OPTIMIZATION OF BIOLOGICAL NITROGEN FIXATON AND YIELD OF GROUNDNUT (*Arachis hypogaea* L.) IN A SAVANNA ALFISOL THROUGH FERTILIZER APPLICATION AND SOIL AMENDMENT

By

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DECLARATION

I declare that the work in this Dissertation entitled "Optimization of Biological Nitrogen Fixation and Yield of Groundnut (*Arachis hypogaea* L.) in a Savanna Alfisol through Fertilizer Application and Soil Amendment" has been carried out by me in the Department of Soil Science. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Dissertation was previously presented for another degree or diploma at this or any other institution.

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CERTIFICATION

This dissertation entitled "OPTIMIZATION OF BIOLOGICAL NITROGEN FIXATION AND YIELD OF GROUNDNUT (*Arachis hypogaea* L.) ON A SAVANNA ALFISOL THROUGH FERTILIZER APPLICATION AND SOIL AMENDMENT" by MUHAMMED MUSTAPHA IBRAHIM meets the regulations governing the award of the degree of Masters in Soil Science of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Productivity of groundnut has been generally low in Nigeria owing to soil fertility problems arising from low soil pH and low organic matter content of the soils leading to deficiency of major nutrients, especially, N and P. Current fertilizer recommendations are based on single nutrient trials and do not take into consideration the use of lime, manure or micronutrients. To this end, this study was designed to identify the major factors that influence biological nitrogen fixation and yield of groundnut in a savanna Alfisol through fertilizer application and soil amendment on an acidic and non-acidic soil so as to optimize productivity. A nutrient omission trial using factorial combinations was used to achieve this aim. The study was carried out in field trials at two locations at the Institute for Agricultural Research (IAR) farm, Samaru, on an acidic (S13) and non-acidic soil (S7) using groundnut genotype, SAMNUT 24 from July to October, 2015. The treatments used on the acidic soil were two levels each of lime, phosphorus, potassium and micronutrients. On the non-acidic soil, there were also two levels each of organic manure, phosphorus, potassium and micronutrients. These levels were zero (0) and the recommended rate for each nutrient. Phosphorus was applied at 54 kg P_2O_5/ha as SSP, K at 25 kg K_2O/ha as MOP, micronutrients with the trade name Agrolyzer at 2 g/L, lime at 250 kg/ha and cow dung at 1.7 tons/ha (equivalent to 10kg N ha^{-1}). The treatments were arranged in a factorial combination and laid out in a randomized complete block design replicated three times on plots of $9m^2$ for each location. The SAMNUT 24 seeds were inoculated with *rhizobial* inoculant, NC 92 to enhance biological nitrogen fixation (BNF) and were planted on all plots. A non-nodulating groundnut genotype, ICGL 5 was included for estimation of BNF. The effects of the main treatments and their interactions were observed on nodule number, nodule dry weight, root and shoot dry weights, nitrogen fixation, 100 seed weight, shelling percentage, pod, grain and haulm yields, as well as harvest index. On the acidic soil, the amount of N_2 fixed and nitrogen derived from the atmosphere (Ndfa) were highest with the application of P while soil N balance was most favoured by the application of P and micronutrients. The combination of lime, P and K was observed to favour pod and grain yields the most. Haulm yield however was most favoured by the application of P only while harvest index was improved mostly by liming. On the non-acidic field, the highest Ndfa, N_2 fixed, grain and haulm yields were obtained by the combination of cow dung, P, K and micronutrients, while the soil N balance was most influenced by addition of P. The combined application of cow dung, K and micronutrients was best for pod yield. Harvest index was highest under the combination of cow dung and K. Stepwise regression and correlation studies showed that the nodule dry weight was important in predicting the grain yield on the acidic soil. On the non-acidic soil, nodulation influenced BNF and yield of groundnut. Similarly, N_2 fixation significantly influenced yield parameters on both soils. These indicate that *rhizobium* inoculation with NC 92 was effective in enhancing BNF and yield in the soils that were characterized with low indigenous *rhizobial* population. This study also showed that groundnut yield was significantly increased by liming and fertilization on the acidic soil. Liming of the acidic soil gave 21% higher pod yield than the non-acidic soil. The positive N balance in both locations indicates improved soil quality and can be beneficial to non-fixing crops grown in rotation.

Micronutrients addition showed no significant difference on pod yield on both locations, indicating sufficiency of inherent micronutrients levels in the soils. This has further shown that poor nutrient management strategies are among the key factors that have affected groundnut productivity in Nigeria. This trend can be reversed through adequate application of fertilizer nutrients and soil amendment.

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CHAPTER ONE

1.0 INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is also known as peanut, earthnut, gobbers, pinders or manila nut (Beghin *et al*., 2003). It is a member of the genus *Arachis* in the family *Fabacaea*. Groundnut is the world's thirteenth most important food crop, fourth most important source of edible oil and the third most important source of vegetable protein (Taru *et al.,* 2008). Its seeds (kernels) contain 40-50% fat, 20-50% protein and 10-20% carbohydrates (FAO, 2006). Its seeds are nutritional source of vitamin E, niacin, falacin, calcium, phosphorus, magnesium, zinc, iron, ribloflavin, thiamine and potassium (FAO, 2006; Kumar and Shankar, 2013).

The uses of groundnut plant make it an excellent cash crop for domestic markets as well as for foreign trade in several developing and developed countries (FAO, 2006). Groundnut yield ranges from 400 kg/ha to several tons/ha depending on production systems and area (Cilliers, 2015). In 2011, total world production was estimated at 38.6 million tons with average yields of 1.8 tons per hectare (FAOSTAT, 2011). In Africa, groundnut production recovered during 2002-2011 showed that yields increased from 600-800 kg/ha in the 1980s to 900-1050 kg/ha during 2002-2011 with Senegal and Nigeria as the major producers (ICRISAT, 2013). Nigeria is the leading producer of groundnut in Africa with production mostly carried out by resource poor farmers (Yusuf *et al.,* 2014).

Groundnut requires a well-drained light coloured, loosed friable sandy loam soil, optimum moisture in pod zone and mean daily temperature of about 30°C (Ajeigbe, 2015). In Nigeria, the Sudan and northern Guinea savanna where the soil and agro-climatological conditions are favourable are major groundnut producing zones (Misari *et al*., 1980). Kano and Niger states account for about 19.6% and 10.7% respectively of groundnut production in Nigeria,

followed by Kaduna, Benue, Zamfara, Taraba, Bauchi, Borno, Katsina and Nasarawa. These top 10 producing states account for nearly 80% of the total area of groundnut for Nigeria (TL II, 2011 inoculation which is a cheaper source of N for groundnut will be of immense importance as well as supp ly of adequa). However, groundnut productivity has significantly reduced owing to cost of production arising from inputs such as fertilizers as smallholder farmers rarely apply N fertilizers despite recommendation for modest application due to low inherent soil N (Yusuf *et al.,* 2011). Hence, *Rhizobial* te organic and inorganic inputs to improve and sustain productivity.

