation can therefore be said to include soil temperature, soil salinity and alkalinity, high soil nitrogen, soil acidity and phosphorus deficiency. These factors are classified as: abiotic and biotic factors.

2.3 Abiotic factors

These factors are non-biological in nature and hinder biological nitrogen fixation in the soil. These factors include:

2.3.1 Soil moisture stress

Under severe environmental conditions such as water stress the growth and population of rhizobia reduce (Zahran, 1999; Ramos *et al.*, 2003) though some rhizobia bacteria (saprophytic) are capable of living in drought stress conditions. Water stress causes the dryness of the land resulting in the evaporation of water and the buildup of salts in the soil (Llangumaran and Smith, 2017). In situations where the water stress condition in the soil is resolved, the rhizobia population increase (Zahran, 1999; Ramos *et al.*, 2003). The level of water stress and the rate at which it inhibits the symbiotic relationship depends on the severity of the stress and the rate of growth and development of the legume plant (Zahran, 1999; Ramos *et al.*, 2003). Temperate and tropical legumes such as Pisum sativum, Medicago sativa, Arachis hypogaea, Glycine max under soil water deficit experience reduce nitrogen fixation. Stages of nitrogen fixation such as nodule initiation and growth of nodules are all influenced by water stress. The effect of water stress to the nodulation and nitrogen gas fixation depends on the growth stage of the plant. The water stress is more detrimental to nodulation and nitrogen fixation at the vegetative stage than at the reproductive stage (Zahran, 1999; Abd-Alla *et al.*, 2014).

2.3.1.2 Salinity and alkalinity

Soils which are rich in salts and have a high pH are not suitable for the cultivation of legumes especially in the semi-arid regions (Rao *et al.*, 2002) where the amount of soil moisture lost through evapotranspiration exceeds the annual rainfall (Monica *et al.*, 2013).

Salinity affects the production of Nod factors (Lira *et al.,* 2015) and both salinity and alkalinity in the soil also reduce the supply of photosynthates to the nodules which hinder the growth and efficiency of already grown legume plants to fix atmospheric nitrogen (Monica *et al*., 2013). High salt content in the soil also reduce the supply of respiratory substrates to the bacteroids (Jia *et al*., 2014) that fix the atmospheric nitrogen and may also affect the metabolism of nodules directly (Sulieman and Tran, 2014; Queiroz *et al*., 2012). The oxygen diffusion barrier in the plant is altered by the presence of salt in the soil (Shrivastava and Kumar, 2015). According to Rao *et al.* (2002), the tolerant limits of Rhizobium and Bradyrhizobium Spp. are much higher than the legume host plant and suggest that it is the host plant that determines the success of the symbiotic relationship between the bacteria and itself.

2.3.1.3 Soil nitrogen quantities

High carbon dioxide (CO_2) in the soil increases the demand for nitrogen in the soil (Berthrong *et al.,* 2014) through mechanisms such as C/N ratio of plant inputs to the soil. However, when there is increase amount of nitrogen in the soil as a result of additions of inorganic nitrogen (Walworth, 2013, Ghaly and Ramakrishnan, 2015), it lowers biological nitrogen fixation rates in the soil by inhibiting the Rhizobium infection process (Carvalho *et al.,* 2014). However, some Rhizobium strains (Azarhizobium caulinodans) are able to fix nitrogen under high soil nitrogen (200 Kg N ha^{-1}). Low supply of nitrogen in the soil (less than 30 N Kg ha^{-1}) stimulates early growth of the plant and also improves biological nitrogen fixation in the soil (Carvalho *et al*., 2014).

2.3.1.4 Drought

Viable strains of Rhizobium usually cannot tolerate or function under high levels of osmotic stress caused by drought (Monica *et al*., 2013). Drought is a major environmental factor affecting the productivity of legumes that fix nitrogen gas in the soil (Monica *et al*., 2013).

Drought causes some changes in the morphology of the Rhizobia such as dehydration of cells, persistence and survival in soil, and root hair colonization (Lebrazi and Benbrahim, 2014).

2.3.1.5 Phosphorus deficiency

Phosphorus supply and availability remains a very serious limitation on nitrogen fixation and symbiotic relationship between the legume plant and Rhizobium (Weisany *et al.*, 2013). The growth of legume plants is most limited by deficiency of phosphorus (Vance, 2001, Jensen *and* Hauggaard, 2003), leading to a reduction in the demand of nitrogen and biological nitrogen fixation (Jensen and Hauggaard, 2003; Mbaga *et al*., 2014).The deficiency of phosphorus hinders root development, the photosynthetic process (Carstensen *et al*., 2018), and translocation of sugars (Lemoine *et al*., 2013) to other parts of the plant which affect the nitrogen fixation process. The production and supply of non - structural carbohydrates to the nodules is affected as well as the activities of nitrogenase in the root nodules when phosphorus is deficient in the soil (Almeida *et al*., 2000). This impairs nodulation and fixation of nitrogen in the plant (Tang *et al.*, 2001). The yield of the plant is reduced of sugars when the formation of nodules is greatly limited in phosphorus deficient soils (Xue *et al*., 2016; Musa *et al.*, 2017).

2.3.1.6 Soil acidity

For plants to grow and yield well, soil pH should be maintained at about 5.5 in the topsoil and 4.8 in the subsoil (Ferguson *et al.*, 2013; Magadlela *et al*., 2016). This will ensure the availability of soil nutrients and also reduce production losses.

Soil acidity alone is responsible for significant losses in global legume production (Ferguson *et al*., 2013) which hinder plant and Rhizobia growth, decrease nodule development and nitrogen fixation (Ferguson *et al*., 2013). Low soil acidity is caused by poor nutrient cycling, soil leaching and acidifying effect of nitrogen fertilizers (Ferguson *et al.*, 2013; Amanullah *et al.*, 2017). Low soil pH causes yield losses of about 50 % or more in wheat, barley and legumes (Ferguson *et al.*, 2013).The concentration of aluminium (Al^{3+}) increases in soils with low pH which serves as a limiting factor on the viability of plants (Ferguson *et al.*, 2013). Aluminium (Al^{3+}) also hydrolyzes in low acidic soils to produce a toxic form of it which inhibit root cell division and elongation. Toxic metals such as Al^{3+} and Cu^{2+} increase as a result of increase in H^{+} concentration which causes intercellular pH instability (Ferguson *et al*., 2013) which limit the growth of the plant. The availability and retention of macro nutrients in low pH soils is reduced when Al^{3+} is present.

The nodulation period is the most sensitive to soil acidity (Ferguson *et al*., 2013; Amanullah *et al*., 2017) in the growth stages of leguminous plants. Ferguson *et al*. (2013), reported that, greater than ninety percent (> 90 %) reduction occur during nodule formation and fifty percent (50 %) reduction of nodule dry weight occurs in crops such as soybean, pea and cowpea.

The reduction in nodule formation in acidic soils affect the fixation of nitrogen in legumes (Unkovich, 2012) as well as the deficiency of molybdenum in soils with low pH hinders nitrogen fixation (Ferguson *et al.,* 2013) because molybdenum is a very important component of the nitrogenase enzyme complex which help in the nitrogen fixation process.

Low soil pH disrupts the signal exchange between the host plant and the microsymbiont which restricts root hair deformation and curling (Jensen *and* Hauggaard, 2003). Reduce flavonoid secretion and rhizobia Nod gene also hinders nitrogen fixation and Nod metabolite excretion (Radutoiu *et al*., 2003; Wasson *et al*., 2006). The attachment to root hairs and colonization of the root by rhizobia is affected in low soil pH (Zahran, 1999; Rodriguez – Navarro *et al*., 2007).

2.3.1.7 Extreme temperatures

Almost all areas of the World experience some period of soil dryness (Kang *et al.,* 2009; Sheffield and Wood, 2008). This period extensively dry up the soil killing the rhizobia bacteria and reducing the population of the rhizobia bacteria (Mohammadi *et al.,* 2012) which should have fixed nitrogen in subsequent crops. High temperatures affect root hair infection (Sita *et al.,* 2017) and the functioning of nodules to fix nitrogen in to the soil (Whittington *et al.,* 2012). The optimal temperature for the fixation of nitrogen in Peanuts is 25^{o}C – 30^{o}C but 35^{o}C – 40^{o}C are critical and do not support the fixation of nitrogen in the soil (Zahran, 1999). Exposure of inoculated seeds to sunlight can also kill the rhizobia bacteria and affect the amount of nitrogen fixed in to the soil. According to Al-Falih (2002), drying reduces about 99 % or more of the viable population of the rhizobia bacteria. Mohammadi *et al.* (2012), reported that, high root temperature influence the infection of the root by the rhizobia bacteria, N_2 fixation, and the growth of the legume plant.

2.3.1.8 Availability of light

The photosynthetic process in plants is controlled by light (Paul and Foyer, 2001) on which biological nitrogen fixation depends (Luca and Hungria, 2014). The growth of most legumes is affected under limited supply of light (Adeyeye *et al*., 2017) which also affect their ability to fix nitrogen in the soil (Magadlela *et al.*, 2016). However, some legumes are able to fix nitrogen under limited amount of light (shade) when inter cropped with other plants (Bhuiyan *et al*., 20080).

2.3.2 Biotic factors

2.3.2.1 Effect of inoculants on the formation of nodules and fixation of nitrogen

Inoculation is an efficient and convenient way of introducing viable rhizobia to the soil for it to reach the rhizosphere of the root of the legume (Sharma *et al.*, 2011). Biological nitrogen fixation is considered as a profitable practice (Badawi *et al*., 2011) in meeting the nitrogen requirement of groundnut. However, native rhizobia can hinder the establishment of inoculant strains which may lead to inoculation failure (Sharma *et al*., 2011). Inoculation is aimed at providing sufficient number of viable and effective rhizobia to induce rapid colonization of the rhizosphere with which nodulation occurs immediately germination occurs (Sharma *et al.*, 2011; Biswas and Gresshoff, 2014) leading to yield increase (Cat roux *et al*., 2001). Studies have shown that there have been increase in yield when inoculants are used (Sajid *et al*., 2011; Gopalakrishnan *et al*., 2015) though the response of groundnut to inoculation has not been consistent (Sharma *et al*., 2011). Increasing the number of viable rhizobia on the seed during seed inoculation will ensure that there is enough of the bacteria in the rhizosphere of the root to enhance formation of nodules in which the bacteroids live (Biswas and Gresshoff, 2014; Cardoso *et al*., 2017). The

bacteroids in the nodules of the host plant directly influence phosphate solubilization (Badawi *et al.*, 2011), iron chelation and fixation of atmospheric nitrogen (Biswas and Gresshoff, 2014; Gopalakrishnan *et al.*, 2015) using the nitrogenase enzyme complex. More bacteroids in the nodules will therefore mean that more nitrogen fixation in the soil. Sajid *et al*. (2010), reported that, Rhizobium inoculation in groundnut had a significant effect on leave number per plant, shoot number per plant, nodule number, plant height, and pod number per plant and yield per plant more than those that were not inoculated. Badaso *et al*. (2011) reported that, peanut seeds inoculated with Bradyrhizobium increased the number and mass of the root nodules, increased the rate of acetylene reduction and all growth parameters more than the uninoculated peanut seeds.

Rhizobium used as a plant growth promoter increase plant protection and the ability of the plant to tolerate abiotic stresses such as temperature, soil pH, salinity, heavy metals, drought and pesticide pollution (Gopalakrishnan *et al*., 2015).

Indigenous rhizobia that already exist in the soil may compete and prevent the establishment of the inoculant strain applied to the soil (Badawi *et al*., 2011) and so hinders the effectiveness of the inoculant (Sharma *et al*., 2011). Basu and Bhadoria (2008) asserts that low yield in groundnut is due to low inoculation and competition from indigenous ineffective strains of inoculants.

2.4 Biological nitrogen fixation in legumes

Biological nitrogen fixation is the process that changes inert nitrogen in to biologically useful ammonia (Downie, 2014; Robert and Idowu, 2015). The atmospheric air contains approximately 80% nitrogen but the nitrogen gas however, is not in a form that plants can

utilize (Flynn and Idowu, 2015) and so scarce in tropical soils (Cardoso *et al.*, 2017). Nitrogen is the most limiting nutrient for plant growth (Mmbaga *et al.*, 2014 and Wagner, 2011) and legumes are capable of converting nitrogen gas (N_2) in the atmosphere in to ammonia (NH_3) a form plants can use (Montiel *et al.*, 2016). The symbiotic relationship between legumes and rhizobium is very significant because it is a major contributor of biological nitrogen fixation in the soil (Bhuiyan *et al.*, 2008). The fixation of N_2 through the relationship ranges between 200 to 300 Kg N ha^{-1} per year (Mohammandi *et al.*, 2012). Both the bacteria and the groundnut plant benefit from the symbiotic relationship, the plant obtain nitrogen fix by the bacteria and intent supply the bacteria with carbohydrates as a source of energy (Prell *et al.*, 2009). The growth rate of the plant determines the rate of nitrogen fixation (Prasad *et al.*, 2009) and so maintaining sufficient leaf area in the plant stand is so critical to ensure the interception of enough sunlight to promote a high growth rate (Evans and Poorter, 2001) leading to a high rate of nitrogen fixation. Factors such as plant nutrients, drought, disease and low temperature reduce the growth rate of the plant and limit nitrogen fixation (Belnap, 2001).

2.4.1 Formation of nodules

The nodules of legumes are organs formed on roots which ranges between 2 – 5 mm in diameter (Downie *et al.*, 2014) and each nodule contain about 10^9 rhizobia in a niche just enough for the conversion of nitrogen gas in to ammonia which plants can use (Biswas and Gresshoff, 2014). Nodule formation in groundnut unlike other legumes, develop only at the sites of lateral root whereas nodulation occurs at the sites of the root – hairs in other legumes (Uheda *et al.*, 2001; Wagner, 2011). The groundnut plant have smaller nodules which have a higher rate of nitrogen fixation as compared to Soybean (Tajima *et al.*, 2004).

In nodule formation, the host plant controls nodulation and the mode of infection (Boogerd *et al.,* 1997; Ferguson *et al.,* 2013). The Rhizobium bacteria normally live in the root nodule or soil (Monica *et al.,* 2013) and can also be introduced in to the soil through inoculation of the legume seed or the soil on which the crop will be planted. These Rhizobia can live in the soil for a long time with the host plant (Gopalakrishnam *et al.,* 2015). However, when nitrogen supply to legume plants is limited, the plants form a symbiotic relationship by releasing the compound flavonoid as a signal to the Rhizobia bacteria for the production of nitrogen (Prell *et al.,* 2009). The Rhizobia also releases a nodulation factor (nod factor), to stimulate nodule formation in the root of the plant especially in soils with limited nitrogen (Biswas and Gresshoff, 2014; Lira *et al.,* 2015). The nod factor triggers the development of deformed root hairs (Esseling *et al.,* 2003) which permit the Rhizobia bacteria to enter the plant. According to Prasad *et al.,* (2009) steps in nodule formation include root colonization and infection, multiplication of bacteria and nodule development.

2.4.1.1 Root Colonization and infection

The beneficial association between legumes and the soil rhizobia bacteria result in the formation of root nodules (Ferguson *et al.,* 2013), where the bacteria develop in to nitrogen fixing bacteroids (Montiel *et al*., 2016). The relationship between the rhizobia bacteria and the legume plant begins in the rhizosphere where there is a cross of specific species between the nodulation factor (Nod factor) produced and secreted by the rhizobia bacteria and the flavonoid released by the root of the legume in the rezosphere (Montiel *et al*., 2016).

In groundnut nodulation is by a wide range of species of rhizobia (Prasad *et al*., 2009) and the development of root nodules is restricted mostly to the first order lateral roots (Uheda, 2001, Tajima, 2006) but will occasionally occur on the tap root (Tajima, 2008). The increase in number of the rhizobia bacteria in the rhizosphere of the groundnut plant is the first step in the formation of nodules in groundnut (Prasad *et al.*, 2009).

There are two main routes of Rhizobium infection for root – nodule formation in legumes: Entry of the Rhizobium bacteria via root hairs which occurs in most legumes and entry of the Rhizobium bacteria via cracks which occur in few legumes (Boogerd *et al.*, 1997; Tajima *et al*., 2008).

In the nodulation process in groundnut, infection is through intercellular spreading (crack entry) mode where the bacteria penetrates the epidermis of the root which occurs at places where the root of the plant protrude (Boogerd *et al*., 1997; Bogino *et al*., 2011). The exposure of the root of groundnut to nod factors as a result of the flavonoid released by the plant (Broughton *et al*., 2003) triggers cellular, physiological and morphological changes (Biswas and Gresshoff, 2014) resulting in the formation of deformed root hairs (Gentili *et al*., 2006) which allow rhizobia to enter the plant (Boogerd *et al*., 1997; Monica *et al*., 2013; Montiel *et al*., 2016) through the infection thread. Prasad *et al.* (2009) reported that, when the rhizobia bacteria gain entry in to the root, it occupies the area between the wall of the root hair and the adjacent epidermal and cortical cells.

The formation of the infection thread and the nodule primordium occur concurrently in the root, where the cortical and the pericycle cells are triggered to go through cell division leading to the formation of the nodule primordium. The rhizobia through the infection thread enter into the nodule primordium and are enclosed in it. The rhizobia bacteria then

increase and the nodule primordium further develop into a mature nodule (Biswas and Gresshoff, 2014). The Rhizobia bacteria aid in the conversion of nitrogen into ammonia (Downie, 2014).

2.4.2 Development of nodules

Nodules have been the structures within which the bacteroids reduce atmospheric nitrogen gas in to ammonia, occur in the soil on the roots within a depth ranging from 5 – 30 cm with few outside the range (Rowland *et al*., 2015). As the main and lateral roots develop, the nodules develop at the junction of the main and lateral roots (Tajima *et al*., 2008; Getahun, 2017). The rhizobium upon entering in to the space between the root hair walls and the adjoining epidermal and the cortical cells, the cells adjacent to the point of penetration in to the root by the rhizobium separate at the middle lamellas creating a space which is filled by bacteria to form an intercellular infection zone (Prasad *et al.*, 2009). The rhizobium further penetrates other cell layers deeply to form the intracellular infection zone. The bacteria after intracellular infection go through cell division and are transformed in to a bacteroid. The number of bacteroids in the nodule of a groundnut is relatively small as compared to cowpea (Prasad *et al.*, 2009). As the nodule matures, its size and structure changes (Tajima *et al.*, 2008). Nodule shape in legumes varies however, Prasad *et al*., (2009) reported that, the nodule shape of a groundnut is spherical throughout. A cross section of a young nodule reveals a whitish colour and mature nodules have a deep pink reddish colour due to the presence of leg hemoglobin (Ott *et al*., 2005). The tissues on the surface of the root nodule are protected by a thin layer (peridermal layer) which is made up of lignin and suberin (Tajima *et al*., 2008). The surface of the nodule shows a light brown colour and reaches senesces where the interior part of the nodule shows a greenish colour

as the nodule ages and become inactive (Sarath *et al*., 1986; Flynn and Idowu, 2015; Prommeresche and Hansen, 2017).

2.3.5 Functions of nodules

The enzyme nitrogenase which reduces nitrogen gas (N_2) in the atmosphere in to ammonia is contained in the bacteroid (Ott *et al.*, 2005; Ferguson *et al.*, 2013) for nodules to effectively fix nitrogen, the nodule must contain leghemoglobin (Ott *et al*., 2005; Wagner, 2011) which give the nodule tissues a pink colouration (Prasad *et al.*, 2011). The host plant provide the energy (carbohydrates) for the bacteria for denitrogen fixation and the carbon skeleton to be used to reduce nitrogen (Mus *et al.*, 2016). The fix nitrogen (o-methylene glutamin) is transported to the shoot of the plant for it to be utilized. However, not all rhizobia that produces nodules are able to fix nitrogen (Prasad *et al.*, 2009). Clayton *et al.* (2004) asserts that seed inoculated pea (*Pisum sativum*) produced less effective nodules to fix nitrogen than soil inoculated pea.

2.5 Specificity of rhizobia

The bacteria of the genera Rhizobium spp., Bradyrhizobium sp., Azorhizobium sp., Mesorhizobium sp., and Sinorhizobium sp. together called Rhizobia (Esseling *et al.*, 2003), which are capable of establishing a symbiotic relationship with compatible legume plants (Mus *et al*., 2016). Not all legumes are infected by Rhizobium (Ferguson *et al.*, 2013), thus a Rhizobium that infect groundnut may not infect common bean. Given strains of Rhizobium will nodulate and fix different amount of nitrogen (N_2) in a symbiotic relationship with a range of host plants of the same species. Groundnut is specifically nodulated by Rhizobium species (Tajima *et al.*, 2008). For a symbiotic relationship between the Rhizobium bacteria and the host plant to be effective and functional it depends

on a genetic determinate in the host plant and the Rhizobium bacteria (Mus *et al*., 2016; Liu and Murray, 2016). The Rhizobium bacteria should be able to recognize the signal released by the host plant (flavonoids and isoflavonoids) in to the rhizosphere (Monica *et al*., 2013) and in response the bacteria carries the signal and bind it to NodD, a transcriptional regulator leading to the activation of a bacterial nodulation genes (Liu and Murray, 2016). The genes then produce the Nod factor which is very essential in the specific host plant – Rhizobium relationship, the infection process and formation of nodules (Mus *et al*., 2016; Liu and Murray, 2016).

2.6 Role of phosphorus (P_2O_5) in nitrogen fixation

Phosphorus is the second most essential macro nutrient (Vance *et al*., 2003) and one of the least accessible nutrients (Balemi and Negisho, 2012; Magadlela *et al*., 2016) in the soil, although its availability in the soil may be high (Vance *et al.,* 2003). The phosphorus needed by legumes is absorbed by the root hairs through diffusion in to the plant (Lambers *et al.,* 2006).

Phosphorus is needed for healthy growth of plants which enhances nodulation and fixation of nitrogen (Gentili and Huss-Danell, 2003). Phosphorus serves as a source of energy for the nodules of legumes (Vance *et al.,* 2003; Magadlela *et al*., 2016) by providing the mechanism for the storage of energy in the form of ATP which is used by plants for nitrogen fixation. Nodule number, volume, and dry weight can be increased when phosphorus is sufficient in the soil (Araujo *et al.,* 2000; Gentili and Huss-Danell, 2003). The initiation and growth of nodules demand high supply of phosphorus (Gentili and Huss-Danell, 2003) which result in increased nitrogen fixation by the bacteroids in the nodules (Geneva *et al.,* 2016).

Mohammed and Abdalla (2013), reported that, phosphorus and nitrogen are essential nutrient element needed for effective production of groundnut. The availability of nitrogen and phosphorus significantly affect nitrogen fixation in legumes (Leidi and Rodriguez - Navarro, 2000; Jensen and Hauggaard. 2003). However, low phosphorus soils limit greatly the growth of legumes more than low nitrogen soils (Magadlela *et al.*, 2016).

2.7 Synergistic effect of phosphorus and rhizobium inoculation in groundnut

Aziz *et al*. (2016) reported that combine rhizobium inoculation and phosphorus fertilization significantly increased nodule number per plant. Mohamed and Abdalla also reported similar finding, that the synergistic effect of rhizobium inoculation and phosphorus fertilizer significantly influence nodule number per plant in groundnut. Mohammad *et al*. (2004) asserts that combine effect of rhizobium inoculation and phosphorus fertilizer significantly increase the nodulation of groundnut. Nodule dry weight of groundnut is increased significantly by rhizobium and phosphorus combination at 6 and 8 WAP (Mahamed and Abdalla, 2013; Muhammad *et al*., 2004). The height and the number of branches of groundnut are significantly increased by the combine effect of rhizobium inoculation and phosphorus fertilization (Mohammad *et al*., 2004). The nitrogen content in the shoot of groundnut plant is increased (Mohamed and Abdalla, 20013) as well as the biological nitrogen fixation by 100 % for Tikolore promiscuous soybean variety (Aziz *et al*., 2016). Geneva *et al*. (2016), asserts that, the combine effect of Rh. Leguminosarum and elevated phosphorus levels in inoculated pea plants significantly increased the nodule number, fresh biomass and photosynthetic rates which result in yield increase. The combine effect of rhizobium and phosphorus fertilizer significantly increased growth parameters

such as plant height, number of branches, leaves, plant biomass and yield (Heisnam *et al.*, 2017). The combine rhizobium inoculation and phosphorus fertilizer significantly increa

2.8 Socio - economic importance of groundnut

According to Nigam (2014) groundnut as an oil seed crop is ranked 6th in the world, 3rd as the most edible crop and 13th as the most utilize food. The lower grade oil produced during the processing of groundnut is used in the production of soap, lubricants and illuminants (Warra *et al.*, 2010). Oil extracted from groundnut is used in some countries such as the United State of America as biofuel to fuel farm machinery (National Geographic Society, 2017).

Groundnut is used as food when eaten fresh, boiled, roasted or used in the preparation of soup (Waele and Swanevelder, 2001).

Nigam (2014) asserts that in West Africa, about 55 % of groundnut produced is utilized as food. It is also a source of high quality protein (22 – 30 %) which is used as butter or mixed in many bakery products. The paste (groundnut cake) obtained after the extraction of oil is cut in to various shapes and fried into what is known locally as “Kulikuli”.

The vine of groundnut (root, stem and flower) produced annually in the World is estimated to be 60 – 65 % of the groundnut produced (Zhao *et al.*, 2012). The vines are rich in dietary fibres and flavonoid compounds which have good health benefits (Du and Fu, 2008).

Groundnut contain vitamin E, K and B complex as well as minerals (Aslam *et al.*, 2017).The shell of groundnut is used as fuel, animal feed, litter for animals and filler in the production of animal feed and fertilizer. The vegetative part of groundnut (haulm) is used as fodder to feed animals or in the preparation of manure (Naab *et al.*, 2009).

The roots of groundnut through the symbiotic relationship with rhizobia bacteria is able to fix nitrogen (100 -152 Kg /ha N) in to the soil and also add organic matter when the roots decay in the soil.

CHAPTER THREE

MATERIALS AND METHODS

The study was conducted in two parts: a field work from planting of inoculated seeds to harvesting to determine impact of inoculation and phosphorus fertilization on growth and yield, and the determination of nitrogen that was biologically fixed by the plant.

3.1 Field location

3.1.1 Site description

The field experiment was conducted during the 2017 cropping season in two groundnut production areas in the Kumbungu and Tolon districts of Northern region of Ghana. The two districts lie within longitudes 0º53’ and 1º25’ west and latitudes 9º 15’ and 10º 02’ north. The Kumbungu and Tolon districts share boundaries with each other to the west and with other districts such as North Gonja, Savelugu, Sagnerigu and central Gonja. (Ghana Statistical Service, 2014).

3.1.2 Climate

Rains in the Tolon and Kumbungu districts begin in May and end in the latter part of October. The peak period of rainfall starts from July to September with a long dry season from November to March. The mean annual rainfall ranges between 950 mm - 1200 mm (Ghana statistical service, 2014). Temperature during February to April is warm, dry and hazy but cool and moist during the wet season.

3.1.3 Vegetation

The vegetation is basically Guinea Savanna which is interspersed with trees and grassland which are drought resistant. Trees such as dawadawa (*Parkia biglobosa*), Shea nut (*Vitellaria paradoxa*), mango (*Mangifera indica*), baobab (*Adansonia digitata*) and neem (*Azadirachta indica*) are found in the area. People in this area depend on trees like sheanut (*Vitellaria paradoxa*), mango (Mangifera indica), and dawadawa (*Parkia biglobosa*), for their livelihood. The land is undulating with a number of scattered depressions. Bush fire is an annual event which usually destroys the vegetation (N'Dri *et al*., 2018; Amoako *et al*., 2018). The fires are noted to result in substantial losses of plant nutrients (Kugbe *et al*., 2012) that have negative impact on the livelihood of the people.

3.1.4 Soil and drainage

The soils in Northern Ghana are Haplic Lixisols (Nketia *et al*., 2018) which are generally sandy loam except the low laying areas where alluvial deposits are found. Soils are highly exposed to erosion due to the perennial burning of the vegetation in these districts leading to the depletion of soil nutrients. The district is drained by a number of rivers and streams, most prominent being the White Volta. Dendrite drainage patterns are exhibited by the rivers and their tributaries, most of which dry up during the dry season (Boakye-Danquah *et al*., 2014).

3.2 Selection of farmers to host the on – farm trials

3.2.1 Choice of farmers

Six farmers' fields (3 in each district) in Gurumanchenyili and Zangbalun Fandu in the Tolon and Kumbungu district respectively were randomly selected as replicates for the study.

3.2.2 Selection of farmers' fields

Fields for the experiment were carefully selected to ensure the existence of minimal soil variations according to Howieson and Dilworth (2016). Soil fertility variability within the fields of smallholder farmers is a common occurrence and so fields with similar soil type, gentle slope, and minimal shading with no history of groundnut cultivation in previous years were selected. Fields that had been cultivated previously with groundnut will have a number of native rhizobia for groundnut that might influence the result of the study (Yates *et al*., 2016). The willingness of the farmers to host the trial, the accessibility of the fields and farmers knowledge about the variation in their fields were key factors that determined the selection of plots for the study.

3.3 Treatments

Eighteen (18) treatments were replicated thrice on the six trial fields in the two selected districts. The treatments for the study were:

1. V1 + P0 Kg /ha + Ino.0g / Kg
2. V1 + P0 Kg /ha + Ino. 3g / Kg
3. V1+P0 Kg /ha + Ino. 6g / Kg
4. V1+P30 Kg /ha + Ino. 0g / Kg

5. V1+P30 Kg /ha + Ino. 3g / Kg
6. V1+P30 Kg /ha + Ino. 6g / Kg
7. V1+P60 Kg /ha + Ino. 0g / Kg
8. V1 +P60 Kg /ha + Ino. 3g / Kg
9. V1 + P60 Kg /ha + Ino. 6g / Kg
10. V2 + P0 Kg /ha + Ino. 0g / Kg
11. V2 + P0 Kg /ha + Ino. 3g / Kg
12. V2 + P0 Kg /ha + Ino. 6g / Kg
13. V2 + P30 Kg /ha + Ino. 0g / Kg
14. V2 + P30 Kg /ha + Ino. 3g / Kg
15. V2 + P30 Kg /ha + Ino. 6g / Kg
16. V2 + P60 Kg /ha + Ino. 0g / Kg
17. V2 + P60 Kg /ha + Ino. 3g / Kg
18. V2 + P60 Kg /ha + Ino. 6g / Kg

Where:

V1 = Chinese groundnut variety, V2 = Nkatie Sari groundnut variety, P0 kg = zero (0) kilogram of phosphorus fertilizer, P30 kg = Thirty (30) kilogram of phosphorus fertilizer, P60 kg = Sixty (60) kilogram of phosphorus fertilizer, Ino.0g / kg = zero (0) gram of inoculant per kilogram of seed, Ino.3 g / kg = 3 gram of inoculant per kilogram of seed, Ino.6 g / kg = 6 gram of inoculant per kilogram of seed.

3.4 Experimental design

All fields were laid out in a factorial arrangement in a randomized complete block design with each lead farmer's field serving as replicate. Variety was at 2 levels (Chinese and Nkatie Sari), phosphorus fertilizer (Triple super phosphate, 46 %) at 3 levels (0 kg/ha, 30 kg/ha and 60 kg P/ha) and inoculant application at 3 levels (0 g / kg of seed, 3 g / kg of seed and 6 g / kg of seed). The phosphorus fertilizer (Triple superphosphate) was applied by side placement at sowing (Kamara *et al*., 2011).

3.5 Plot dimensions

Eighteen plots each 4 m x 2.4 m and 4 m x 1.8 m for Nkatie Sari and Chinese variety respectively were prepared and bounded to minimize error due to P and inoculant drifting.

The groundnut varieties were spaced at 20 cm x 30 cm for the erect variety (Chinese) and 20 cm x 40 cm for the creeping variety (Nkatie – Sari). To reduce drifting of phosphorus and inoculants to adjacent sites during excessive rainfall, the two levels of variety were used as the main plot, three levels of phosphorus as the sub-plot and three levels of inoculant as the sub-sub plot.

3.6 Data collection and analysis

3.6.1 Soil sampling and analysis

Prior to treatment application and after planting, representative soil samples were collected from depth of 0 – 20 cm using soil auger. The sub – samples were then mixed thoroughly and combined into a composite sample of approximately 1 kg per plot. The soil samples were then air – dried and then sieved with a 2mm sieve. About 250 g of each sample was then used for the analysis.

The soil samples were analyzed for nitrogen, available phosphorus, potassium and pH.

3.6.1.1 Total nitrogen (N) determination in the soil

The Kjeldahl method was used in the determination of nitrogen of the soil. The method involved three processes namely:

a) Digestion

About 1 g of the soil sample was put into the digestion tubes and 5 ml of sulfuric acid added to the soil sample. The mixture was then digested until it became clear and colourless and allowed to cool. The clear digest was then totally transferred into a 100 ml volumetric flask and made up to the mark with distilled water.

b) Distillation

An aliquot of 20 ml of the digest was transferred into the Kjeldahl distillation flask and 20 ml of 40 % sodium hydroxide (NaOH) was added. A 75 ml distillate was then collected over 10 ml of 5 % boric acid in a 100 ml conical flask.

c) Titration

The pink boric acid, after the addition of the distillate turns green, indicating the concentration of nitrogen in the sample. However, the collected distillate was titrated with 0.1 N HCl till the green colouration changes to pink.