Biological nitrogen fixation is becoming more attractive and economically viable nitrogen input, substitute of inorganic fertilizers for resource poor farmers, and is an environmentally friendly agricultural input (Bekere and Hailemariam, 2012). Although, there is generally an inter and intra-specific variation in the amount of N_2 fixed by legumes (Yusuf *et al.,* 2008) due to several reasons including nodulation efficiencies, genotype and maturity period, etc. The ability of legumes to fix N_2 allows farmers to grow them with minimal inputs of N fertilizer (Lupwayi *et al.,* 2011). The use of appropriate strains of inoculants in nitrogen deficient soils of the northern Guinea savanna of Nigeria can offer an excellent opportunity for improving groundnut growth and development. This is of importance in most tropical soils with low nitrogen status (Yakubu *et al*., 2010). Fortunately, groundnut offers an opportunity to increase the supply of N from symbiotic association with effective strains of *Rhizobia* (Yusuf *et al.,* 2011). Woomer (2010) reported that N fixation in groundnut is about 150 kg N/ha which offers strong residual benefits to following crops.

Soil acidity is a major factor that affects plant growth in many countries (Ndakidemi and Semoka 2006; Godsey *et al.,* 2007). It may affect all stages of growth and specifically the legume-*Rhizobium* symbiosis, from strain survival in soil and on the seed, to root-hair

infection, nodule initiation and nitrogen fixation (Wood *et al*., 1984). The most common management practice to ameliorate acid soils is through the surface application of lime (Bolan *et al*., 2003). The major influence of lime when applied in the soil is on its ability to supply Ca^{2+} which is essential for plant growth (White and Broadley, 2003).

Phosphorus deficiency is the most frequent nutrient stress for growth and development of grain legumes including groundnut (Kamara *et al.,* 2008). The deficiency of P supply and availability remains a severe limitation to nitrogen fixation and symbiotic interactions, as nodules themselves are strong sinks for P. The absence of the required *Rhizobia* species and optimal phosphorus level can limit legume production. Hence, inoculation with compatible and suitable *Rhizobia* with application of optimal phosphorus level may be essential and a key strategy by which groundnut farmers can optimize yields.

The role of K in agricultural production is intimately connected with photosynthesis (Atkin and Macherel, 2009). The need for K has been demonstrated for some *Rhizobia* strains (Vincent, 1977), who showed restricted growth of *R. trifolii* and *R. meliloti* when K was omitted from a defined medium.

Nutrient limitations to legume production result from deficiencies of not only major nutrients but also micronutrients (Bhuiyan *et al.,* 1999). However, as a general practice, optimal supply of macronutrients to crops is usually ensured while that of micronutrients is ignored. Ashraf *et al.,* (2012) observed that the involvement of micronutrients in different physiological and biochemical activities of the legume plants is well documented because correlations between micronutrient supply and crop growth and productivity have often been observed. Problem soils such as acid, alkaline or sandy soils are often deficient in one or more micronutrient elements (Ashraf *et al.,* 2012). Thus, application of micronutrients as

foliar or soil amendment is paramount to achieving optimum groundnut productivity from the soils of the northern Guinea savanna.

Cattle manures are a source of N and other nutrients for plants (such as phosphorus, potassium, calcium, iron, zinc and copper) that can make valuable contributions to soil's organic matter, can improve soil physical fertility, and are a centre for biological activities (Khalid and Shafei, 2005; Najm *et al.,* 2012). Organic amendments can improve plant health beyond the nitrogen fertility value (Atiyeh *et al.,* 2002). They contribute to plant growth through their favourable effects on the physical, chemical and biological properties of soil.

1.1 Statement of the Problem

Nearly all the crops grown in the northern Guinea savanna of Nigeria are produced mainly on peasant small farm-holdings with low levels of input and management, so yields have remained low in spite of ample experimental evidences that up to three to four times the present yield levels are attainable (Chude *et al*., 2012). ICRISAT (2013) reported that groundnut yield in Africa has generally been poor due to a combination of factors, including unreliable rains, little technology available to small scale farmers, poor seed varieties, and increased cultivation on marginal land. In Africa (including Nigeria), groundnut is grown mostly by smallholder farmers under rain fed conditions with limited inputs (Samson, 2012) so, yields have remained low. Nitrogen and P have been identified as the most important mineral nutrient limiting increased crop productivity in the Nigerian savanna (Yusuf *et al.,* 2014). In addition, majority of the soils of the Guinea savanna of Nigeria are inherently low in fertility especially organic matter, phosphorus and nitrogen (Odunze and Kureh, 2009, Oluwasemire and Alabi, 2004) and fertility of these soils have decreased with continuous cultivation. This has led to the inability of farmers to obtain the average world optimum pod yield of groundnut (1.8 tons) in the Nigerian savanna. This large gap between actual and

potential yields is due to several factors, including non-availability of seeds of improved varieties for a particular ecology, poor soil fertility, inappropriate crop management practices, pests and diseases (Ahmed *et al.*, 2010).

1.1.Justification of the Study

Recommended fertilizer rate of NPK ha^{-1} for groundnut is 25 kg of N, 50 kg of P_2O_5 and 100 kg of K_2O (Ajeigbe, 2015). Current fertilizer recommendations are based on single nutrients thereby necessitating a factorial experiment which is useful in the study of interaction of nutrients on plant growth. Moreover, these recommendations do not take into account the use of lime, organic manure or micronutrients in single or combined applications. However, modest applications of these nutrients based on the single recommendation have not yielded the average optimum pod yield of groundnut in the Nigerian savanna (Yusuf *et al.*, 2011). The beneficial effects of combined organic and inorganic sources on soil nutrients, crop yields, and maintenance of soil organic matter have been reported in field trials (Nandwa, 2003; Vanlauwe *et al*., 2002). Owing to this fact, this practice is worth adopting in groundnut production so as to increase productivity while maintaining and improving soil organic matter content.

1.3. Aim of the study

The general aim of the study seeks to identify the major factors that influence biological nitrogen fixation and yield of groundnut in a savanna Alfisol through fertilizer application and soil amendments on acidic and non-acidic soils so as to reduce the yield gap between potential yield and actual yield of the crop.

The specific objectives therefore are;

1. To determine the effects of combined application of lime, phosphorus, potassium and micronutrients on nodulation, biomass, biological nitrogen fixation and soil N balance of groundnut in an acidic soil.

2. To determine the effects of combined application of lime, phosphorus, potassium and micronutrients on yield and yield components of groundnut in an acidic soil.