The nitrogen in the soil was calculated as follows:

14 g of N is contained in one equivalent of ammonia

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

Where:

A = volume of standard HCl used in the sample titration, mL

B = volume of standard HCl used in the blank titration, mL

N = normality of standard HCl

Weight of sample used, in grams considering the dilution and the aliquot taken for distillation $= \frac{wt.*10\ mls}{100\ ml}$

$= \frac{wt.in\ g}{10}$

Thus, the percentage of nitrogen in the sample was calculated as

$$\% \text{ Nitrogen} = \frac{14*(A-B)*N*100}{1000*wt.in\ g} \longrightarrow \text{Equation 1}$$

3.6.1.2 Determination of available phosphorus (P) in the soil

The bray No.1 method was used to analyze the P content in the Soil. Five grams of air-dried soil which was sieved with a 2 mm sieve was weighed and put into a shaking bottle and 35 mL of the extracting solution made up of ammonium fluoride (0.03M NH_4F) and hydrochloric acid (0.5M Hcl) was added to it. The mixture was shaken on a mechanical shaker for 8 minutes at 3000 rpm then filtered through whatman No. 42 filter paper. One ml of the clear extract was pipetted into a set of clean test tubes and 6 mL of distilled water was added. Two ml of molybadate-ascorbic acid was added as a colour reagent and mixed well. One mL of ascorbic solution was also added and mixed thoroughly again and the colour water length measured at 650 nm on the UV/VIS Spectrophotometer after 30 minutes.

The phosphorus (P) Content was calculated using the equation:

$$P\ mg/kg = \frac{(\mathrm{A}-\mathrm{B})*\mathrm{V.E}*\mathrm{d.f}}{\mathrm{Wt.}} \longrightarrow \text{Equation 2}$$

Where

P mg/kg = Available phosphorus in the soil

A= Absorbance of Sample from the machine reading

B = Absorbance of Blank from the machine reading

V.E = Volume of extract

d.f = Dilution Factor

Wt. = weight of sample taken

3.6.1.3 Determination of exchangeable potassium (K) in the soil

Potassium is an exchangeable cation which was extracted with 1 M ammonium acetate at pH 7.0. Five g of a 2 mm sieved and air-dried soil was weighed and kept into a 100 mL shaking bottle and 50 ml of 1.0 M ammonium acetate solution was added. The mixture was cocked and shook on a mechanical shaker for 2 hours. The soil solution was then filtered through whatman filter paper No. 42 for a clean and clear filtrate. The potassium was then measured from the filtrate by using the flame photometer.

The exchangeable potassium (K) was calculated as follows:

$$\text{Exc. K}, mg/kg = \frac{(\text{A}-\text{B})*\text{V.E}*\text{d.f}}{\text{Wt.}} \rightarrow \text{Equation 3}$$

Where

Exc. K mg/kg = Exchangeable potassium in the soil

A= Absorbance of sample from the machine reading

B = Absorbance of Blank from the machine reading

V.E = Volume of extract, mL

d.f = Dilution Factor

3.6.1.4 Determination of soil pH

The soil pH was determined using the electrometric method (Adebiyi *et al*., 2006). Ten grams of air – dried soil was weighed and put into a 50 ml beaker and 25 ml of distilled water was added. The Suspension was stirred vigorously for 20 minutes and allowed to stand for 30 minutes by which time most of the suspended clay had settled out from the suspension. The pH meter was calibrated with standard solutions at pH of 7 and 4. The electrode of the pH meter was inserted into the partial settled suspension to read the value and the result recorded.

3.6.2 Nodulation assessment

Due to the destructive nature of the sampling method, nodulation assessment was taken only at the flowering stage and not at two weeks intervals. The number of nodules, size and distribution can indicate the effectiveness or ineffectiveness of the Rhizobia strain. Nodulation assessment was done on the following parameters: Nodule number per plant, nodule colour and vegetative growth (health, vigour and colour of plants). Nodules were classified as effective and ineffective (Kukkamalla and Vardhan, 2016) and plants that were healthy, green and vigorous were considered to be effective in nitrogen fixation with such nodules centered around the tap root (Flynn and Idowu, 2015).

Effective nodules were considered to be those with pinkish or red colour internally, whiles ineffective nodules were considered to be those with whitish or greenish internal colour

when dissected (Ishizawa and Toyoda, 1955). Five plants were randomly selected per plot and examined. Selected plants were carefully dug up with a hoe and collected when the soil was moist to avoid shedding or desiccation of the nodules under dry conditions.

The selected plants were carefully dug out at a distance of 15 cm to the bare of the plant and 30 cm deep in to the soil using a hoe. The plants were then placed in a plastic bucket containing water for 20 minutes to loosen adhering soil on the roots of the plants. The soils were carefully removed from the roots to avoid losing some nodules after the 20 minutes had elapsed. The plants were separated thereafter into shoots and roots by cutting from the first node. The roots were then taken to the University for Development Studies Agssip laboratory where the roots were washed under running water. Nodules from the roots were removed and counted with 270 nodules from each rep assessed for nodule colour. The nodules were then weighed to obtain fresh weight and then oven dried for 72 hours at 70 ^{0}C and weighed again for nodule dry matter assessment.

The nodule number and nodule dry matter were also assessed. These parameters were very imperative to be taken to avoid relying on nodule dry matter because it is hard to completely clean the nodules from soil particles which can increase the weight of the nodules.

The nodule number, nodule colour, vigor and growth of plant were determined by the Zaychuk (2009) procedure below.

The nodule number ratings are:

- Super – nodulated (> 50 nodules)..... 1
- Abundant (> 20 nodules) ……... 2

- Moderate (11 – 20 nodules) …… 3
- Few (5 – 10 nodules) ……..…... 4
- Root nodules absent (0 – 4 nodules) ……..…5

The nodule internal colour ratings are:

- Predominantly red in colour …… 1
- Some pink or reddish colour …… 2
- Some pink or greenish colour ……..... 3
- Some white or greenish colour ……. 4
- Predominantly white or greenish colour ……... 5

The final rating scores for nodulation were done using a scale of 1 to 15 to rate nodulation effectiveness, taking into account nodulation number and nodule colour.

3.6.3 Canopy spread

Five plants were tagged and canopy spread measurement was taken on each at 2, 4, 6, and 8 weeks after planting (WAP). Measurement was made at the widest width for each of the five plants on each plot. The mean canopy spread for each plot was estimated from the five tagged plants.

3.6.4 Plant biomass at flowering stage

Plants selected for nodulation were also used for biomass assessment, nodulation and tissue P, K and N analysis. The shoots and roots were separated and weighed using an electronic scale with a precision of 0.01 g on the field to obtain the shoot weight (fresh above ground dry matter) and root weight (below groundnut dry matter) respectively. The samples from

each plot were packed into brown envelops and labelled. Labelled samples were then transported to the University for Development Studies laboratory on Nyankpala campus for the determination of shoot and root dry matter. The samples were air-dried to constant mass and then oven-dried at 70^0C for 72 hours before the shoots and roots of individual plants were weighed and recorded as in Okogun *et al*. (2005) and Yates *et al*. (2016).

3.6.5 Plant chemical analysis for N, P and K

Plants shoots and roots were cut into pieces and then oven dried at 70 ^{0}C for two days for the chemical analysis of N, P and K (48 hours). The dry samples were then milled and analyzed chemically for N using the Kjeldahl method (Amin and Flowers, 2004; Sáez-plaza *et al*., 2013) as described in page 31.

The same digest was used for the determination of K using the flame photometer (Amin and Flowers, 2004), whiles P was determined by developing a colour for P in the digest and quantifying the concentration of P with the UV/VIS Spectrophotometer through colorimetric determination (Gerdel, 1928).

3.6.6 Determination of biological nitrogen fixation

Maize plants were grown on soils that received the same soil treatments as the groundnuts. The whole plant of both groundnut and maize materials were sampled by treatments and prepared for the quantitative determination of total nitrogen using the Kjeldahl method. The Kjeldahl method involves three steps which are:

i. Digestion

   The plant samples were digested in boiling concentrated sulphuric acid at a temperature of 360^{o}C together with copper sulphate and selenium as catalyst at a

ratio of 10:1 respectively. This was done to break down the carbon chains in the plant material and releases them into solution giving it a brown colour until the whole solution is completely dissolved and oxidized (colourless) resulting in the formation of ammonium sulphate (N).

$$\text{Protein (N)} + H_2SO_4 \xrightarrow{\text{catalyst}} (NH_4)_2SO_4 + CO_2 + H_2O$$

ii. Distillation

Excess sodium hydroxide solution was added to the digested solution to release ammonium ions in the form of ammonia which was then distilled and received on a boric acid solution. The boric acid which has a pink colour changes the colour of the solution into light or deep green depending on the concentration of nitrogen in the solution.

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3\,(gas) + Na_2SO_4 + 2H_2O$$

iii. Titration

The amount of nitrogen in the plant material was then quantified by changing the green colour of the solution to pink by adding a known concentration of hydrochloric acid to the solution. The result was expressed in N% or NH_3.

The amount of nitrogen fixed in the plant was then calculated by using the nitrogen – difference technique (Mweetwa *et al*., 2014: Anglade *et al., 2015*).

Q = N yield (groundnut) - N yield (maize) + [N soil (groundnut) - N soil (maize)]

Where:

Q = Quantity of legume N derived from N_2 fixation

N yield (groundnut) = Total N in groundnut plant

N yield (maize) = Total N in maize plant

N soil (groundnut) = Soil mineral N for groundnut plots after harvest

N soil (maize) = Soil mineral N for maize plots after harvest

The nitrogen difference method however, has some limitations:

- The method may be problematic in soils with moderate to high levels of mineral N which suppresses symbiotic nitrogen fixation activities
- When the root morphology of both the fixing and non-fixing plants is different it can result in different rates background soil nitrogen uptake

3.6.7 Number of pods per plant and grain yield

At physiological maturity of the Chinese variety (95 days after planting, DAP) and Nkatie Sari (120 DAP), the groundnut were harvested from a net plot of 0.6 m x 4 m (2.4 m^2) and 0.8 m x 4 m (3.2 m^2) measured within the two middle rows for each plot for the Chinese and Nkatie Sari varieties respectively. The detached pods were air dried and then oven dried at a temperature of 60 ^{0}C for three days (72 hours).The dried grains were then weighed and recorded. The grain dried weights were used to estimate the yield of the grain per hectare as reported by Yol *et al*. (2018).

3.6.8 Harvest index

The harvest index which is the ratio of the seed yield to the total dry matter (Mukhtar *et al*., 2013) was calculated at harvest using the formula:

$$H = \frac{\text{Grain yield from sample of TDM}}{Xg\ TDM\ Sample} \times 100 \longrightarrow \text{Equation 4}$$

Where H = Harvest index, TDM=Total dry matter (g)

3.7 Data Analysis

The data collected were subjected to analysis of variance (ANOVA) using the GENSTAT statistics package 12th edition. All count data were transformed (O'Hara and Kotze, 2014) before being subjected to ANOVA. Means of treatments were separated by the least significant difference (LSD) test at 5 % probability level (Saville, 2014). The relationship between parameters that were measured were established by using simple correlation.

CHAPTER FOUR

4.0 RESULTS

4.1 Soil Properties

The results of the physio – chemical properties of the soil samples taken from the study area before and after planting are presented in tables 1, 2, 3, 4 and 5 respectively.

Table 1: Chemical and physical properties of soil at the experimental sites prior to planting

	Gurumanchenyili			Zangbalun Fandu		
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Parameters						
pH	5.13± 0.2	5.12± 0.6	5.14± 0.7	5.12±1.4	5.13±1.7	5.13±0.9
OC (%)	1.09± .04	1.09± .02	1.09± .01	1.09±.02	1.09±.05	1.09±.07
Available P (mg/kg)	2.84± 0.1	2.81± .01	2.93± .08	2.83±1.0	2.88±0.9	2.84±0.7
Exchangeable K (mg/kg)	5.04± 0.2	5.05± 0.3	5.06± 0.6	5.02±0.9	5.04±1.2	5.03±0.8
TN (%)	0.05± .01	0.04±.01	0.07± .01	0.05±.01	0.06±.03	0.05±.01
Particle size distribution						
Sand (%)	56.2±47.5	56.2±44.8	56.2±50.0	56.2±49.1	56.2±47.8	56.2±45.4
Clay (%)	5.33±2.34	5.33± 0.7	5.33±3.33	5.33±2.51	5.33±1.55	5.33±2.0
Silt (%)	34.8±20.5	34.8±18.7	34.8±19.7	34.8±22.9	34.8±19.0	34.8±16.6
Texture	Sandy loam					

The soil at the study area was sandy loam with a slightly acidic pH (Table 1). The organic carbon level was moderate (1.09±.07). The available phosphorus recorded was below the critical range (10.0 – 14.0 mg/kg). The exchangeable potassium ranges between 5.02 – 5.06 mg/kg. The total nitrogen recorded before planting at the study areas was generally very low.

4.1.1 Effect of groundnut variety, phosphorus fertilizer and inoculant on soil pH

The highest mean change in soil pH was recorded at 0.32 and the lowest was 0.04 for Nkatie Sari variety in the Gurumanchenyili fields (1, 2, and 3) whiles the highest mean change in soil pH was 0.4 and the lowest mean change was 0.05 for the Chinese variety in the Zangbalun Fandu fields (4, 5, and 6) as shown in (Table 2). The 30 kg/ha of phosphorus fertilizer plus 0 g/kg of inoculant, 60 kg/ha of phosphorus fertilizer plus 6 g/kg inoculant and 30 kg/ha of phosphorus fertilizer plus 6 g/kg inoculant, 0 kg/ha of phosphorus fertilizer plus 3 g/kg inoculant gave the highest pH values among the treatments for Nkatie Sari and Chinese varieties respectively.

Table 2: Effect of groundnut variety, phosphorus fertilization and inoculation on pH of soils in Gurumanchenyili and Zangbalun in Northern Ghana

		Treatments																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	15	17	18
Site 1	Before										5.13								
	After	5.18	5.22	5.2	5.17	5.19	5.21	5.117	5.16	5.17	5.2	5.19	5.21	5.17	5.21	5.18	5.16	5.18	5.17
	Change	0.05	0.09	0.07	0.04	0.06	0.08	0.04	0.03	0.04	0.07	0.06	0.08	0.04	0.08	0.05	0.03	0.05	0.04
Site 2	Before										5.12								
	After	5.19	5.2	5.16	5.18	5.16	5.18	5.16	5.19	5.2	5.22	5.17	5.19	5.16	5.17	5.19	5.16	5.17	5.2
	Change	0.07	0.08	0.04	0.06	0.04	0.06	0.04	0.07	0.08	0.1	0.05	0.07	0.04	0.05	0.07	0.04	0.05	0.08
Site 3	Before										5.14								
	After	5.19	5.21	5.18	5.2	5.17	5.16	5.21	5.17	5.19	5.21	5.18	5.2	5.17	5.2	5.22	5.19	5.17	5.17
	Change	0.05	0.07	0.04	0.06	0.03	0.02	0.07	0.03	0.05	0.07	0.04	0.06	0.03	0.06	0.08	0.05	0.03	0.03
Mean change in pH																			
Site 4	Before										5.12								
	After	5.16	5.18	5.21	5.18	5.16	5.2	5.19	5.13	5.17	5.2	5.18	5.2	5.21	5.19	5.17	5.2	5.18	5.13
	Change	0.04	0.06	0.09	0.06	0.04	0.08	0.07	0.01	0.05	0.08	0.06	0.08	0.09	0.07	0.05	0.08	0.06	0.01
Site 5	Before										5.13								
	After	5.18	5.14	5.2	5.19	5.16	5.21	5.17	5.19	5.2	5.17	5.19	5.14	5.21	5.18	5.14	5.19	5.17	5.21
	Change	0.05	0.01	0.07	0.06	0.03	0.08	0.04	0.06	0.07	0.04	0.06	0.01	0.08	0.05	0.01	0.06	0.04	0.08
Site 6	Before										5.13								
	After	5.15	5.19	5.2	5.17	5.21	5.18	5.21	5.19	5.17	5.21	5.14	5.18	5.19	5.17	5.2	5.16	5.19	5.2
	Change	0.02	0.06	0.07	0.04	0.08	0.05	0.08	0.06	0.04	0.08	0.01	0.05	0.06	0.04	0.07	0.03	0.06	0.07
Mean change in PH		0.05	0.32	0.06	0.05	0.04	0.06	0.07	0.04	0.1	0.4	0.2	0.1	0.06	0.1	0.06	0.05	0.09	0.05

Legend

Nkatie Sari Variety

1 = 30 kg of Phosphorus/ha and 3 g of Inoculant/kg

2 = 30 kg of Phosphorus/ha and 6 g of Inoculant/kg

3 = 30 kg of Phosphorus/ha and 0 g of Inoculant/kg

4 = 0 kg of Phosphorus/ha and 0 g of Inoculant/kg

5 = 0 kg of Phosphorus/ha and 6 g of Inoculant/kg

6 = 0 kg of Phosphorus/ha and 3 g of Inoculant/kg

7 = 60 kg of Phosphorus/ha and 3 g of Inoculant/kg

8 = 60 kg of Phosphorus/ha and 6 g of Inoculant/kg

9 = 60 kg of Phosphorus/ha and 0 g of Inoculant/kg

Chinese Variety

10 = 60 kg of Phosphorus/ha and 6 g of Inoculant/kg

11 = 60 kg of Phosphorus/ha and 0 g of Inoculant/kg

12 = 60 kg of Phosphorus/ha and 3 g of Inoculant/kg

13 = 30 kg of Phosphorus/ha and 3 g of Inoculant/kg

14 = 30 kg of Phosphorus/ha and 0 g of Inoculant/kg

15 = 30 kg of Phosphorus/ha and 6 g of Inoculant/kg

16 = 0 kg of Phosphorus/ha and 6 g of Inoculant/kg

17 = 0 kg of Phosphorus/ha and 0 g of Inoculant/kg

18 = 0 kg of Phosphorus/ha and 3 g of Inoculant/kg

4.1.2 Effect of groundnut variety, phosphorus fertilizer and inoculant on soil exchangeable potassium in Gurumanchenyili and Zangbalun Fandu in Northern Ghana

The highest mean change in soil K was 0.11 and the lowest was 0.01 for Nkatie Sari variety in the Gurumanchenyili fields (1,2, and 3) whiles the highest mean change in

soil K was 0.14 and the lowest mean change was 0.04 for the Chinese variety in the Zangbalun Fandu fields (4,5, and 6) as shown in (Table 3). The 60 kg/ha of phosphorus fertilizer plus 6 g/kg of inoculant, 60 kg/ha of phosphorus fertilizer plus 0 g/kg of inoculant, gave the highest soil K values among the varieties.