3. To determine the effects of combined application of organic manure, phosphorus, potassium and micronutrients on nodulation, biomass, biological nitrogen fixation and soil N balance of groundnut in a non-acidic soil.

4. To determine the effects of combined application of organic manure, phosphorus, potassium and micronutrients on yield and yield components of groundnut in a non-acidic soil.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Groundnut

The productivity of groundnuts varies from 3500 kg/ha in the United States of America to 2500 kg/ha in South America, 1600 kg/ha in Asia, and less than 800 kg/ha in Africa (Vara Prasad, 2009) with world average production of 1800 kg/ha (FAOSTAT, 2011). However, groundnut production recovered during 2002-2011 in Africa showed yields increased from 600-800 kg/ha in the 1980s to 900-1050 kg/ha during 2002-2011 with Senegal and Nigeria as the major producers ICRISAT (2013). The low productivity in Africa is mainly due to various abiotic and biotic constraints. Abiotic stresses of prime importance include; temperature extremes, drought stress, soil factors such as acidity, alkalinity, poor soil fertility and nutrient deficiencies (Cilliers, 2015). Groundnut grows best in light textured sandy loam soils with neutral pH (Vara-Prasad, 2009). Optimum temperature for their growth and development ranges from 28 to 30 ºC; the crop requires about 500-600 mm of well distributed rainfall (Ajeigbe *et al.,* 2015). The stages of reproductive development prior to flowering, at flowering and at early pod development, are particularly sensitive to these constraints. Apart from N, P and K, other nutrient deficiencies causing significant yield losses are Ca, Fe and B. Biotic stresses mainly include pests, diseases and weeds. Among insects pests, pod borers, aphids and mites are of importance. The most important diseases are leaf spots, rusts and the toxin-producing fungus *Aspergillus* (Vara-Prasad, 2009). Mineral nutrient deficiencies are the major constraints limiting legume N_2 fixation and yield (O'Hara *et al.,* 1987). In leguminous crops such as groundnut, these deficiencies can be

overcome by applying *Rhizobium* strain and providing essential nutrients in the soil (Fatima *et al.,* 2007). In addition to N, other essential nutrient elements which might be needed to enhance growth, optimum nodulation, N_2 fixation and yield of groundnut are P, K and calcium (Ojiem *et al.,* 2007; Graham and Vance, 2000). Nutrient limitations in legume production result from deficiencies of not only major nutrients but also micronutrients such as molybdenum (Mo), zinc (Zn), boron (B) and iron (Fe) (Graham, 1981).

2.2 Nitrogen

Nitrogen is the fifth most abundant element in the universe and makes up about 78% of the earth's atmosphere, which contains an estimated 4,000 trillion tonnes of the gas and it is usable by most living organisms (Kramer, 1999). Plants, animals and microorganisms can die as a result of nitrogen deficiency (Lindemann and Glover, 2003). Nitrogen is the most limiting nutrient for increasing crop productivity. It is the nutrient that is most commonly deficient in soils, contributing to reduced agricultural yields throughout the world. Nitrogen is essential for the function of biochemical agents like chlorophyll (which make photosynthesis possible), enzymes (which helps organisms carry out biochemical processes and assimilate nutrients) and nucleic acids such as DNA and RNA (which are involved in reproduction) (Kramer, 1999). Plants lacking nitrogen show stunted growth and their foliage is pale green to yellowish (Marschner and Marschner, 2012).

Nitrogen ranks first among external inputs required to maximise output in agriculture. Input efficiency of N fertilizer is one of the lowest among the plant nutrients and in turn, contributes substantially to environmental pollution (Bohlool *et al.,* 1992). Nitrates in ground water are of major health concern. The nitrates can accumulate in ground water, and ultimately in drinking water, because nitrates can be reduced to highly reactive nitrites by microorganisms in anaerobic conditions in the gut and binds to haemoglobin reducing its

oxygen-carrying capacity. Nitrogen in runoff and surface waters has led to extensive pollution and eutrophication of rivers and lakes and other fresh water sources (Stout *et al.,* 2000).

Most legumes have potential of fixing their own nitrogen when they come in symbiotic association with nitrogen fixing bacteria such as *Rhizobia* which are special bacteria that can live in the soil or in nodules formed on the roots of legumes. In root nodules, they form a symbiotic association with the legumes, obtaining nutrients from the plant and producing nitrogen in a process called biological nitrogen fixation (BNF) (Uchida, 2000). Nitrogen fixation is one of the ways through which soil fertility can be improved (Mclaughlin *et al.,* 1990).

2.2.1 Biological nitrogen fixation

Despite the abundance of Nitrogen in the atmosphere, it is not available to plants in a form that is suitable for metabolism until it is converted to ammonia or other reduced forms (Bohlool *et al.,* 1992). Nitrogen in the atmosphere occurs mostly in the form of dinitrogen, essentially inert, with a very strong triple bond (N≡N). In order for nitrogen to be used for growth it must be "fixed" (combined) in the form of ammonium (NH_4^+) or nitrate (NO_3^-). Atmospheric N can be fixed industrially, through Haber Bosch process, or through biological nitrogen fixation (BNF). Nitrogen can be supplied to crops by BNF, a process which is becoming more important for not only reducing energy costs, but also in seeking more sustainable agricultural production. Augmenting nitrogen supply through BNF is a viable option for resource-poor farmers of developing countries (Rattan, 1995). Nitrogen fixing systems offer an economically and ecologically attractive means of reducing external inputs and improving internal resources (Albareda *et al.,* 2009). Legumes play an important role in farming systems, fixing atmospheric N that contributes nitrogenous compounds to the soil,

either directly, by nodule excretion, or indirectly, by decomposition of root nodules and tissues (Giller, 2001). This primary role of fixation of atmospheric N leads to two dependent or consequential roles of legumes: (1) their capacity to increase soil fertility and (2) the generally high levels of protein in the herbage and hence its high forage or mulching quality. Biological nitrogen fixation helps in maintaining and/or improving soil fertility by using N_2 which is in abundance in the atmosphere. Plant-associated nitrogen fixation currently contributes 50–70 million tonnes annually to the global agricultural N budget (Unkovich *et al.*, 2008), this account for from 40 to 70% of total global nitrogen input (Kahindi and Karanja, 2009). This illustrates the importance of BNF in the context of the global N cycle.

2.2.2 Mechanisms of biological nitrogen fixation

Biological nitrogen fixation, a process utilized only by certain prokaryotes, is catalysed by a two-component nitrogenase complex (Yan *et al*., 2010). Nitrogenase catalyzes the simultaneous reduction of one N_2 and $2H^+$ to ammonia and a molecule of hydrogen gas. The nature of BNF is that the dinitrogenase catalyzes the reaction, splitting triple-bond inert atmospheric nitrogen (N_2) into organic ammonia molecule (Cheng, 2008).

$N_2 + 8H_2 + 16\ ATP \longrightarrow 2NH_3 + 2H_2 + 16ADP + 16Pi$

The immediate electron donor is the potent reducing agent ferredoxin and the reaction is driven by the hydrolysis of 2 ATP for each electron transferred (Wheelis, 2008).The best known BNF system occurs between legumes and *Rhizobia* (Carvalho *et al*., 2011). The symbiotic association between the roots of legumes and certain soil bacteria, generally known as *Rhizobia*, accounts for the development of a specific organ, the symbiotic root-nodule, whose primary function is nitrogen fixation. Root nodules make a crucial contribution to the nitrogen content of the soil playing a key role in agricultural practices (Alla *et al*., 2010). Perception of legume root exudates triggers the production of *Rhizobial*

nod factor signals which are recognized by compatible plant receptors leading to the formation of root nodules, in which differentiated bacteria (bacteroids) fix atmospheric nitrogen (Oldroyd and Downie, 2008). In the nodule, maintenance of nitrogenase activity is subject to a delicate equilibrium. Firstly, a high rate of oxygen respiration is necessary to supply the energy demands of the Nitrogen reduction process (Sanchez *et al*., 2011), but oxygen also irreversibly inactivates the nitrogenase complex. These conflicting demands are reconciled by control of oxygen flux through a diffusion barrier in the nodule cortex and by the plant oxygen carrier, leghemoglobin, which is present exclusively in the nodule (Minchin *et al*., 2008).

2.2.3 Nature of the symbiosis

The legume-*Rhizobium* symbiosis is a classic example of mutualism. *Rhizobia* supplies ammonia or amino acids to the plant and in return receive organic acids (principally as the dicarboxylic acids malate and succinate) as a carbon and energy source. However, because several unrelated strains infect each individual plant, cheater strains may hoard plant resources such as polyhydroxybutyrate for the benefit of their own reproduction without fixing an appreciable amount of nitrogen (Ratcliff *et al.,* 2008). There are two competing hypotheses for the mechanism that maintains legume-*Rhizobium* symbiosis (though both may occur in nature). The 'sanctions hypothesis' suggests the plants police cheating *Rhizobia*. Sanctions could take the form of reduced nodule growth, early nodule death, decreased carbon supply to nodules, or reduced oxygen supply to nodules that fix less nitrogen (Denison, 2000). The 'partner choice hypothesis' proposes that the plant uses pre-nodulation signals from the *Rhizobia* to decide whether to allow nodulation, and chooses only non-cheating *Rhizobia*. There is evidence for sanctions in soybean plants, which reduce

Rhizobium reproduction (perhaps by limiting oxygen supply) in nodules that fix less nitrogen (Kiers *et al.,* 2003). However, other studies have found no evidence of plant sanctions, and instead support the partner choice hypothesis (Heath and Tiffin, 2009; Marco *et al.,* 2009). While both mechanisms no doubt contribute significantly to maintaining *Rhizobial* cooperation, they do not in themselves fully explain the persistence of the mutualism.

2.2.4 Ecological benefits of biological nitrogen fixation

Atmospheric nitrogen fixed symbiotically by the association between legumes and *Rhizobium* species represents a renewable source of N for agriculture (Peoples *et al.,* 1995). Values estimated for various legume crops and pasture species are often impressive ranging from 200-300 kg N ha^{-1} $year^{-1}$ (Peoples *et al.,* 1995). Yield increases of crops planted after harvesting of legumes are often equivalent to those expected from application of 30-80kg fertilizer N ha^{-1} (Rascio and Rocca, 2008). Inputs of fixed N for alfalfa, red clover, pea, soybean, cowpea and vetch were estimated to be about 65-335 kg N ha^{-1} $year^{-1}$ (Tate, 1995) or 23-300 kg N ha^{-1} $year^{-1}$ (Wani *et al.,* 1995). Therefore, BNF is regarded as a renewable resource for sustainable agriculture as it helps to reduce fertilizer N requirements and thus increases economic returns to producers (Walley *et al*., 2007). The fixation of N_2 by legumes has the potential to contribute greatly to more economically viable and environmentally friendly agriculture (Odair *et al*., 2006). Economically, BNF reduces costs of production. Field trials have shown that the N captured by crops due to the use of *Rhizobial* inoculants costing $3/ha is equal to fertilizer N costing $87 (Silva and Uchida, 2000). Environmentally, the use of inoculants as alternatives to N fertilizer avoids problems of contamination of water resources from leaching and runoff of excess fertilizer. It has been demonstrated that fertilization with ammonia leads to increase soil acidity (Werner *et al.,* 2005). It has been

estimated that the 80-90% of the nitrogen available to plants in natural ecosystems originates from biological nitrogen fixation (Rascio and Rocca, 2008).

Numerous genera of non-leguminous angiosperms, such as *Alnus, Casuarina, Coriaria, Myrica*, etc., form root nodules in response to infection by the actinobacteria *Frankia* (Hubbel and Gerald, 2003). *Actinorhizal* infections are major contributors to nitrogen inputs in forests, wetlands, fields and disturbed sites of temperate and tropical regions (Tate, 1995). The contributions of fixed N_2 to native as well as managed ecosystems by the *actinorhizal* symbiosis are comparable to those of the more extensively studied *Rhizobium*-legume interactions (Zahran, 1999). Typical contributions by *Alnus* associations are 12-200 kg N ha^{-1} $year^{-1}$ and *Hippophae* associations are 27-179 kg N ha^{-1} $year^{-1}$ (Baker and Mullin, 1992).

2.2.5 Factors affecting biological nitrogen fixation

Establishment of effective N_2 fixing symbioses between legumes and their N_2 fixing bacteria is dependent upon many environmental factors, and can be greatly influenced by farm management practices (Peoples *et al.*, 1995). Environmental factors affecting nitrogen fixation include temperature, moisture, acidity and several chemical components of the soil such as nitrogen, phosphorus, calcium and molybdenum content (FAO, 1984). The efficiency of symbiotic BNF is markedly dependent on the mutual compatibility of both partners, and is influenced by a number of environmental factors which are macrosymbionts which comprises of variety, nodulin, photosynthate availability and tolerance of stress; microsymbiont which comprises of ineffectiveness, effectiveness, competitive ability and saprophytic competence and environmental factors which comprises of combined nitrogen, light, temperature, water, aeration, salinity and biotic agents (Vincent, 1980). It is often difficult to isolate the effect of the above factors on inoculation success from their influence on symbiosis and nitrogen fixation. For example, acidity, as well as calcium, aluminium and

manganese concentrations will interact and affect bacterial proliferation, root-hair infection and plant growth (Panchali, 2011). Numerous (micro)-climatic variables, soil physical properties and agronomic management factors also play a part in controlling N_2 fixation. The degree of specificity of certain species of tropical legumes and of tropical strains of *Rhizobia* has been previously discussed (Thies *et al.,* 1991; Simões-Araújo *et al.,* 2008). However, for groundnut, the ease with which this plant is nodulated by several types of *Rhizobia* has discouraged the selection of strains for crop. From the point of view of the crop, the strains in the soil responsible for nodulation are very competitive in the formation of nodules, but evidence suggests they are less efficient in N_2 fixation (Rumjanek *et al.,* 2005).