Table 3: Effect of groundnut variety, phosphorus fertilization and inoculation on exchangeable potassium (K) of soils in Gurumanchenyili and Zangbalun Fandu

		Treatments																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Site 1	Before									5.04									
	After	5.01	5.01	5.02	5.04	5.03	5.04	5.06	5.05	5.05	5.05	5.05	5.07	5.06	5.04	5.01	5.01	5.01	5.01
	Change	0.03	0.03	0.02	0	0.01	0	0.02	0.01	0.01	0.01	0.01	0.03	0.02	0	0.03	0.03	0.03	0.03
Site 2	Before									5.05									
	After	5.07	5.06	5.06	5.08	5.08	5.07	5.07	5.08	5.08	5.07	5.01	5.07	5.06	5.08	5.07	5.04	5.04	5.04
	Change	0.02	0.01	0.01	0.03	0.03	0.02	0.02	0.03	0.03	0.02	0.04	0.02	0.01	0.03	0.02	0.01	0.01	0.01
Site 3	Before									5.06									
	After	5.06	5.05	5.05	5.05	5.06	5.06	5.04	5.07	5.05	5.05	5.07	5.08	5.06	5.06	5.04	5.04	5.05	5.05
	Change	0	0.01	0.01	0.01	0	0	0.02	0.01	0.01	0.01	0.01	0.02	0	0	0.02	0.02	0.01	0.01
Mean change in K																			
Site 4	Before									5.02									
	After	5.01	5.01	5.04	5.03	5.03	5.04	5.04	5.03	5.02	5.02	5.04	5.03	5.02	5.02	5.04	5.04	5.03	5.04
	Change	0.01	0.01	0.02	0.01	0.01	0.02	0.02	0.01	0	0	0.02	0.01	0	0	0.02	0.02	0.01	0.02
Site 5	Before									5.04									
	After	5.04	5.03	5.05	5.04	5.06	5.06	5.05	5.07	5.05	5.04	5.07	5.06	5.06	5.04	5.05	5.05	5.05	5.04
	Change	0	0.01	0.01	0	0.02	0.02	0.01	0.03	0.01	0	0.03	0.02	0.02	0	0.01	0.01	0.01	0
Site 6	Before									5.03									
	After	5.03	5.03	5.02	5.04	5.05	5.02	5.04	5.01	5.02	5.04	5.06	5.04	5.04	5.02	5.02	5.04	5.02	5.01
	Change	0	0	0.03	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.02
Mean change in K		0.01	0.07	0.1	0.06	0.09	0.07	0.1	0.11	0.07	0.05	0.14	0.11	0.06	0.04	0.11	0.1	0.08	0.09

Legend

Nkatie Sari Variety

1 = 30 kg of Phosphorus/ha and 3 g of Inoculant/kg

2 = 30 kg of Phosphorus/ha and 6 g of Inoculant/kg

3 = 30 kg of Phosphorus/ha and 0 g of Inoculant/kg

4 = 0 kg of Phosphorus/ha and 0 g of Inoculant/kg

5 = 0 kg of Phosphorus/ha and 6 g of Inoculant/kg

6 = 0 kg of Phosphorus/ha and 3 g of Inoculant/kg

7 = 60 kg of Phosphorus/ha and 3 g of Inoculant/kg

8 = 60 kg of Phosphorus/ha and 6 g of Inoculant/kg

9 = 60 kg of Phosphorus/ha and 0 g of Inoculant/kg

Chinese Variety

10 = 60 kg of Phosphorus/ha and 6 g of Inoculant/kg

11 = 60 kg of Phosphorus/ha and 0 g of Inoculant/kg

12 = 60 kg of Phosphorus/ha and 3 g of Inoculant/kg

13 = 30 kg of Phosphorus/ha and 3 g of Inoculant/kg

14 = 30 kg of Phosphorus/ha and 0 g of Inoculant/kg

15 = 30 kg of Phosphorus/ha and 6 g of Inoculant/kg

16 = 0 kg of Phosphorus/ha and 6 g of Inoculant/kg

17 = 0 kg of Phosphorus/ha and 0 g of Inoculant/kg

18 = 0 kg of Phosphorus/ha and 3 g of Inoculant/kg

4.1.3 Effect of groundnut variety, fertilizer and inoculant on soil phosphorus (P)

The highest mean change in soil P was recorded at 0.19 and the lowest was 0.08 for Nkatie Sari variety in the Gurumanchenyili fields (1, 2, and 3) whiles 0.19 is the highest mean change in soil P and the lowest mean change was 0.09 for the Chinese variety in the Zangbalun Fandu fields (4, 5, and 6) as shown in (Table 4). The 60 kg /ha of phosphorus

fertilizer plus 6 g/ha of inoculant, 60 kg/ha of phosphorus fertilizer plus 3 g/ha of inoculant and 30 kg/ha of phosphorus plus 3 g/kg inoculant produced the highest values for mean change in soil P among the varieties.

Table 4: Effect of groundnut variety, phosphorus fertilization and inoculation on phosphorus (P) of soils in Gurumanchenyili and Zangbalun Fandu in Northern Ghana

		Treatments																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Site 1	Before									2.84									
	After	2.88	2.92	2.88	2.85	2.86	2.82	2.88	2.86	2.91	2.87	2.98	2.91	2.86	2.89	2.86	2.88	2.81	2.78
	Change	0.04	0.08	0.04	0.02	0.02	0.02	0.04	0.02	0.07	0.03	0.14	0.07	0.02	0.05	0.02	0.04	0.03	0.06
Site 2	Before									2.81									
	After	2.81	2.85	2.85	2.81	2.84	2.79	2.84	2.92	2.87	2.83	2.94	2.98	2.91	2.84	2.84	2.82	2.82	2.84
	Change	0	0.04	0.04	0	0.03	0.02	0.03	0.11	0.06	0.02	0.13	0.17	0.1	0.03	0.03	0.01	0.01	0.03
Site 3	Before									2.93									
	After	2.97	2.93	2.94	2.94	2.88	2.89	2.97	2.95	2.98	3.03	2.96	2.99	2.96	2.94	2.95	2.9	2.92	2.94
	Change	0.04	0	0.01	0.01	0.05	0.04	0.04	0.02	0.05	0.1	0.03	0.06	0.03	0.01	0.02	0.03	0.01	0.01
Mean change in P																			
Site 4	Before									2.83									
	After	2.85	2.83	2.84	2.83	2.81	2.84	2.93	2.91	2.85	3.02	2.87	2.92	2.85	2.84	2.86	2.84	2.84	2.87
	Change	0.02	0	0.01	0	0.02	0.01	0.1	0.08	0.02	0.19	0.04	0.09	0.02	0.01	0.03	0.01	0.01	0.04
Site 5	Before									2.88									
	After	2.89	2.86	2.89	2.81	2.88	2.82	2.89	2.9	2.94	2.92	2.89	2.97	2.89	2.89	2.88	2.91	2.89	2.84
	Change	0.01	0.02	0.01	0.01	0	0.06	0.01	0.02	0.06	0.04	0.01	0.09	0.01	0.01	0	0.03	0.01	0.04
Site 6	Before									2.44									
	After	2.47	2.49	2.46	2.38	2.41	2.45	2.5	2.52	2.49	2.51	2.47	2.51	2.46	2.48	2.46	2.42	2.42	2.43
	Change	0.03	0.05	0.02	0.04	0.03	0.01	0.06	0.08	0.05	0.07	0.03	0.07	0.02	0.04	0.02	0.02	0.02	0.01
Mean change in P		0.14	0.19	0.13	0.08	0.15	0.16	0.19	0.33	0.31	0.36	0.38	0.55	0.2	0.15	0.12	0.14	0.09	0.19

Legend

Nkatie Sari Variety

1 = 30 kg of Phosphorus/ha and 3 g of Inoculant/kg

2 = 30 kg of Phosphorus/ha and 6 g of Inoculant/kg

3 = 30 kg of Phosphorus/ha and 0 g of Inoculant/kg

4 = 0 kg of Phosphorus/ha and 0 g of Inoculant/kg

5 = 0 kg of Phosphorus/ha and 6 g of Inoculant/kg

6 = 0 kg of Phosphorus/ha and 3 g of Inoculant/kg

7 = 60 kg of Phosphorus/ha and 3 g of Inoculant/kg

8 = 60 kg of Phosphorus/ha and 6 g of Inoculant/kg

9 = 60 kg of Phosphorus/ha and 0 g of Inoculant/kg

Chinese Variety

10 = 60 kg of Phosphorus/ha and 6 g of Inoculant/kg

11 = 60 kg of Phosphorus/ha and 0 g of Inoculant/kg

12 = 60 kg of Phosphorus/ha and 3 g of Inoculant/kg

13 = 30 kg of Phosphorus/ha and 3 g of Inoculant/kg

14 = 30 kg of Phosphorus/ha and 0 g of Inoculant/kg

15 = 30 kg of Phosphorus/ha and 6 g of Inoculant/kg

16 = 0 kg of Phosphorus/ha and 6 g of Inoculant/kg

17 = 0 kg of Phosphorus/ha and 0 g of Inoculant/kg

18 = 0 kg of Phosphorus/ha and 3 g of Inoculant/kg

4.1.4 Effect of groundnut variety, phosphorus fertilizer and inoculant on soil nitrogen

Mean change in soil N was highest at 0.18 and the lowest was 0.02 for Nkatie Sari variety in the Gurumanchenyili fields (1,2, and 3) whiles the highest mean change in soil N was recorded at 0.16 and the lowest 0.04 for the Chinese variety in the Zangbalun

Fandu fields (4,5, and 6) as shown in (Table 5). The 60 kg/ha of phosphorus fertilizer plus 6 g/ha of inoculant, 30 kg/ha of phosphorus fertilizer plus 0 g/kg inoculant and 60 kg/ha of phosphorus plus 3 g/kg inoculant produced the highest mean values for soil N among the varieties.

Table 5: Effect of groundnut variety, phosphorus fertilization and inoculation on total nitrogen (N) of soils in Gurumanchenyili and Zangbalun Fandu in Northern Ghana.

		Treatments																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Site 1	Before									0.05									
	After	0.06	0.06	0.05	0.05	0.07	0.06	0.06	0.07	0.06	0.08	0.07	0.06	0.05	0.05	0.07	0.08	0.05	0.06
	Change	0.01	0.01	0	0	0.02	0.01	0.01	0.02	0.01	0.03	0.02	0.01	0	0	0.02	0.03	0	0.01
Site 2	Before									0.04									
	After	0.06	0.08	0.05	0.04	0.06	0.05	0.06	0.07	0.06	0.08	0.05	0.06	0.05	0.04	0.06	0.06	0.03	0.04
	Change	0.02	0.04	0.01	0	0.02	0.01	0.02	0.03	0.02	0.04	0.01	0.02	0.01	0	0.02	0.02	0.01	0
Site 3	Before									0.07									
	After	0.07	0.08	0.07	0.06	0.08	0.07	0.07	0.09	0.08	0.09	0.07	0.08	0.07	0.06	0.09	0.08	0.07	0.06
	Change	0	0.01	0	0.01	0.01	0	0	0.02	0.01	0.02	0	0.01	0	0.01	0.02	0.01	0	0.01
Mean change in N																			
Site 4	Before									0.05									
	After	0.06	0.07	0.06	0.06	0.08	0.05	0.05	0.09	0.06	0.08	0.06	0.05	0.07	0.07	0.05	0.06	0.03	0.06
	Change	0.01	0.02	0.01	0.01	0.03	0	0	0.04	0.01	0.03	0.01	0	0.02	0.02	0	0.01	0.02	0.01
Site 5	Before									0.06									
	After	0.07	0.09	0.05	0.06	0.06	0.05	0.06	0.09	0.06	0.09	0.05	0.06	0.08	0.08	0.06	0.07	0.05	0.05
	Change	0.01	0.03	0.01	0	0	0.01	0	0.03	0	0.03	0.01	0	0.02	0.02	0	0.01	0.01	0.01
Site 6	Before									0.05									
	After	0.06	0.07	0.04	0.05	0.06	0.05	0.06	0.09	0.07	0.06	0.06	0.07	0.07	0.05	0.06	0.07	0.05	0.06
	Change	0.01	0.02	0.01	0	0.01	0	0.01	0.04	0.02	0.01	0.01	0.02	0.02	0	0.01	0.02	0	0.01
Mean change in N		0.06	0.13	0.04	0.02	0.09	0.03	0.04	0.18	0.07	0.16	0.06	0.06	0.08	0.05	0.07	0.1	0.04	0.05

Legend

Nkatie Sari Variety

1 = 30 kg of Phosphorus/ha and 3 g of Inoculant/kg

2 = 30 kg of Phosphorus/ha and 6 g of Inoculant/kg

3 = 30 kg of Phosphorus/ha and 0 g of Inoculant/kg

4 = 0 kg of Phosphorus/ha and 0 g of Inoculant/kg

5 = 0 kg of Phosphorus/ha and 6 g of Inoculant/kg

6 = 0 kg of Phosphorus/ha and 3 g of Inoculant/kg

7 = 60 kg of Phosphorus/ha and 3 g of Inoculant/kg

8 = 60 kg of Phosphorus/ha and 6 g of Inoculant/kg

9 = 60 kg of Phosphorus/ha and 0 g of Inoculant/kg

Chinese Variety

10 = 60 kg of Phosphorus/ha and 6 g of Inoculant/kg

11 = 60 kg of Phosphorus/ha and 0 g of Inoculant/kg

12 = 60 kg of Phosphorus/ha and 3 g of Inoculant/kg

13 = 30 kg of Phosphorus/ha and 3 g of Inoculant/kg

14 = 30 kg of Phosphorus/ha and 0 g of Inoculant/kg

15 = 30 kg of Phosphorus/ha and 6 g of Inoculant/kg

16 = 0 kg of Phosphorus/ha and 6 g of Inoculant/kg

17 = 0 kg of Phosphorus/ha and 0 g of Inoculant/kg

18 = 0 kg of Phosphorus/ha and 3 g of Inoculant/kg

4.2 Climatic data for the cropping season

The data for the study site on rainfall, temperature, sunshine hours, and relative humidity were obtained from the savannah research institute as shown in Table 6. The total rainfall during the peak period (July to September) was good for the growth and development of

peanuts. The temperature for the cropping period was within the optimal temperature (25-30^{o}C) for the growth and fixation of nitrogen by the groundnut plant.

Table 6: Rainfall, sunshine, temperature and relative humidity during cropping season, 2017

Month	Total rainfall (mm)	Number of rain days	Mean sunshine hours	Mean temperature (^{0}C)	Mean relative humidity (%)
June	186.9	11	7.2	28.7	80
July	300.8	11	6.8	26.8	84
August	120	11	6.2	26.4	84
September	147.6	12	5.7	26.9	84
October	40.3	7	8.1	27.9	75
Total	525.6	52	34.0	136.7	407
Mean	105.2	10.40	6.80	27.34	81.40

(Source: SARI-Nyankpala, 2017)

4.3 Canopy spread

The second order interaction of variety, phosphorus fertilizer and inoculant did not significantly ($P > 0.05$) affect canopy spread at 2 and 6 WAP. However, the main effect of variety did significantly ($P < 0.002$) increase canopy spread at 4 WAP. The Nkatie Sari

gave the highest canopy spread (0.206) at 4 WAP whiles the Chinese variety gave the lowest canopy spread (0.172) at 4 WAP. There was no significant different between the canopy spread of Nkatie Sari and the Chinese variety for 4 WAP.

The first order interaction of variety and inoculant significantly ($P < 0.002$) increased canopy spread at 8 WAP. The 6 g/kg of inoculant gave the highest canopy spread (0.227) in Nkatie Sari whiles the Chinese variety gave the lowest canopy spread (0.189) at 8 WAP.

Table 7: Canopy spread as influenced by variety, phosphorus fertilizer and inoculant in Gurumanchenyili and Zagbalun Fandu

Treatment	Week 2	Week 4	Week 6	Week 8
Variety				
Chinese	0.1090	0.1715	0.1643	0.2013
Nkatie Sari	0.1089	0.2057	0.1723	0.2141
LSD (0.05)	0.0108	6.215E-03	9.874E-03	4.978E-03
P-Value	0.9882	0.0027	0.4566	0.0499
Phosphorus fertilizer rate (kg/ha)				
0	0.1097	0.1852	0.1664	0.2042
30	0.1079	0.1929	0.1638	0.2045
60	0.1092	0.1877	0.1748	0.2144
LSD (0.05)	8.683E-03	0.0142	0.0114	9.097E-03
P-Value	0.9770	0.8603	0.6137	0.4605
Inoculant rate (g/kg)				
0	0.1066	0.1858	0.1728	0.2029
3	0.1075	0.1831	0.1680	0.2078
6	0.1127	0.1969	0.1642	0.2123
LSD (0.05)	9.368E-03	0.0138	0.0101	7.132E-03
P-Value	0.7831	0.5750	0.6983	0.4233
V x PR				
P-Value	0.0681	0.2605	0.1368	0.1502
V x IR				
P-Value	0.9008	0.7371	0.6636	0.0020
PR x IR				
P-Value	0.1612	0.5472	0.3392	0.6927
V x PR x IR				
P-Value	0.5907	0.8599	0.3455	0.3931

4.4 Haulm weight

The interaction between variety, phosphorus fertilizer and inoculant did not significantly affect haulm weight. However, the main effect of inoculant significantly ($P < 0.003$) increased haulm weight (Figure1). Zero gram per kilogram of inoculant significantly produced the highest haulm weight (5607.6 Kg/ha) in Nkatie Sari variety whiles the 6 g/kg of inoculant produced the lowest haulm weight (4748.3 kg/ha) in Chinese variety (Appendix 5). Zero gram per kilogram of inoculant was statistically different from the three gram per kilogram of inoculant applied to the groundnut varieties.

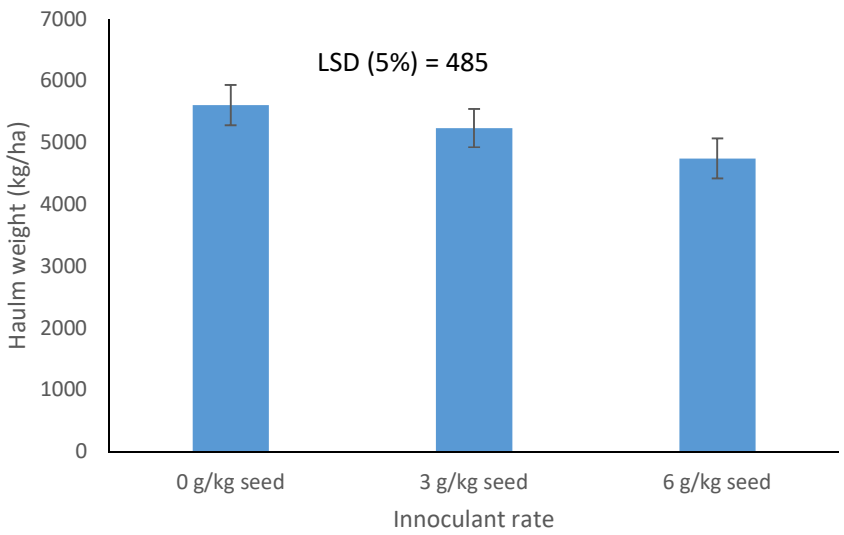

Figure 1: Effect of variety and inoculant on haulm weight of groundnut

4.5 Biomass

The first and second order interactions of variety, phosphorus fertilizer and inoculant did not significantly ($P > 0.05$) affect biomass (Appendix 6). However, the main effect of inoculant significantly ($P < 0.0311$) increased biomass production. The control produced

the lowest biomass (842.0 kg/ha) whiles the 6 g/kg of inoculant produced the highest biomass (1033.0kg/ha) as shown in (Figure 2).

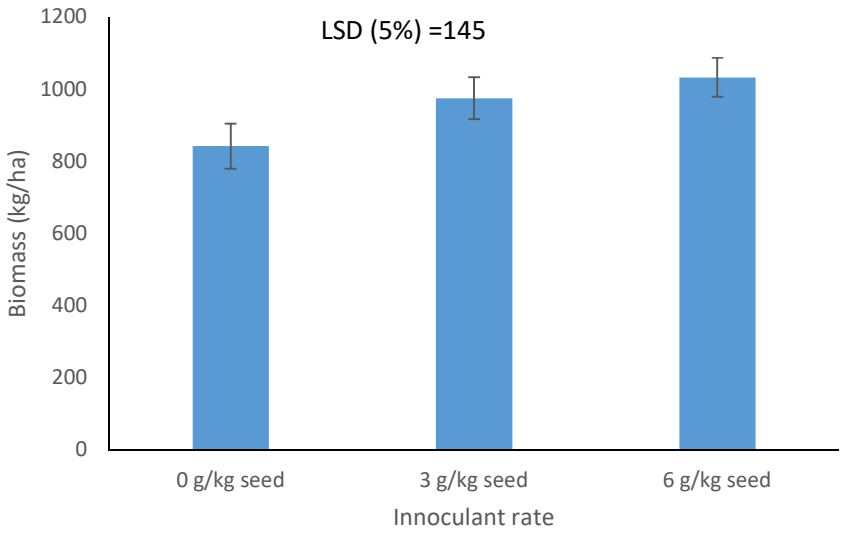

Figure 2: Effect of inoculant on biomass of groundnut

4.6 Plant height

The first and second order interactions of groundnut variety, phosphorus fertilizer and inoculant did not significantly ($p > 0.05$) affect plant height at 2, 4, 6 and 8 WAP. However, the main effect of variety for 2, 4, 6 and 8 WAP did significantly ($p < 0.05$) affect plant height (Appendix 7, 8, 9 and 10). In terms of variety effect, the Nkatie sari variety gave the highest plant height for all weeks of interest (Table: 8).

Table 8: Effect of variety, phosphorus fertilizer and inoculant on plant height

Treatment	Week 2	Week 4	Week 6	Week 8
Variety				
Chinese	0.0342	0.0629	0.0780	0.1132
Nkatie Sari	0.0401	0.1211	0.1301	0.1484
LSD (0.05)	3.972E-03	0.0285	0.0414	0.0212
P-Value	0.0128	0.0033	0.0233	0.0079
Phosphorus fertilizer rate (kg/ha)				
0	0.0370	0.0850	0.1029	0.1360
30	0.0370	0.0984	0.1100	0.1347
60	0.0370	0.0927	0.0993	0.1217
LSD (0.05)	3.972E-03	0.0143	0.0133	0.0212
P-Value	0.8104	0.1737	0.2553	0.5344
Inoculant rate (g/kg)				
0	0.0374	0.0884	0.1000	0.1204
3	0.0378	0.0923	0.1059	0.1407
6	0.0363	0.0954	0.1062	0.1313
LSD (0.05)	2.065E-03	8.606E-03	0.01113	0.0290
P-Value	0.3457	0.2724	0.4700	0.3828
V x PR				
P-Value	0.2358	0.1715	0.0850	0.1595
V x IR				
P-Value	0.6758	0.2089	0.1075	0.0907
PR x IR				
P-Value	0.0675	0.2632	0.6224	0.5125
V x PR x IR				
P-Value	0.7906	0.0617	0.7426	0.6599

4.7 Nodule number per plant at flowering

The second order interactions among variety, phosphorus fertilizer and inoculant did not show any significant ($p > 0.05$) differences on nodule number per plant. However, the interaction between variety and inoculant did significantly ($p < 0.03$) increased the number of nodules produced per plant (Appendix 11). The Nkatie Sari variety produced the greatest mean number of nodules (42) per plant at 0 g/kg of inoculant whiles the Chinese variety produced the least mean number of nodules (37) per plant at 6 g/kg of inoculant (Figure 3)

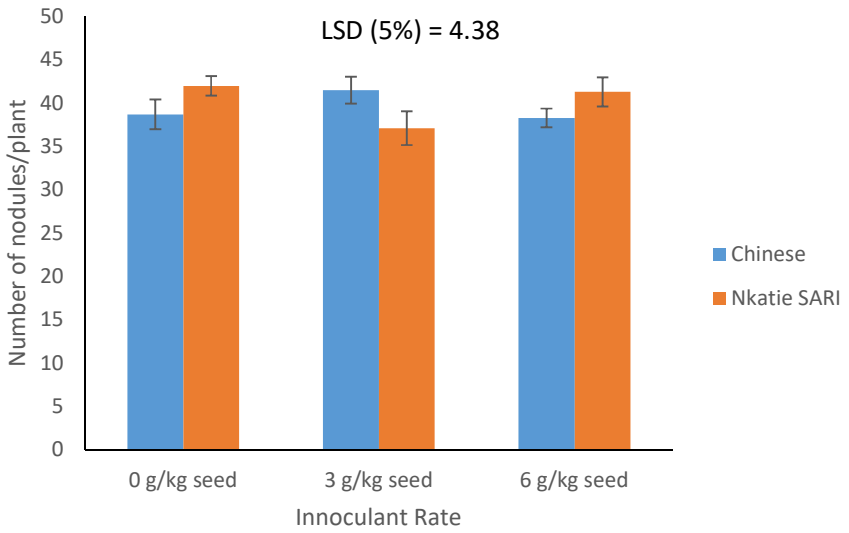

Figure 3: Effect of variety by inoculant on nodule number per plant at flowering

4.8 Effective nodules

The second order interactions of variety, phosphorus fertilizer and inoculant were not significant ($p > 0.05$) at flowering. However, the interaction between variety and phosphorus did significantly ($p < 0.001$) increase effective nodules at flowering (Appendix12). Nkatie Sari gave the highest effective nodules (13.44) whiles the Chinese variety gave the lowest effective nodules (11.50) as shown in (Figure 4).

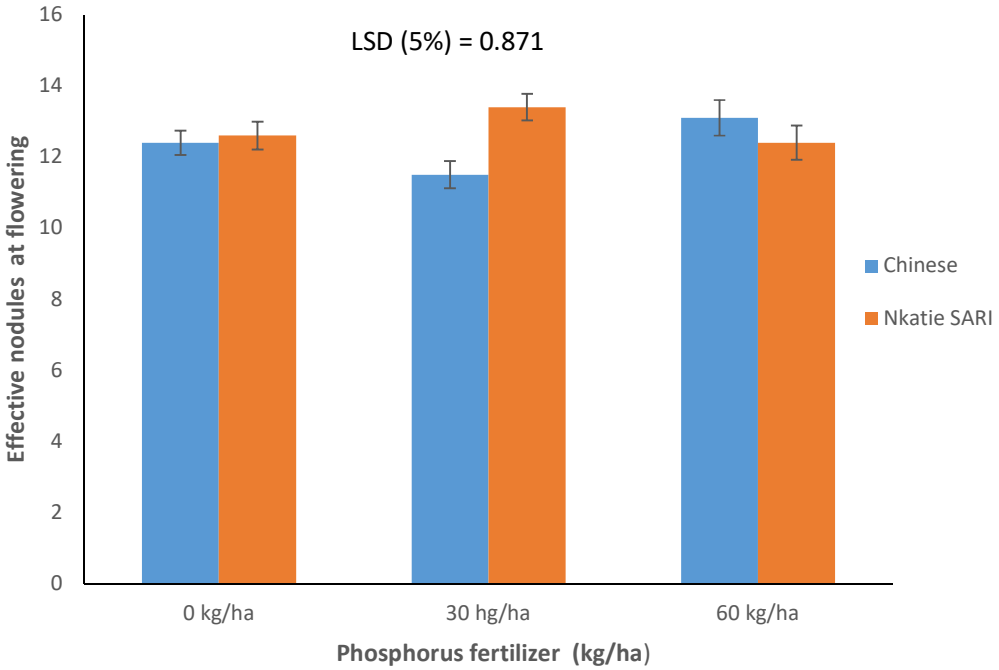

Figure 4: Effect of variety by phosphorus on effective nodules at flowering

4.9 Ineffective nodules

The interaction between variety and phosphorus were significant ($p < 0.005$) regarding ineffective nodules (Appendix 13). The Chinese variety produced the highest ineffective nodules (3.56) and Nkatie Sari produced the lowest ineffective nodules (1.56) as shown in (Figure 5). The highest ineffective nodules produced by 60 kg/ha of phosphorus fertilizer were significantly different from the ineffective nodules produced by the 30 kg/ha of phosphorus fertilizer applied.

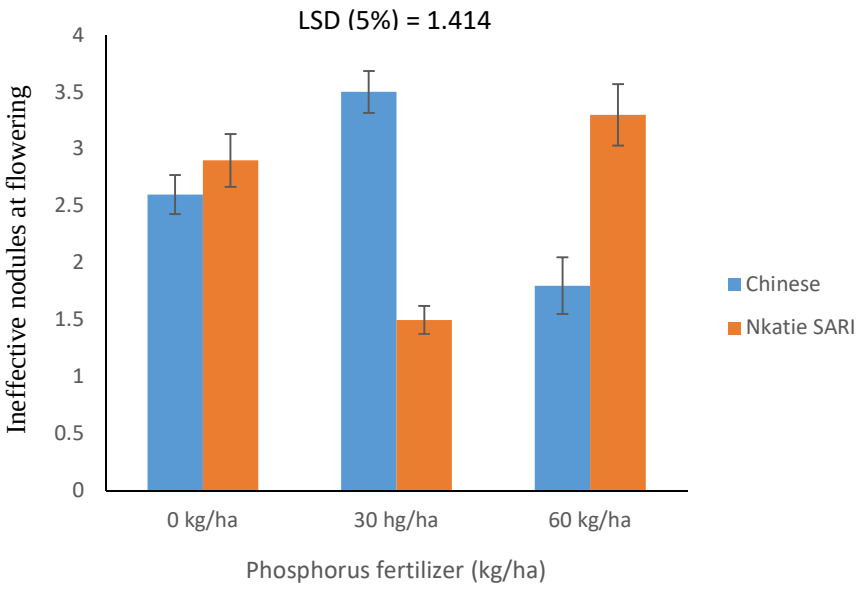

Figure 5: Effect of variety by phosphorus on ineffective nodules at flowering

4. 10 Number of branches per plant

The main effect, the first and second order interactions between variety, phosphorus fertilizer and inoculant were not significant ($p > 0.05$) on number of branches per plant at all weeks of interest (Appendix 14, 15, 16 and 17). However, Nkatie sari produced the highest number of branches (3.04) and the 3 g/kg of inoculant produced the highest number of branches (2.98) per plant (Table 9).