2.2.6 Need to inoculate

The need to inoculate arises when a legume is being introduced into an area for the first time, where compatible *Rhizobia* are absent, where the native *Rhizobia* is ineffective in fixing Nitrogen and lastly where the population of compatible *Rhizobia* is too small to improve nodulation (Herridge *et al*., 2002). Although *Rhizobia* seem to be as widely distributed as the legumes, many soils used for legume cultivation do not contain adequate numbers of highly effective *Rhizobia*. High N_2 fixation requires the presence of adequate numbers of highly effective *Rhizobia* in the soil (Thies *et al.,* 1991). However, most soils may be devoid of the *Rhizobia,* may contain low numbers of effective strains or they may contain high numbers of ineffective or partially effective strains, thus not capable of satisfying the N requirements of the crop, leading to a high dependence on soil or fertilizer N for optimum yields. The question "When to inoculate?" is critical and has been pondered at length, although it should be stated that there are far less problems with inoculating when not needed (i.e. over-inoculating) than not using inoculants and producing N-deficient crops. The

definitive indicator is the numbers of *Rhizobia* in the soil. Published data suggest a population of *Rhizobia* of >1000/g soil is required for optimum nodulation and N_2 fixation (Singleton *et al.,* 1992; Nazih and Weaver 1994). The numbers can be readily established and maintained in good quality soils through inoculation and continuing cultivation of the legume, but won't be achieved if the legume or near relatives have never been grown on the land, or if the land is severely degraded or perturbed. The primary aim of inoculation is to increase the number of desirable strains of *Rhizobia* at the rhizosphere (Lupwayi *et al*., 2000) and consequently increase biological nitrogen fixation and grain yield. Sometimes inoculation is applied as a form of insurance against crop failures (Deaker *et al*., 2006) as there is less problem associated with over inoculation than not inoculating at all (Herridge *et al*., 2002).

2.3 Soil Acidity and Liming

Acidity is believed to rank among the top hindrances to good yields (CIMMYT, 1998; Donovan *et al.* 2002). Approximately 30% of the world's total land area consists of acidic soils, and as much as 50% of the world's potentially arable lands are acidic (Von Uexkull and Mutert, 1995). Raising pH by adding suitable quantity of lime can eliminate the harmful effects of soil acidity. The fixation of phosphorus in acid soils has been one of the most important factors limiting the plant growth. Applied P is rapidly fixed as insoluble and unavailable compounds of Al and Fe in acidic conditions. This is so because acid soils are highly weathered and contain large quantities of Al and Fe hydrous oxides that have the ability to adsorb major elements onto their surfaces such that much of added nutrients are fixed instead of being made available for crop use (Akinrinade *et al.,* 2006).

Soils, especially, in the humid tropics, become acidic when basic cations are removed through leaching, plant uptake and plant harvest (Wild, 1993), carbon dioxide from

decomposing organic matter and root respiration dissolving in soil water to form a weak organic acid and formation of strong organic and inorganic acids, such as nitric and sulphuric acid, from decaying organic matter and oxidation of ammonium and sulphur fertilizers (Donald, 2011**).**

According to Chude *et al.* (2005), soils with pH values of less than 5.5 are considered as acidic. Soil acidification is an on-going natural process which can be enhanced by human activities or can be controlled by appropriate soil management practices (Fageria and Baligar, 2008). Acid soils can be managed in two ways, i.e., either by growing suitable crops for a particular soil pH or by ameliorating the soils through application of amendments, which counteract the soil acidity (Biswas and Mukherjee, 1994). The usual agricultural practice for most crops is to maintain a soil pH of 6.0- 6.5 by the addition of lime. Soil pH is used to determine whether or not to lime a soil (TSO, 2010). Liming materials include $CaCO_3$, $Ca,Mg(CaCO_3)_2$, $Ca(OH)_2$, CaO and others, which vary according to their neutralizing value and degree of fineness (TSO, 2010). When lime is applied to the soil, Ca^{2+} and Mg^{2+} ions displaces H^+, Fe^{2+}, Al^{3+}, Mn^{4+} and Cu^{2+} ions from soil adsorption site resulting in increase in soil pH. Other than increasing soil pH, lime also supplies significant amounts of Ca and Mg, depending on the type. Indirect effects of lime include increased availability of P, Mo and B, and more favourable conditions for microbially mediated reactions such as nitrogen fixation and nitrification, and in some cases improved soil structure (Nekesa *et al*., 2005). Calcium deficiency is known to restrict the amount of N_2 fixed in legumes, hence resulting into reduced plant growth due to inadequate nitrogen which is required as building blocks of proteins (Dutta, 2002).

Calcium is required by groundnut plants from the time when pegs begin to appear, fruit formation, until the pods are mature and it has been demonstrated that supply of Ca^{2+} through

lime significantly increased pod yield in groundnut (Dutta *et al*., 2004; Rangit, 2007). Calcium deficiency leads to high percentage of aborted seeds (empty pods), improperly filled pods (Ntare *et al.* 2008). In addition, there are also indications that high level of soil Ca are associated with reduced incidence of various pod and root rots. The yield benefits have also been shown to be evident especially when in combination with fertilizer (GART, 2001; Mitchell *et al.*, 2005).

2.3.1 Farming practices that contribute to soil acidity

Use of legume crops continuously or in rotation can increase soil acidity. Bolan and Hedley (2003) found that continuous cultivation of legume crops decreased the pH of agricultural soils. Other researchers have found that legume based pastures also increases soil acidification (Williams, 1980). Williams (1980) reported that even the normal growth of clover pasture for 50 years decreased the pH of an Australian soil from 6.0 to 5.0 at a depth of 30 cm. Legumes also increase soil acidification in arable cropping systems due to their high absorption of basic cations and the release of H^+ ions by the roots to maintain ionic balance (Bolan and Hedley, 2003). According to these authors, for different legume species, about 0.2–0.7 mole of H^+ were released per mole N_2 fixed. In addition, they stated that the amount of H^+ ions released during N_2 fixation is a function of carbon assimilation and hence depends mainly on the form and amount of amino acids and organic acids synthesized within the plants. Soil acidification is also caused by the release of protons (H^+) during the transformation and cycling of carbon, nitrogen and sulphur in the soil-plant-animal system (Bolan and Hedley, 2003).

2.3.2 Impact of soil acidity on plant growth and soil microorganisms

Soil microbiological properties can serve as soil quality indicators because soil microorganisms are the second most important (after plants) biological agents in the agricultural ecosystem (Fageria, 2002). Soil microorganisms provide the primary driving force for many chemical and biochemical processes and thus affect nutrient cycling, soil fertility, and carbon cycling (He *et al.,* 2003). Plant roots and rhizosphere are colonized by many plant beneficial microorganisms such as symbiotic and non-symbiotic N_2 fixing bacteria, plant growth promoting rhizobacteria, saprophytic microorganisms, bio-control agents, mycorrhizae and other free-living fungi. Soil acidity restricts the activities of these beneficial microorganisms, except fungi, which grow well over a wide range of soil pH (Brady and Weil, 2002).

Acid soils affect plants in several ways. For instance, Al prevents plant root elongation due to its direct effect on metabolism or indirectly by rendering the phosphate in the soil unavailable by binding it to form aluminium phosphates thereby leading to overall low crop yields (Mora *et al.,* 2005). Plant species and varieties differ in their sensitivity to the conditions in acid soils (Wild, 1993). Acidity produces complex interactions of plant growth-limiting factors involving physical, chemical, and biological properties of soil. Among biological properties, activities of beneficial microorganisms are adversely affected by soil acidity, which has profound effects on the decomposition of organic matter, nutrient mineralization, and immobilization, uptake and utilization by plants, and consequently on crop yields (Huber, 2006). Various studies have demonstrated that changes in soil microbial communities across space are often strongly correlated with differences in soil chemistry (Lauber *et al.,* 2008; Jenkins *et al.,* 2009). In particular, it has been shown that the composition, and in some cases diversity, of soil bacterial communities is often strongly correlated with soil pH (Hartman *et al.,* 2008; Lauber *et al.,* 2009). This pattern holds both

for overall bacterial community composition (Fierer and Jackson, 2006) and for the composition of individual bacterial groups (Jenkins *et al.,* 2009).

2.3.3 Liming as a soil acidity management strategy

Application of lime at an appropriate rate brings several chemical and biological changes in the soils, which are beneficial or helpful in improving crop yields on acid soils. Adequate liming eliminates soil acidity and toxicity of Al, Mn, and H; improves soil structure and thus, aeration; improves availabilities of Ca, P, Mo, and Mg, and N_2 fixation; and reduces the availabilities of Mn, Zn, Cu, and Fe and leaching loss of cations. For several crops, liming results in some chemical changes in the soil such as, increase in pH, effective cation exchange capacity (ECEC), and exchangeable Ca, decrease in toxic elements for example Al^{3+} and Mn^{2+} and changes in the proportion of basic cations in CEC sites (Ezekiel, 2006). Lime is usually added to acid soils to increase soil pH. Its addition not only replaces hydrogen ions and raises soil pH, thereby eliminating most major problems associated with acid soils but it also provides calcium, and/or magnesium to the soil. Lime also makes phosphorus that is added to the soil to be more available for plant growth and increases the availability of nitrogen by hastening the decomposition of organic matter (Donald, 2011). Liming materials are relatively inexpensive, comparatively mild to handle and leave no objectionable residues in the soil. Over-liming, however, can significantly reduce the bioavailability of micronutrients (Zn, Cu, Fe, Mn and B), which decrease with increasing pH (Fageria *et al.,* 2002). This can produce plant nutrient deficiencies, particularly that of Fe which is made available at medium acidic conditions.

2.4 Phosphorus

Phosphorus is among the 17 essential nutrients for plant growth. Its functions cannot be performed by any other nutrient, and an adequate supply of phosphorus is required for

optimum growth and reproduction (Uchida, 2000). Despite the considerable amount of total phosphorus in soils, many soils throughout the world are phosphorus deficient because the free P concentration (the form available to plants) even in fertile soils is generally not sufficient (Haru and Ethiopia, 2012). The importance of phosphorus in biological nitrogen fixation is well known, as it is an energy driven process (Haru and Ethiopia, 2012). Phosphorus is involved in several key plant functions, including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from one generation to the next (Uchida, 2000). Generally, phosphorus is vital to plant growth and is found in every living plant cell. *Rhizobium* use phosphorus as an essential ingredient in converting atmospheric N_2 to ammonium (NH_4), a form useable by plants (Dakora and Keya, 1997).

Inadequate P restricts root growth, the process of photosynthesis, translocation of sugars, and other such functions, which directly or indirectly influence nitrogen fixation by legume plants (Olivera *et al*., 2004). When P is limiting, the most prominent effects are a reduction in leaf expansion, leaf surface area and the number of leaves (Bekere *et al*., 2012). Shoot growth is more susceptible than root growth, which leads to a decline in the shoot-root dry weight ratio. However, root growth is also reduced by P deficiency, leading to fewer roots mass to attain water and nutrients (Uchida, 2000). Since phosphorus is readily mobilized in the plant, when a deficiency occurs, it is translocated from older tissues to active meristematic tissues, resulting in foliar deficiency symptoms emerging on the lower part of the plant (Weisany *et al*., 2013). Other effects of P deficiency on plant growth include delayed maturity, reduced quality seeds, fruit and decreased disease resistance. Plants need phosphorus for growth throughout their life cycle, especially during the early stages of growth and development for proper and well-built roots. The primary role of phosphorus

compounds in plants is to store and transfer energy produced by photosynthesis to be used for growth and reproduction (Leidi *et al*., 2000).

During various chemical reactions, P is integrated into organic compounds, including nucleic acids (DNA and RNA), phosphoproteins, phospholipids and sugar phosphate compounds like adenosine triphosphate (ATP) (Bashir *et al*., 2011). The ATP is then available as an energy source for many other reactions that occur within the plant, and the sugars are used as building blocks to produce other cell structural and storage components (Marschner and Marschner, 2012)

2.4.1 Phosphorus need in legumes

Phosphorous is among the important element needed for growth and production of legumes in many tropical soils (Buerkert *et al.*, 2001; Nekesa *et al.*, 2007)**.** Symbiotic legumes have a high requirement for phosphorus. This high required amount is essential for stimulating root and shoot growth and also influences the efficiency of the *Rhizobium*-legume symbiosis through facilitation of energy transfer reactions which involves ATP in nitrogenase activity (Leidi *et al*., 2000). Legumes such as groundnut need phosphorus for adequate growth and nitrogen fixation. Sufficient phosphorus levels are also required to enhance different plant organs growth and promote nodulation and early maturity (Kamara *et al*., 2007). Studies done by Ndakidemi *et al*. (2006) and Shahid *et al*. (2009) provided proofs that increased phosphorus application enhances plant growth significantly. Supplementing legumes with nutrients, especially phosphorus has great potential for increasing yields, as it not only promotes plant growth but also enhances symbiotic establishment for increased N_2 fixation (Gangasuresh *et al*., 2010). Phosphorus is a crucial elements in crop production which plays important role for many characteristics of plant growth such as sugar and starch utilization,

photosynthesis use, cell division and organization, nodule formation, root development, flower initiation and seed and fruit development (Gangasuresh *et al*., 2010).