Table 9: Number of branches as influenced by variety, phosphorus fertilizer and inoculant

Treatment	Week 2	Week 4	Week 6	Week 8
Variety				
Chinese	3.0411	4.1741	5.3185	5.4000
Nkatie Sari	2.8456	4.2815	5.5074	5.5741
LSD (0.05)	0.4503	0.3445	0.4360	0.4351
P-Value	0.3150	0.4592	0.3161	0.3510
Phosphorus fertilizer rate (kg/ha)				
0	2.9772	4.4333	5.3611	5.4611
30	2.9461	4.0778	5.3444	5.4056
60	2.9067	4.1722	5.5333	5.5944
LSD (0.05)	0.1939	0.3169	0.44448	0.43088
P-Value	0.7517	0.0760	0.6251	0.6590
Inoculant rate (g/kg)				
0	2.9472	4.1778	5.2889	5.3833
3	2.9811	4.2333	5.5056	5.6000
6	2.9017	4.2722	5.4444	5.4778
LSD (0.05)	0.1744	0.2202	0.3353	0.3254
P-Value	0.6600	0.6911	0.4166	0.4153
V x PR				
P-Value	0.2114	0.6605	0.7078	0.6590
V x IR				
P-Value	0.9103	0.7004	0.8193	0.8894
PR x IR				
P-Value	0.8288	0.11887	0.0674	0.0625
V x PR x IR				
P-Value	0.8197	0.2293	0.7319	0.7174

4. 11 N, P and K contents of plants

The result for the analyzed plant material for nitrogen, potassium and phosphorus content is recorded in (Table 10) below. The interaction of variety, Phosphorus fertilizer and inoculant did significantly ($p < 0.007$) influence the amount of nitrogen content in the whole plant material (Appendix 18). The Nkatie sari accumulated more nitrogen (1.93%) at 60 kg/ha of phosphorus fertilizer and 6 g/kg inoculant whiles the Chinese variety accumulated less nitrogen (1.56%) at 0 kg/ha of phosphorus fertilizer and 0 g/kg inoculant.

The interaction of phosphorus fertilizer and inoculant significantly ($p < 0.014$) affected the accumulation of phosphorus in the plant material (Appendix 19). The control less influenced the accumulation of phosphorus in the Nkatie sari variety (766.8 mg/kg) whiles 60 kg /ha of phosphorus fertilizer plus 6 g/kg inoculant increased the accumulation of phosphorus (7319.7 mg/kg) in Chinese variety. The phosphorus content in plant material at 60 kg/ha of phosphorus fertilizer plus 6 g/kg inoculant applied was significantly different from the phosphorus content in plant material at 0 kg/ha of phosphorus fertilizer plus 0 g/kg of inoculant applied.

The first order interaction of variety and inoculant significantly ($p < 0.002$) aided in the accumulation of potassium in the plant material (Appendix 20). The 6 g/kg inoculant influenced the accumulation of potassium (10484 mg/kg) more in Nkatie sari variety than the accumulation of potassium (4753 mg/kg) in the Chinese variety at 0 g/kg of inoculant applied. The interaction of variety and phosphorus fertilizer significantly ($p < 0.029$) influenced the potassium content in the plant material. The 60 kg/ha of phosphorus fertilizer gave the highest potassium content (11568 mg/kg) in the Chinese variety and also

the lowest potassium content (3456 mg/kg) in the Chinese variety at 0 kg/ha of phosphorus fertilizer applied.

Table 10: N, P, K contents of plants

Treatment	N	P	K
Variety			
Chinese	1.5604	3393.1	7191.9
Nkatie Sari	1.9333	3238.7	7176.3
LSD (0.05)	0.0388	2285.7	80277
P-Value	0.0002	0.8690	0.9620
Phosphorus fertilizer rate (kg/ha)			
0	1.2444	919..6	3607
30	1.7122	2938.4	7426
60	2.2839	6089.7	10519
LSD (0.05)	0.1741	2370.0	1394.2
P-Value	0.0000	0.0008	0.0000
Inoculant rate (g/kg)			
0	1.3367	2756.5	5256.7
3	1.6839	3107.3	6450.2
6	2.2200	4083	9845.5
LSD (0.05)	0.1433	389.85	1095.2
P-Value	0.0000	0.0000	0.0000
V x PR			
P-Value	0.7142	0.8582	0.0295
V x IR			
P-Value	0.0740	0.4039	0.0023
PR x IR			
P-Value	0.0002	0.0143	0.0473
V x PR x IR			
P-Value	0.0073	0.4550	0.1422

4.12 Biological nitrogen fixation

The second order interaction of variety, phosphorus and inoculant did significantly **(***p* < 0.0015) influence the amount of nitrogen fixed (Appendix 21). The highest nitrogen (1.68%) was fixed at 60 kg/ha of phosphorus plus 6 g/kg of inoculant applied in Nkatie sari whiles the 0 kg/ha of phosphorus plus 0 g/kg of inoculant applied fixed the lowest amount of nitrogen (0.11%) in Chinese variety. The 60 kg/ha of phosphorus fertilizer plus 6 g/kg inoculant applied was significantly different from the 30 kg/ha of phosphorus fertilizer plus 3 g/kg inoculant applied regarding the amount of nitrogen fixed (Table 11).

Table 11: N fixation as affected by variety, phosphorus and rhizobium inoculation rates

Treatment	N Fixed (%)
Variety	
Chinese	0.2013
Nkatie Sari	0.2141
LSD (0.05)	0.1393
P-Value	0.1025
Phosphorus fertilizer rate (kg/ha)	
0	0.2042
30	0.2045
60	0.1741
LSD (0.05)	0.1878
P-Value	0.0000
Inoculant rate (g/kg)	
0	0.2029
3	0.2078
6	0.2123
LSD (0.05)	0.1832
P-Value	0.0000
V x PR	
P-Value	0.0252
V x IR	0.8075
P-Value	0.0020
PR x IR	
P-Value	0.0002
V x PR x IR	
P-Value	0.0015

4. 13 Number of pods per hectare

The main effects, the first and second order interactions between variety, phosphorus fertilizer and inoculant were not significant ($p > 0.05$) with regards to number of pods per hectare (Appendix 23). However, the Chinese variety produced the highest number of pods per hectare (179630) whiles Nkatie Sari produced the lowest pod number (160648) per hectare. The control and the 60 kg/ha of phosphorus fertilizer gave the highest pod number (171528) and 6 g/kg of inoculant gave the highest pod number (179861) more than the control (Table 12)

Table 12: Number of pods per hectare as influenced by variety, phosphorus fertilizer and inoculant

Treatment	Means
Variety	
Chinese	179630
Nkatie Sari	160648
LSD (0.05)	41448
P-Value	0.2921
Phosphorus fertilizer rate (kg/ha)	
0	171528
30	167361
60	171528
LSD (0.05)	18761
P-Value	0.8676
Inoculant rate (g/kg)	
0	161806
3	168750
6	179861
LSD (0.05)	15830
P-Value	0.0790
V x PR	
P-Value	0.0797
V x IR	
P-Value	0.5658
PR x IR	
P-Value	0.1779
V x PR x IR	
P-Value	0.2050

4. 14 Pod weight (kg/ha)

The main effect of phosphorus significantly **(***p* < 0.0000) increased pod weight (Appendix 24). Sixty kilogram of phosphorus fertilizer gave the highest pod weight (4817.7 kg/ha) in Nkatie sari whiles the 0 kg/ha gave the lowest pod weight (1597.2 kg /ha) in the Chinese variety (Figure 10). There were significant difference between pod weight produced by 60 kg/ha of phosphorus and that produced by the 30 kg/ha of phosphorus fertilizer.

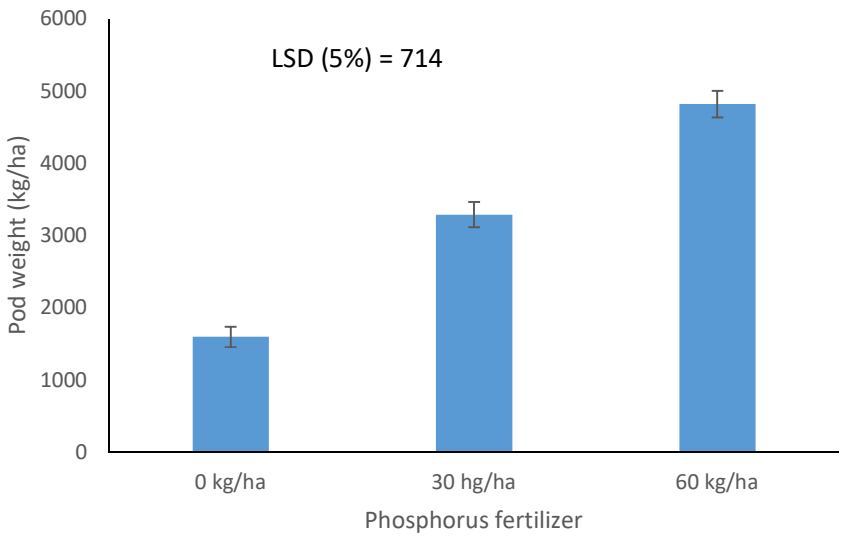

Figure 6: Pod weight as affected by phosphorus fertilizer

The main effect of inoculant also significantly (*p* < 0.004) increased pod weight. Nkatie sari produced the highest pod weight (3550.3 kg/ha) at 6 g/kg inoculant whiles the Chinese produced the lowest pod weight (3064.2 kg/ha) at 3 g/kg inoculant. The 6 g/kg of inoculant is significantly different from the 3 g/kg of inoculant (Figure 7).

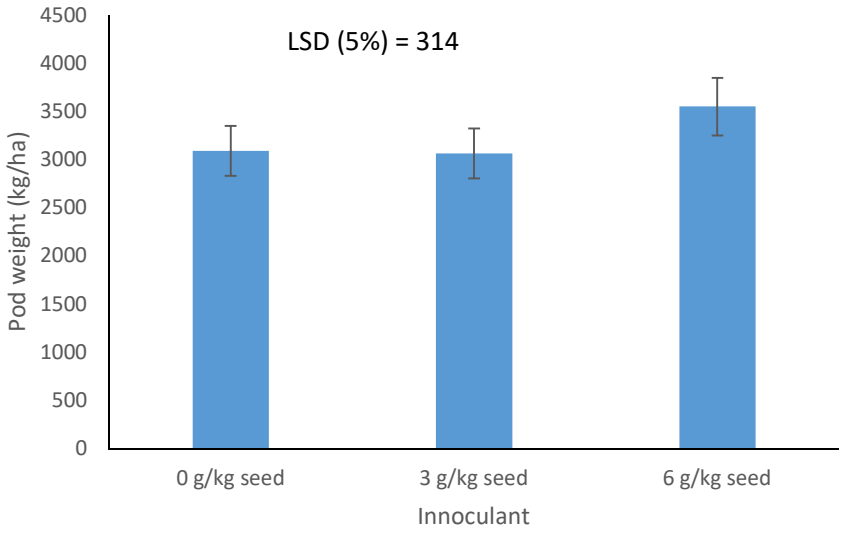

Figure 7: Pod weight as affected by inoculant

4.15 Harvest index

The results indicate that the main effect of phosphorus fertilizer ($p < 0.0001$) and inoculant ($p < 0.033$) significantly affected harvest index except variety (Appendix 25). Sixty kilogram per hectare of phosphorus fertilizer gave the highest harvest index (51.07) whiles the zero kilogram per hectare of phosphorus fertilizer gave the lowest harvest index (19.76). There was no significant difference between the 60 kg/ha of phosphorus fertilizer and 30 kg/ha of phosphorus fertilizer applied (Figure 12).

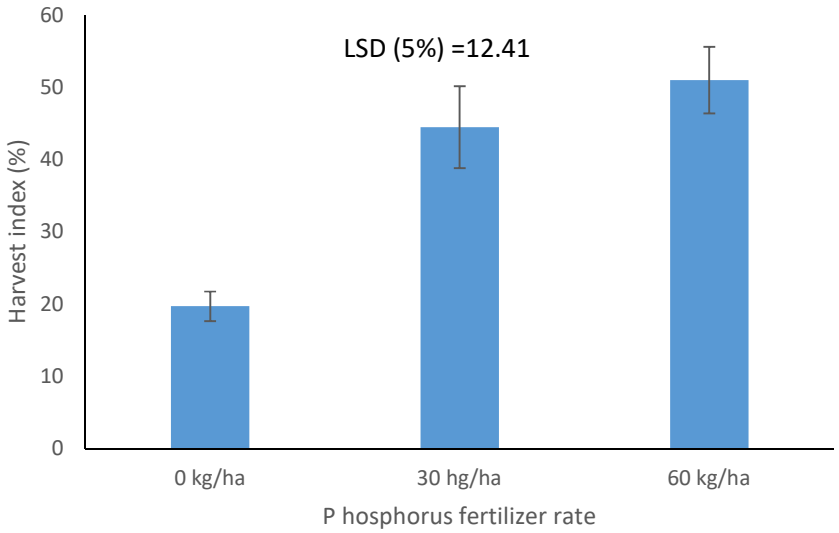

Figure 8: Effect of phosphorus fertilizer rates on harvest index of groundnut

The main effect of inoculant quantities significantly ($p < 0.033$) influenced harvest index (Appendix 25). Three gram per kilogram of inoculant produced the highest harvest index (43.30) whiles the control produced the lowest harvest index (31.05). The six gram per kilogram of inoculant was not significantly different from the 3 g/kg of inoculant applied (Figure 9).

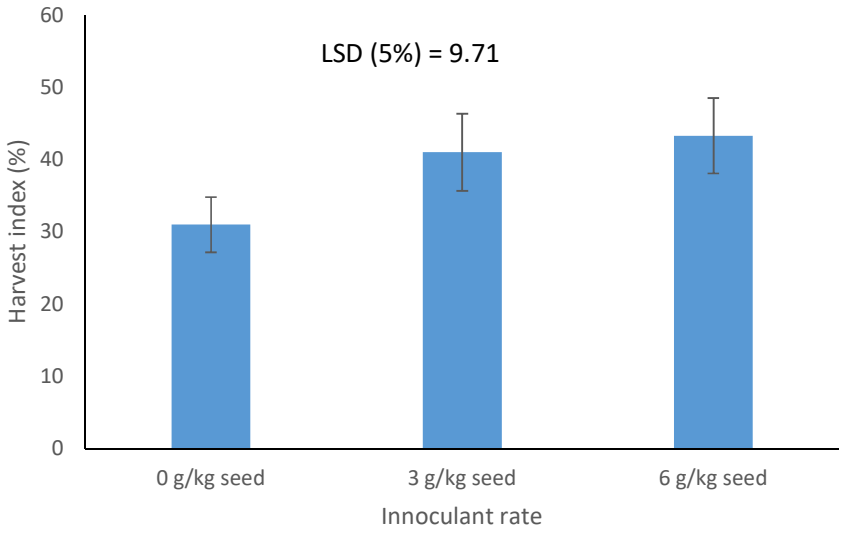

Figure 9: Effect of inoculant rates on harvest index of groundnut

CHAPTER FIVE

5.0 Discussion

5.1 Soil analyses

Soil pH

The pH of the soil at the study area was slightly acidic. This promotes the growth and development of the plant leading to the fixation of nitrogen in the soil. Such pH values are needed for effective nitrogen fixation by rhizobia in most leguminous plants (Simon *et al*., 2014). Ferguson *et al*. (2013), reported that, slightly acidic soils ensure the availability of soil nutrients. Ferguson *et al*. (2014), further reported, that low soil pH result in a reduction in plant and rhizobia growth leading to about 50 % yield losses. The formation of nodules which lead to nitrogen fixation was not affected in these findings by the low pH as reported by Unkovich (2012).

Total nitrogen content in the soil

The total nitrogen was generally low in the experimental site before and after harvest though there were slight increase in the nitrogen levels among the treatments. The total nitrogen in the soil was below the critical limit (0.25 %) for crop production which can lead to reduced nitrogen fixation by the plant. Low nitrogen in the soil stimulates biological nitrogen fixation in the soil (Simon *et al.,* 2014) which was the case in this experiment. The low nitrogen in the soil was as a result of ineffective indigenous rhizobia interfering with the establishment of the applied inoculant strain to effect the nitrogen fixation process (Koskey *et al*., 2017).

Badawi *et al*. (2011) reported that, indigenous rhizobia in the soil can hinder the effectiveness of inoculant applied to the soil.

Available phosphorus

Low available phosphorus (2.84 – 3.57 mg/kg) was recorded in the soil before planting and after harvest respectively. The value for the available phosphorus was below the critical range (10.0 – 14.0 mg/kg) though it increased after harvest. This might have affected root development of the plant and the fixation of nitrogen. The low phosphorus in the soil was as a result of unavailability of phosphorus in the soil for the plant. This could have been the reason why phosphorus fertilizer was applied so that the nutrient could be made more available to plants.

This finding agrees with Carstensen *et al*. (2018) who stated that, deficiency of phosphorus hinders root development which affects biological nitrogen fixation. The activities of nitrogenase in root nodules, which plays an important role in nitrogen fixation, is also affected in soils with low phosphorus (Almeida *et al*., 2000). Weisany *et al*. (2013) reported that, deficiency of phosphorus can seriously limit biological nitrogen fixation. The growth of legumes is most limited by phosphorus deficiency (Vance, 2001; Jensen and Hauggard, 2003) leading to reduction in biological nitrogen fixation (Mbaga *et al*., 2014).