2.4.2 Effect of phosphorus on nodulation and nitrogen fixation in legumes

Phosphorus is second only to nitrogen as an essential mineral fertilizer for crop production. At any given time, a substantial component of soil P is in the form of poorly soluble mineral phosphates which is not readily available to plants (Marschner and Marschner, 2012). Scientific evidence suggests that a high phosphorus supply is needed for nodulation (Elkoca *et al.*, 2007) and nitrogen fixation in legumes (Abdul-Jabbar and Saud, 2012). When legumes depend on symbiotic nitrogen and receive an inadequate supply of phosphorus, they may suffer from nitrogen deficiency (Weisany *et al.*, 2013) as a result of poor N_2 fixation. Generally, the influence of P on symbiotic nitrogen fixation in leguminous plants has received considerable attention (Al-Falih, 2002; Tsvetkova and Georgiev, 2003; Yusuf *et al.*, 2011). Phosphorus promotes early root formation and the formation of lateral, fibrous and healthy roots, which play an important role in N_2 fixation, nutrient and water uptake (Bhuiyan *et al.*, 2008; Niu *et al.*, 2012). Several studies have reported that application of P along with *Rhizobium* inoculant influenced nodulation and N fixation of legume crops (Lal and Sanchez, 1992; Saini *et al.*, 2004). It has been reported that nodulated legumes require high levels of P for optimal symbiotic performance (Zahran, 1999). Phosphorus contributes in improving the activity of bacteroids inside the root nodules and often results in large amounts of N_2 fixed, in most of tropical grain legumes (Dakora and Keya, 1997). A study conducted in the Usambara Mountains in northern Tanzania also showed a strong response to 26 kg/ha of P fertilizer by increasing the percentage of N_2 fixed by BNF from 25-27% to 48-51% (Giller *et al.*, 1998).

2.5 Potassium

Potassium is the main osmotic solute in plants and its accumulation in the cell favours the activation of a wide range of enzyme systems regulating photosynthesis, water use efficiency and movement, nitrogen uptake and protein building (Nguyen *et al.,* 2002). It is documented that potassium may "activate" at least 60 different enzymes involved in plant growth (Marschner and Marschner, 2012). Potassium functions by changing the physical shape of the enzyme molecule which exposes the appropriate chemical active sites for reaction (Ball, 2008). Potassium also neutralizes various organic anions and other compounds within the plant, helping to stabilize pH between 7 and 8 which is considered to be optimum for most enzyme reactions (Wakeel *et al.,* 2011). The amount of K present in the cell determines how many of the enzymes can be activated and the rates at which chemical reactions can proceed. Thus, the rate of a given reaction is controlled by the rate at which K enters the cell (Munson, 1985).

Potassium plays significant roles in enhancing crop quality. High levels of available K improve the physical quality, disease resistance, and shelf-life of fruits and vegetables used for human consumption and the feeding value of grain and forage crops (Sangakkara *et al.,* 1996). Potassium influences the water economy and crop growth through its effects on water uptake, root growth, maintenance of turgor, transpiration and stomatal regulation (Khurana and Sharma, 2000; Singh and Kataria, 2012). The "reading" of the genetic code in plant cells to produce proteins and enzymes that regulate all growth processes would be impossible without adequate K. When plants are deficient in K, proteins are not synthesized despite an abundance of available nitrogen (N). Instead, protein "raw materials" (precursors) such as amino acids, amides and nitrate accumulates in the tissue. The enzyme nitrate reductase catalyzes the formation of proteins and K is likely responsible for its activation and synthesis (Chandok *et al.,* 2003). However, K has other important roles in major plant processes such

as photosynthesis, respiration, osmoregulation, growth and yield of plants but however, does not enter into the composition of any product unlike nitrogen and phosphorus (Khurana and Sharma, 2000).

2.5.1 Effect of potassium on nodulation and nitrogen fixation in legumes

Adequate supply of K is important for the symbiotic relationship that enables bacteria to fix nitrogen from the atmosphere for use by legumes (Weisany *et al.,* 2013). Sangakkara *et al.* (1996) reported that high potassium supply had positive effect on nitrogen fixation and shoot and root growth. Studies have revealed that increasing the level of K fertilizer increased dry matter production and total N_2 fixed in faba bean (Zahran, 1999; Al-Falih, 2002). Generally, high potassium supply is required in symbiotic system to ensure an optimal growth and N_2 fixation (Kurdali *et al.,* 2002). Imas and Magen, (2007) studied the effect of K on Soybean and concluded that plants growing at higher K level have better development of nodules and consequently higher N_2-fixation. Potassium application increased the nodule activity, the number of nodules formed, fresh weight of nodules, and amount of N_2 fixed per nodule. Studies by Mengel *et al.* (1974) on the effect of K on the N_2 fixation by root nodules of faba bean, *Vicia faba* showed that plants supplied well with potassium had better carbohydrate supply to the nodules, and a higher carbohydrate turnover in the nodules thus improving the provision of ATP and reducing electrons required by the nitrogenase enzyme and finally enhanced the N_2 fixation.

Kurdali *et al.* (2002) studied the effects of different rates of K fertilizer on nodulation, dry matter production, and N_2 fixation by chickpea and faba bean subjected to different soil moisture levels at the beginning of flower bud initiation stage. They found that plant species differed in their response to K fertilizer as a means of enhancing growth and overcoming the stress conditions. The higher level of K fertilizer increased both dry matter production and

total N_2 fixed in faba bean, but did not have any impact on chickpea. From the above background, potassium is an important element in legume nutrition. Although several legume varieties have been developed for smallholder production in Africa, the effectiveness of supplying optimum levels of K to enhance N_2 fixation is not widely documented.