5.2 Canopy spread

The result indicated that the main effect of variety and the interaction between variety and inoculant significantly ($P < 0.05$) affected canopy spread at 4 and 8 WAP respectively (Table 7). The difference in canopy spread among the two varieties due to their genetic makeup and the influence of the phosphorus fertilizer on the growth of the plant. Mukhtar

et al. (2013) reported genetic differences among groundnut varieties. Dapaah *et al.* (2014) also observed that, the creeping type of groundnut (Nkatie Sari) produce more branches per plant as compared to the erect type of groundnut (Chinese). The Nkatie Sari variety produced the highest canopy closure at the early reproductive stage more than the Chinese variety which is important for higher solar radiation interception and higher yield (Rahman and Hossain, 2011). Leon *et al*. (2016) stated that difference in canopy among cultivars was significant which confirms the findings of this research. Mukhtar (2011) also reported similar findings where canopy differed significantly among varieties of groundnut. However, Drammeh (2015) asserts that there are no significant differences among varieties in all the phosphorus rates applied.

5.3 Biomass

The findings showed that inoculant had a significant ($p < 0.0311$) influence on biomass of Nkatie Sari and the Chinese varieties of groundnut (Figure 2). The difference in biomass among the two varieties was as a result of inoculant increasing the number of branches per plant, shoots and the variations in the genetic makeup of the two varieties of groundnut. Sharma *et al*. (2014) reported that inoculant produced a higher plant biomass, longer roots, and shoots and also increased the number of branches per plant. Mandou *et al*. (2017) also gave a similar report that, rhizobia inoculant increased significantly the biomass of the plant. Oktaviani *et al*. (2017) assert that rhizobia inoculation increased the dry weight of the plant more than the control. Rhizobia inoculation also increased shoot biomass among varieties of legumes (Chemining'Wa *et al*., 2017). Nkatie Sari displayed superiority regarding dry matter production. The result agrees with Olayinka and Etejere (2015) who reported that, biomass among two groundnut varieties differed significantly ($p \leq 0.01$).

Mukhtar *et al*. (2013) also reported that, significant differences were exhibited by groundnut varieties regarding their total dry matter. Dapaah *et al*. (2014) observed higher significant differences of total dry matter accumulation in two groundnut varieties. Chemining'Wa *et al*. (2017) observed that, lablab, common bean and lima bean were superior in shoot biomass more than the other legumes. However, total dry matter did not differ significantly among groundnut varieties as reported by Camilotti *et al.* (2012).

5.4 Haulm weight

The main effects of variety, phosphorus fertilizer and inoculant did not significantly **(***p* > 0.05**)** affect haulm weight**.** However, the main effect of inoculant significantly (*p* > 0.003) increased haulm weight. There was a significant increase in haulm weight because inoculant increased the branches, leaf and height of the groundnut plant. Dey *et al.* (2004) reported that, the application of plant growth promoting rhizobacteria in peanuts increased plant haulm weight over the control. Tarekegn and Kibret (2017) observed that seed inoculation of Bradyrhizobium japonicum significantly increased haulm weight per plant over the uninoculated control which agrees with this finding. Didagbe *et al*. (2014) assert that, the use of rhizobial inoculant increased haulm weight in groundnut varieties. Asante *et al*. (2020) also observed that inoculants increased haulm weight of groundnut. However, Mweetwa *et al*. (2014) reported that, the use of inoculant did not result in a significant change in haulm weight of groundnut. Chemining'Wa *et al*. (2007) also gave a similar report that Rhizobia inoculant did not significantly improve haulm weight per plant in lablab and common beans.

5.5 Plant height

The main effect of variety did significantly ($p < 0.05$) affect plant height at 2, 4, 6 and 8 WAP (Table 8). The significant increase in plant height is as a result of the variation in genetic composition of the two varieties of groundnut. Kamara *et al.* (2011) reported significant differences in plant height among two varieties of groundnut. This observation agrees with the findings of Canavar and Kaynak (2008) who reported significant differences in plant height among peanut varieties. A similar result was also reported by Arruda *et al. (*2015) that, main effect of cultivar showed significant ($p \leq 0.05$) difference regarding plant height in peanuts. Golakia *et al*. (2005) also observed significant difference regarding plant height among the Virginia runner type of groundnut and the Spanish bunch type of groundnut which gave the highest plant height over the former. Kakahy *et al*. (2012) recorded variation in plant height among varieties of beans and attributed the cause to variation in environmental factors among the varieties.

5.6 Nodule number per plant at flowering

The first order interaction between variety, phosphorus fertilizer and inoculant did not significantly ($p > 0.05$) increase nodule number per plant except the interaction between variety and inoculant ($p < 0.0274$) as shown in (Figure 3). The Nkatie sari and the Chinese varieties were different regarding the number of nodules they produced (Appendix 11). This is because some groundnut varieties produce more nodules then other varieties. Solomon *et al.* (2012) reported significant differences among varieties regarding the number of nodules produced per plant. Asante *et al*. (2020) reported that, the addition of rhizobium inoculant helped in increasing the number of nodules per plant more than the uninoculated control. Lira *et al.* (2015) observed that, inoculants stimulate nodule

formation in legumes especially in soils with limited nitrogen content. Sharma *et al.* (2011) also recorded similar finding that, rhizobia cultures showed significant differences regarding nodule number per plant. Solomon *et al.* (2012) again reported that, inoculant significantly increased nodule numbers per plant.

5.7 Effective nodules

The interaction between variety and phosphorus fertilizer did significantly ($p < 0.001$) influence effective nodules at flowering (Figure 4). This is due to varietal difference, the enhancement of nodule development by phosphorus and the slightly acidic soils on the research fields (Table 1). Leguminous plants are able to auto regulate the number of nodules they produce (Downie, 2014) and so the Nkatie Sari variety produced the highest effective nodules because it produced a high number of nodules per plant than the Chinese variety which were effectively colonized by rhizobia for nitrogen fixation. Phosphorus fertilizer is also needed for initiation and development of effective nodules in plants (Yakubu *et al.*, 2010). The varietal differences of the two groundnut varieties to absorb phosphorus aided in the growth of the whole plant which controls the nodulation process in plants. Gentili *et al*. (2006) reported that, phosphorus fertilizer supplied in medium concentration influences initial nodule developmental process. However, Gentili and Huss-Danell (2003) observed that application of phosphorus fertilizer in high concentration inhibited plant growth. Phosphorus provides the mechanism for the storage of energy which is required for nitrogen fixation (Wagner, 2011) and for the growth of the bacteria that converts nitrogen (N_2) into ammonium (NH_3).

The slightly acidic soils on the research fields promoted the growth and nodulation of the groundnut varieties that were cultivated on it. Highly acidic soils inhibit nodule formation and growth of rhizobia and the host plant (Ferguson *et al*., 2013).

5.8 Ineffective nodules

The interactive effect of variety and phosphorus fertilizer did significantly ($p < 0.05$) affect ineffective nodules (Figure 5). This is because of genetic differences of the two varieties and increasing rate of phosphorus in the soil. The Chinese variety produced more ineffective nodules than the Nkatie Sari variety due to genetic differences. Kukkamalla and Vardhan (2016) reported that, generally leguminous plants control the number of nodules they produce and depending on the variety the number of ineffective nodules formed varies. Ishizawa and Toyoda, (1955) observed that, nodule effectiveness differ and this may be due to their genetic makeup as being reported in the case of Nkatie Sari and the Chinese variety in this research work. The increase in the rate of phosphorus fertilizer increased the number of ineffective nodules (Figure 13) containing rhizobium which were ineffective in utilizing the phosphorus fertilizer in order to fix nitrogen. The ineffective nodules contained poorly developed bacteroid tissues which do not contain rhizobium (Kukkamalla and Vardhan, 2016) to utilize the phosphorus applied as a source of energy (ATP) for the fixation of nitrogen.

5.9 Plant chemical analysis for N, K and P content

The interaction of variety, Phosphorus fertilizer and inoculant did significantly ($p < 0.007$) influence the amount of nitrogen content in the whole plant material (Appendix 18). This is because of nutrient uptake variation among plants and phosphorus and inoculant aid in the

growth and development of plants. Gastal *et al*. (2002) assert that, the uptake of nitrogen varies among crops. A health growth of a plant increases its ability to absorb and accumulate nutrient (Castro *et al*., 2006). The combine effect of rhizobium inoculant and phosphorus fertilizer increased significantly plant height, number of branches, leaves and plant biomass (Geneva *et al*., 2016; Heisnam *et al*., 2017).

The interaction of phosphorus fertilizer and inoculant significantly ($p < 0.014$) affected the accumulation of phosphorus in the plant material (Appendix 19). This is because combine phosphorus and inoculant promotes growth of the plant. Sharma *et al*. (2012) assert that, the combine effect of phosphorus fertilizer and inoculant increased significantly the uptake and accumulation of phosphorus in plants. Aliyu *et al*. (2019) also reported that, phosphorus is one of the main determinants of plant growth.

The interaction of variety and inoculant significantly ($p < 0.002$) influenced the accumulation of potassium in the two varies of groundnut (Appendix 20). This is as a result of a well-developed root which aids in the uptake and accumulation of potassium and variation in nutrient uptake among crops. The ability of a plant to absorb potassium depends on its longer roots with denser root hairs (Hafsi *et al*., 2014). Hafsi *et al*. (2014) also further reported that, the ability of plants to absorb potassium differs from one plant to the other.

5.10 Effect of variety, phosphorus fertilizer and inoculant on biological nitrogen fixation

The interaction of variety, phosphorus and inoculant did significantly ($p < 0.0015$) affect the amount of nitrogen fixed (Appendix 21). This is because of the synergistic effect of phosphorus and inoculant on the groundnut varieties regarding nitrogen fixation. Gentili and Huss-Danell (2003) reported that, phosphorus is needed by plants for healthy growth, nodulation and enhancement of nitrogen fixation. The combine effect of rhizobium inoculation and phosphorus fertilization significantly enhanced formation of nodules and fixation of nitrogen (Siyeni, 2016). Mohammed and Abdalla (2013) also recorded significant increase in nitrogen fixation as a result of the combine effect of phosphorus and inoculant among groundnut varieties.

5.11 Number of pods per hectare

The main effect, first and second order interactions of variety, phosphorus fertilizer and inoculant were not significant ($p < 0.05$) regarding pod number per hectare (Table 12). This is because the podding capacities of the two varieties of groundnut were not different from each other and the lack of influence of phosphorus fertilizer on the formation of effective nodules and pods. The result agrees with Asante *et al*. (2020) who reported that, there were no significant ($p > 0.05$) interaction of variety, phosphorus and rhizobium inoculants with respect to pod number. This suggests that the varieties responded similarly to phosphorus and inoculant quantities (Kamara *et al*., 2011). The inability of phosphorus to aid in the formation of effective nodules and mature pods also affected pod number per hectare. Sibhatu *et al.* (2016) attributed significant increase in pod number per plant to the application of phosphorus fertilizer. Sharma *et al*. (2011) assert that significant increase in

pod number is due to increase in mature pods and number of effective nodules produced per plant which translates in to pods per hectare.

5.12 Pod weight (kg/ha)

The main effect of phosphorus fertilizer and inoculant did significantly ($p < 0.0000$, $p < 0.004$) influence pod weight respectively (Appendix 24). Nkatie Sari variety significantly produced the highest (4817.7 kg/ha) pod weight at 60 kg/ha of phosphorus fertilizer whiles the Chinese variety significantly produced the lowest (1597.2 kg/ha) pod weight. This is because of varietal differences and the influence of phosphorus fertilizer in promoting pod development leading to a higher pod weight in Nkatie Sari than the Chinese variety.

Kombiok *et al*. (2012) reported that, Nkatie Sari variety produced a higher (3156 kg/ha) pod weight more than the Chinese variety (2501 kg/ha) produced. CSIR (2012) also reported that, Nkatie Sari yields more than the Chinese variety and has a potential yield of 2.0 t/ha but the actual yield is less than the potential yield which may be due to soil and environmental factors. Ha (2003), asserts that phosphorus fertilizer significantly increased the yield of groundnut and recommended that 60 kg/ha of phosphorus fertilizer will increase yield in poor alluvial soils more than the untreated control. Aziz *et al*. (2016) like the authors above agree with these research findings that phosphorus fertilizer and varietal difference significantly increased pod number.

5.13 Harvest index

The similar treatments given to the two groundnut varieties and similar environmental conditions under which they were cultivated resulted in the varietal effect not being significant. However, the main effect of phosphorus fertilizer and inoculant significantly

influenced harvest index. The significant influence of phosphorus fertilizer on harvest index is as a result of the effect of phosphorus fertilizer in increasing pod number in the two groundnut varieties. Jan *et al*. (2014) reported that, phosphorus fertilizer significantly affected yield and that plots that were treated with phosphorus fertilizer produced the highest harvest index than untreated plots. Noonari *et al.* (2016) asserts that, phosphorus fertilizer rates significantly increased grain yield. However, Iqbal and Chauhan (2003) reported that, phosphorus fertilizer rates did not significantly influence harvest index.

Inoculant also had a significant influence on harvest index. This is because inoculants initiates the development of root nodules (Ntambo *et al.*, 2017) leading to increase growth rate of the plant as well as the fixation of nitrogen in the plant which can result in yield increase. Soe *et al*. (2010) had reported significant increase in grain yield as a result of crops inoculated with Bradyrhizobia isolates. Mohamed and Hassan (2015) assert that, inoculated plants produced higher pod yield as well as grain yield more than the uninoculated plants. The result of this research also agrees with Ntambo *et al*. (2017) who reported that, inoculation with rhizobium sp. had a significant effect on the growth and yield of plants.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The findings of this study have revealed the importance of rhizobia inoculation and phosphorus fertilizer application on the growth and yield of groundnuts in soils with low nutrients in the Guinea savanna zone of Ghana.

Growth parameters that were significantly improved by the application of rhizobia inoculation and phosphorus fertilizer more than the control included biomass, haulm weight, nodule number per plant, harvest index and biological nitrogen fixation.

Pod weight and harvest index were the yield parameters that were significantly improved with the application of the rhizobia inoculation and phosphorus fertilizer to the two groundnut varieties. The interaction between groundnut variety, phosphorus fertilizer and rhizobia inoculation were not significant in increasing the pod number per hectare. However, Chinese variety produced the highest pod number per hectare at 60 kg/ha of phosphorus fertilizer and 6 g/kg of inoculant more compared to the control.

The interaction of variety, phosphorus and rhizobia inoculation increased N, P and K content in plant material and biological nitrogen fixation. The increase in biological nitrogen fixation improved the fertility of the soil but did not lead to significant increase in yield.

The actual yield that was obtained in this study was below the potential yield. However, the combine effect of rhizobia inoculation and phosphorus fertilizer will help to increase yield and bridge the wide gap that exist between the actual and potential yield of groundnut.

6.2 Recommendation

The research work evaluated the use of rhizobia strains as a cheaper source of soil nitrogen for the cultivation of legumes. The combined effect of rhizobia inoculation and phosphorus fertilizer increased the performance of the crop. Hence it is recommended that the use of rhizobia strains and phosphorus fertilizer application could be used by farmers to increase groundnut production on their farms as well as maintaining the fertility of the soil in the Guinea savanna zone of Ghana. This will increase farmers' income and alleviate poverty in Africa.

The following recommendations are made based on the outcome of the research work.

i. Farmers in the Guinea savanna zone are advised to use the rhizobia strains for groundnut production.
ii. Farmers are also advised to add small amount of phosphorus fertilizer to improve nodulation and growth of the plant.

Some issues that will lead to increase in groundnut production were addressed by the research work. However, there is the need for further work to be carried out on the following areas:

i. The study should be replicated in other areas within the agro-ecological zones to determine variability that exists in the soil.
ii. An investigation into the residual effect of rhizobia inoculation and phosphorus fertilizer should also be conducted.
iii. The persistence of the rhizobia strains introduced into the soil should be investigated after one cropping season to determine whether or not to continue to inoculate the soil with the rhizobia strain.

iv. Research should be conducted to find out the factors that contribute in widening the actual and potential yield of groundnut.

v. Further studies can also be conducted to determine the efficiency, appropriate rate and shelf life of the rhizobia strain.

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APPENDICES

Appendix 1: Analysis of variance for Canopy spread at 2 weeks

Source Of Variations	DF	SS	MS	Vr	F Pr
Rep. Stratum	5	0.02978	5.956E-30		
Variety (V)	1	7.500E-07	7.500E-07	0.00	0.9882
Phosphorus rate (PR)	2	6.313E-05	3.156E-05	0.02	0.9770
Inoculants rate (IR)	2	7.756E-04	3.878E-04	0.25	0.7831
V×PR	2	8.364E-03	4.182E-03	3.08	0.0681
V×IR	2	3.307E-04	1.653E-04	0.10	0.9008
PR×IR	4	0.01076	2.690E-03	1.70	0.1612
V×PR×IR	4	4.464E-03	1.116E-03	0.71	0.5907
Residual	60	0.09479	1.580E-03		
Total	107	0.19211			

Appendix 2: Analysis of variance for Canopy spread at 4 weeks

Source Of Variations	DF	SS	MS	Vr	F Pr
Rep. Stratum	5	0.00710	0.00142		
Variety (V)	1	0.03172	0.03172	30.42	0.0027
Phosphorus rate (PR)	2	0.00110	0.00055	0.15	0.8603
Inoculants rate (IR)	2	0.00382	0.00191	0.56	0.5750
V×PR	2	0.01042	0.00521	1.44	0.2605
V×IR	2	0.00210	0.00105	0.31	0.7371
PR×IR	4	0.01056	0.00264	0.77	0.5472
V×PR×IR	4	0.004450	0.00111	0.33	0.8599
Residual	60	0.20504	0.00342		
Total	107	0.35387			

Appendix 3: Analysis of variance for Canopy spread at 6 weeks

Source Of Variations	DF	SS	MS	Vr	F Pr
Rep. Stratum	5	0.00831	1.662E-03		
Variety (V)	1	0.00171	1.712E-03	0.65	0.4566
Phosphorus rate (PR)	2	0.00236	1.178E-03	0.50	0.6137
Inoculants rate (IR)	2	0.00133	6.662E-04	0.36	0.6983
V×PR	2	0.001036	5.182E-03	2.20	0.1368
V×IR	2	0.00152	7.615E-04	0.41	0.6636
PR×IR	4	0.00853	2.133E-03	1.16	0.3392
V×PR×IR	4	0.00843	2.107E-03	1.14	0.3455
Residual	60	0.11067	1.845E-03		
Total	107	0.21347			

Appendix 4: Analysis of variance for Canopy spread at 8 weeks

Source of variation	DF	SS	MS	Vr	F Pr
Rep. Stratum	5	0.01285	2.5070E-03		
Variety (V)	1	0.00442	4.421E-03	6.61	0.0499
Phosphorus rate (PR)	2	0.00240	1.201E-03	0.81	0.4605
Inoculants rate (IR)	2	0.00160	7.986E-04	0.87	0.4233
V×PR	2	0.00622	3.109E-03	2.09	0.1502
V×IR	2	0.01265	3.109E-03	6.91	0.0020
PR×IR	4	0.00205	6.324E-03	0.56	0.6927
V×PR×IR	4	0.00382	5.126E-04	0.56	0.3931
Residual	60	0.05494	9.544E-04		
Total	107	0.13408	9.157E-04		

Appendix 5: Analysis of variance for haulm weight

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	1.306E+08	2.612E+07		
Variety(V)	1	1333333	1333333	0106	0.8195
Phosphorus rate (PR)	2	1.584E+07	7921332	3.00	0.0728
Inoculant rate (IR)	2	1.338E+07	6689779	6.31	0.0032
V x PR	2	4814019	2407010	0.91	0.4185
V x IR	2	178602	89301.2	0.08	0.9193
PR x IR	4	4898220	1224555	1.16	0.3394
V x PR x IR	4	9316623	2329156	2.20	0.0799
Residual	60	6.358E+07	1.059643		
Total	107	4.121E+08			

Appendix 6: Analysis of variance for biomass

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	2370515	474103		
Variety(V)	1	11718.8	11719	0.03	0.8679
Phosphorus rate (PR)	2	208623	104311	1.15	0.3378
Inoculant rate (IR)	2	691479	345739	3.68	0.0311
V x PR	2	32118.1	16059	0.18	0.8395
V x IR	2	289280	144640	1.54	0.2228
PR x IR	4	708044	177011	1.88	0.1249
V x PR x IR	4	342014	85503	0.91	0.4639
Residual	60	5635851	93931		
Total	107	1.402E+07			

Appendix 7: Analysis of variance for plant height at 2 weeks

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	2.52E-04	4.104E-05		
Variety (V)	1	9.246E-04	9.246E-04	14.34	0.0128
Phosphorus rate (PR)	2	6.685E-06	3.34E-06	0.21	0.8104
Inoculant rate (IR)	2	4.146E-05	2.07E-05	1.08	0.3457
V x PR	2	4.891E-05	2.445E-05	1.55	0.2358
V x IR	2	1.513E-05	7.565E-06	0.39	0.6758
PR x IR	4	1.776E-04	4.441E-05	2.32	0.0675
V x PR x IR	4	3.254E-05	8.134E-06	0.42	0.7906
Residual	60	1.151E-03	1.918E-05		
Total	107	3.240E-03			

Appendix 8: Analysis of variance for plant height at 4 weeks

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	0.01930	0.00386		
Variety (V)	1	0.09164	0.09164	27.56	0.0033
Phosphorus rate (PR)	2	0.00324	0.00162	1.91	0.1737
Inoculant rate (IR)	2	0.00089	0.00044	11.33	0.2724
V x PR	2	0.00326	0.00163	1.93	0.1715
V x IR	2	0.00107	0.00054	1.61	0.2089
PR x IR	4	0.00180	0.00045	1.35	0.2632
V x PR x IR	4	0.00317	0.00079	2.38	0.0617
Residual	60	0.01999	0.00033		
Total	107	0.17791			

Appendix 9: Analysis of variance for plant height at 6 weeks

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	0.02885	0.00577		
Variety (V)	1	1.07306	1.07306	10.42	0.0233
Phosphorus rate (PR)	2	0.00215	0.00107	1.46	0.2553
Inoculant rate (IR)	2	0.00081	0.00044	15.16	0.4700
V x PR	2	0.00410	0.00205	2.80	0.0850
V x IR	2	0.00211	0.00105	1.84	0.1675
PR x IR	4	0.00151	0.00038	0.66	0.6224
V x PR x IR	4	0.00112	0.00028	0.49	0.7426
Residual	60	0.03430	0.00057		
Total	107	0.19779			

Appendix 10: Analysis of variance for plant height at 8 weeks

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	0.06058	0.01212	5.76	
Variety (V)	1	0.03343	**0.03343**	18.24	0.0079
Phosphorus rate (PR)	2	0.00451	0.00225	0.65	0.5344
Inoculant rate (IR)	2	0.00737	0.00369	0.98	0.3828
V x PR	2	0.01405	0.00702	2.01	0.1595
V x IR	2	0.00953	0.00477	1.26	0.2907
PR x IR	4	0.01252	0.00313	0.83	0.5125
V x PR x IR	4	0.00916	0.00229	0.61	0.6599
Residual	60	0.22671	0.00378		
Total	107	0.45672	0.02160		

Appendix 11: Analysis of variance for Nodule number per plant at flowering

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	243.86	48.772	0.66	
Variety(V)	1	10.08	10.083	0.61	0.4698
Phosphorus rate (PR)	2	122.06	61.028	1.46	0.2562
Inoculant rate (IR)	2	20.06	10.028	0.23	0.7937
V x PR	2	121.06	60.528	1.45	0.2589
V x IR	2	330.39	165.194	3.82	0.0274
PR x IR	4	356.22	89.056	2.06	0.0973
V x PR x IR	4	252.22	63.056	1.46	0.2261
Residual	60	2593.78	143.230	3.863	
Total	107	4968.92			

Appendix 12: Analysis of variance for effective nodules at flowering

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	41.861	8.3722		
Variety (V)	1	7.787	7.7870	4.91	0.0776
Phosphorus rate (PR)	2	2.056	1.0278	0.66	0.5301
Inoculant rate (IR)	2	9.389	4.6944	1.33	0.2700
V x PR	2	31.241	15.6204	9.96	0.0010
V x IR	2	12.796	6.3981	1.81	0.1719
PR x IR	4	3.889	0.9722	0.28	0.8926
V x PR x IR	4	14.259	3.5648	1.01	0.4093
Residual	60	211.667	3.5278		
Total	107	374.250			

Appendix 13: Analysis of variance for ineffective nodules at flowering

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	59.111	11.8222		
Variety(V)	1	0.148	0.1481	0.03	0.8694
Phosphorus rate (PR)	2	1.167	0.5833	0.14	0.8693
Inoculant rate (IR)	2	20.056	10.0278	1.83	0.1687
V x PR	2	56.796	28.3981	6.87	0.0054
V x IR	2	17.907	8.9537	1.64	0.2031
PR x IR	4	28.111	7.0278	1.29	0.2860
V x PR x IR	4	13.148	3.2870	0.60	0.6633
Residual	60	328.111	5.4685		
Total	107	632.000			

Appendix 14: Analysis of variance for number of branches per plant at 2 WAP

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	4.4163	0.88326		
Variety(V)	1	1.0325	1.03253	1.25	0.3150
Phosphorus rate (PR)	2	0.0900	0.04501	0.29	0.7517
Inoculant rate (IR)	2	0.1144	0.05721	0.42	0.6600
V x PR	2	0.5227	0.26134	1.68	0.2114
V x IR	2	0.0258	0.1288	0.09	0.9103
PR x IR	4	0.2026	0.05066	0.37	0.8288
V x PR x IR	4	0.2097	0.05242	0.38	0.8197
Residual	60	8.2051	0.13675		
Total	107	22.0708			

Appendix 15: Analysis of variance for number of branches per plant at 4 WAP

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	4.4922	0.89844		
Variety (V)	1	0.3115	0.31148	0.64	0.4592
Phosphorus rate (PR)	2	2.4422	1.22111	2.94	0.0760
Inoculant rate (IR)	2	0.1622	0.08111	0.37	0.6911
V x PR	2	0.3519	0.17593	0.42	0.6605
V x IR	2	0.1563	0.07815	0.36	0.7004
PR x IR	4	1.6756	0.41889	1.92	0.1187
V x PR x IR	4	1.2637	0.31593	1.45	0.2293
Residual	60	13.0889	0.21815		
Total	107	34.6767			

Appendix 16: Analysis of variance for number of branches per plant at 6 WAP

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	15.8152	3.16304		
Variety(V)	1	0.9633	0.96333	1.24	0.3161
Phosphorus rate (PR)	2	0.7874	0.39370	0.48	0.6251
Inoculant rate (IR)	2	0.8985	0.44926	0.89	0.4166
V x PR	2	0.5756	0.28778	0.35	0.7078
V x IR	2	0.2022	0.10111	0.20	0.8193
PR x IR	4	4.6859	1.17148	2.32	0.0674
V x PR x IR	4	1.0222	0.25556	0.51	0.7319
Residual	60	30.3378	0.50563		
Total	107	75.5419			

Appendix 17: Analysis of variance for number of branches per plant at 8 WAP

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	16.1796	3.23593		
Variety(V)	1	0.8181	0.81815	1.06	0.3510
Phosphorus rate (PR)	2	0.6785	0.33926	0.43	0.6590
Inoculant rate (IR)	2	0.8496	0.42481	0.89	0.4153
V x PR	2	0.4385	0.21962	0.28	0.7622
V x IR	2	0.1119	0.05593	0.12	0.8894
PR x IR	4	4.5170	1.12926	2.37	0.0625
V x PR x IR	4	1.0015	0.25037	0.53	0.7174
Residual	60	28.5867	0.47644		
Total	107	72.9819			

Appendix 18: Analysis of variance for nitrogen content (%) in plant material

Source Of Variations	DF	SS	MS	Vr	F Pr
Rep. Stratum	5	12.6457	2.52914		
Variety (V)	1	3.7557	3.75574	92.21	0.0002
Phosphorus rate (PR)	2	19.5128	9.75638	77.80	0.0000
Inoculants rate (IR)	2	14.2591	7.12954	77.13	0.0000
V×PR	2	0.0859	0.04294	0.34	0.7142
V×IR	2	0.5029	0.25147	2.72	0.0740
PR×IR	4	2.4809	0.62021	6.71	0.0002
V×PR×IR	4	1.4320	0.35800	3.87	0.0073
Residual	60	5.5463	0.09244		
Total	107	62.9331			

Appendix 19: Analysis of variance for phosphorus content (%) in plant material

Source Of Variations	DF	SS	MS	Vr	F pr
Rep. Stratum	5	5.468E+08	1.094E+08		
Variety (V)	1	643724	321862	0.03	0.8690
Phosphorus rate (PR)	2	4.888E+08	2.441E+08	10.52	0.0008
Inoculants rate (IR)	2	3.407E+07	8517095	24.91	0.0000
V×PR	2	7159876	1789969	0.15	0.8582
V×IR	2	1258632	314658	0.92	0..4039
PR×IR	4	92936229	1161704	3.40	0.0143
V×PR×IR	4	2532156	316519	0.93	0.4550
Residual	60	4.102E+07			
Total	107	1.703E+09			

Appendix 20: Analysis of variance for potassium content (%) in plant material

Source Of Variations	DF	SS	MS	Vr	F Pr
Rep. Stratum	5	2.893E+07	5786242		
Variety (V)	1	6595.70	6595.70	0.00	0.9620
Phosphorus rate (PR)	2	8.631E+08	4.316E+08	53.67	0.0000
Inoculants rate (IR)	2	4.081E+08	2.041E+08	37.82	0.0000
V×PR	2	6.791E+07	3.395E+07	4.22	0.0295
V×IR	2	7.268E+07	3.634E+07	6.74	0.0023
PR×IR	4	5.534E+07	1.383E+07	2.56	0.0473
V×PR×IR	4	3.869E+07	9671735	1.79	0.1422
Residual	60	3.238E+08	5395850		
Total	107	2.033E+09			

Appendix 21: Analysis of variance for biological nitrogen fixation (%)

Source Of Variations	DF	SS	MS	Vr	F Pr
Rep. Stratum	5	0.3784	0.07		
Variety (V)	1	0.3158	569	3.98	0.1025
Phosphorus rate (PR)	2	5.0882	0.31584	17.45	0.0000
Inoculants rate (IR)	2	5.7011	2.85053	18.88	0.0000
V×PR	2	1.2973	0.64866	4.45	0.0252
V×IR	2	0.0648	0.03240	0.21	0.8075
PR×IR	4	4.0023	1.00059	6.63	0.0002
V×PR×IR	4	3.0374	0.75936	5.03	0.0015
Residual	60	9.0591	0.15098		
Total	107	32.2577			

Appendix 22: Analysis of variance for number of pods per plant

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	35.861	7.1722		
Variety(V)	1	15.565	15.5648	1.39	0.2921
Phosphorus rate (PR)	2	0.667	0.3333	0.14	0.8676
Inoculant rate (IR)	2	9.556	4.7778	2.65	0.0790
V x PR	2	13.407	6.7037	2.88	0.0797
V x IR	2	2.074	1.0370	0.57	0.5658
PR x IR	4	11.778	2.9444	1.63	0.1778
V x PR x IR	4	11.037	2.7593	1.53	0.2050
Residual	60	108.222	1.8037		
Total	107	310.917			

Appendix 23: Analysis of variance for pod number per hectare

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	2.241E+10	4.483E+09		
Variety (V)	1	9.728E+09	9.728E+09	1.39	0.2921
Phosphorus rate (PR)	2	4.167E+08	2.083E+08	0.14	0.8676
Inoculant rate (IR)	2	5.972E+09	2.986E+09	2.65	0.0790
V x PR	2	81380E+19	4.190E+09	2.88	0.0797
V x IR	2	1.296E+09	6.481E+8	0.57	0.5658
PR x IR	4	7.361E+09	1.840E+09	1.63	0.1778
V x PR x IR	4	6.898E+19	1.725E+09	1.53	0.2050
Residual	60	6.764E+10	1.127E+09		
Total	107	1.943E+11			

Appendix 24: Analysis of variance for pod weight (Kg/ha)

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	2.241E+10	4.483E+09		
Variety(V)	1	9.728E+09	9.728E+09	1.39	0.2921
Phosphorus rate (PR)	2	4.167E+08	2.083E+08	0.14	0.8676
Inoculant rate (IR)	2	5.972E+09	2.986E+09	2.65	0.0790
V x PR	2	8.380E+09	4.190E+09	2.88	0.0797
V x IR	2	1.296E+09	6.481E+08	0.57	0.5658
PR x IR	4	7.361E+09	1.840E+09	1.63	0.1778
V x PR x IR	4	6.898E+09	1.725E+09	1.53	0.2050
Residual	60	6.764E+10	1.127E+09		
Total	107	1.943E+11			

Appendix 25: Analysis of variance for harvest index of groundnut varieties

Source of variation	DF	SS	MS	Vr	F pr
Rep. Stratum	5	17837.6	3567.51		
Variety(V)	1	2616.6	2616.55	1.19	0.3246
Phosphorus rate kg/ha (PR)	2	19638.4	9819.21	15.4	0.0001
Inoculant rate g/kg (IR)	2	3052.2	1526.10	3.50	0.0334
V x PR	2	687.1	343.55	0.54	0.5912
V x IR	2	81.8	40.90	0.10	0.9082
PR x IR	4	428.9	107.23	0.25	0.9068
V x PR x IR	4	857.5	214.38	0.51	0.7317
Residual	60	25440.7	424.01		
Total	107	94341.8			