2.6 Organic Amendment

Soil microorganisms especially bacteria and fungi have been shown to be sensitive to organic amendments (Magdoff, 2001). Organic amendments are known to increase the abundance of various components of the soil food web, including the soil fungal and bacterial communities (Forge *et al.,* 2008). In addition they supply other macro and micro nutrient elements which are contituents of organic matter and are beneficial to plants and microorganisms

2.6.1 Nitrogen supply by organic amendment

It has been observed that the nitrogen content of organically amended soils is higher than soils receiving inorganic fertilization (Sullivan *et al.,* 2003; Rodriguez *et al.,* 2005). This is likely due to higher rates of total nitrogen applied in organic wastes than in inorganic fertilizers in order to meet plant nutrient needs. Researchers have observed that composted organic amendments can improve plant health beyond the nitrogen fertility value (Buckerfield *et al.,* 1999; Atiyeh *et al.,* 2002). Of particular interest is the apparent ameliorating effect of organic amendments on drought-stressed crops. HuiLan *et al.* (1998) noted that the application of organic amendments increased water stress resistance of sweet corn leaves. In particular, stomatal and curticular conductance of the leaves were lower in these plants than in inorganically fertilized plants. In five-week old water stressed maize seedlings, Xu (2000) recorded higher photosynthetic rates when the soils were organically amended. Heckman *et al.* (1987) found that field grown soybeans fertilized with sewage

sludge had increased drought resistance and nitrogen fixation than the control treatment. The phytohormonal activity of humic substances in composted organic amendments is thought to play a causal role in drought stress amelioration (Xu, 2000).

Nitrogen mineralization is controlled by compost properties including organic carbon content, C:N, total nitrogen, and plant available nitrogen; soil moisture; microbial activity; and soil texture (Eghball *et al.,* 2002; Agehara and Warnke, 2005). Compost C:N greater than 25:1 have been shown to immobilize nitrogen in soils (Nishio and Oka, 2003; Flavel *et al.,* 2005). Although often used to estimate nitrogen mineralization (Khalil *et al.,* 2005), it was concluded that C:N did not accurately estimate nitrogen mineralization and that total nitrogen was a better estimator of nitrogen mineralization (Kessel and Reeves, 2003; Cabrera *et al.,* 2005).

2.6.2 Phosphorus supply by organic amendment

The application of organic amendments to soils has been shown to increase soil phosphorus content and plant available phosphorus (Gao and Chang, 1996; Eghball *et al.,* 2004). Compost applied on a nitrogen basis may significantly increase soil phosphorus levels (Park *et al.,* 2004; Singer *et al.,* 2004). Eghball *et al.* (2004) observed continued plant phosphorus uptake up to four years after nitrogen-based compost and manure applications ceased without the addition of phosphorus fertilizers. Excessive soil phosphorus levels are not detrimental to plant health, but can cause serious environmental degradation if the soil is subject to runoff and erosion. It was proven that many microorganisms in soil produce organic acids like carbonic acids, acetic acids, citric acids, etc. These acids create favourable environment for the enhancement of P solubility and uptake by plants (Sharif *et al*., 2011). Kucey *et al*., (1989) have shown from liquid medium studies that the microbial solubilization of soil phosphate has often been due to excretion of organic acids. The availability of P for

plant uptake can therefore be increased by treatment with mineral acids, organic acids, and a mixture of organic materials, biological treatment, etc. Incorporating organic manures and P materials has been shown to enhance the solubility (Sharif *et al*., 2011).

2.6.3 Potassium, secondary and micronutrients in organic amendments

Compost may contain appreciable levels of potassium, calcium, magnesium, and micronutrients. Animal feed is often supplemented with minerals (e.g., copper, zinc, manganese) to maximize animal growth and development (Kegley, 2001). The ability of animals to utilize these minerals is generally less than the amount of mineral provided in the feed. It is consequently excreted as waste and incorporated into the organic amendment matrix (Kingery *et al.,* 1994). Researchers have documented increases in soil potassium (Khatik and Dikshit, 2001; Rodriguez *et al.,* 2005) calcium and magnesium (Eghball *et al.,* 2002; Walker and Bernal, 2004), and micronutrients (Negm and Zaki, 2004; Yogananda *et al.,* 2004) after the addition of composted materials.

2.6.4 Effects of organic amendment on soil physical properties

A reduction in soil water holding capacity is considered by some to be the major factor contributing to reduced yields in eroded, low organic matter soils (Bauer and Black, 1992). The addition of organic amendments has been observed to increase soil water holding capacity (Curtis and Claassen, 2005; Konomi *et al.,* 2005). The addition of organic material increases the number of smaller pores and surface area of sandy soils; thus, more water is held in amended soils at lower tensions. The amount of water retained in organically-amended soils is greater than that retained in unamended soils as soil-water tension increases (Elsharawy *et al.,* 2003). Increased plant water availability has been considered the causal factor for increased crop yields in organically amended soils (Curtis and Claassen, 2005).

2.7 Micronutrients in Nitrogen Fixation

2.7.1 Molybdenum

Molybdenum is a micronutrient specifically for plants that form root nodules with nitrogen-fixing bacteria, though plants that do not form nodules also use trace amounts of it in a protein involved with nitrogen metabolism and uptake (Wiedenhoeft, 2006). The Mo-Fe protein contains two atoms of molybdenum and has oxidation–reduction centres of two distinct types: two iron-molybdenum cofactors called FeMoCo and four Fe-S (4Fe-4S) centres. The Fe–Mo cofactor (FeMoCo) of nitrogenase constitutes the active site of the molybdenum-containing nitrogenase protein in N_2-fixing organisms (Allen *et al,* 1999). Although at low supply, molybdenum is preferentially transported into the nodules (Brodrick and Giller, 1991). Molybdenum deficiency-induced nitrogen deficiency in legumes relying on N_2 fixation is widespread, particularly in acid mineral soils of the humid and sub humid tropics. There are reports that foliar applications of Mo to grain legumes in field conditions increase levels of N_2 fixation and nodule mass, resulting in higher overall N content and seed yield (Vieira *et al.,* 1998). It is also reported that a *B. japonicum* strain deficient in molybdenum transport showed impaired nitrogen fixation activity when inoculated to soybean roots (Delgado *et al.,* 2006).

2.7.2 Iron

Iron is required for several key enzymes of the nitrogenase complex as well as for the electron carrier ferredoxin and for some hydrogenases. A particular high iron requirement exists in legumes for the heme component of hemoglobin. Therefore, in legumes, Fe is required in a greater amount for nodule formation than for host plant growth, for example in lupins (Tang *et al.,* 1990). A reduction in specific rates of nitrogenase activity has been

observed in Fe limited groundnut nodules (O'Hara *et al.*, 1988), indicating a possible direct limitation by Fe deficiency on nodule function. Leghaemoglobin is an oxygen-binding protein. The single most abundant protein that the plant host makes in the nodule is leghaemoglobin, an iron protein. In the bacteria, nitrogenase and nitrogenase reductase contain FeS clusters and the former has the cofactor FeMoCo at the active site for N_2 reduction. Furthemore, bacteroids have a very high respiratory demand, requiring abundant cytochromes and other electron donors, each with their own Fe centers (Delgado *et al.*, 1998). Although iron deficiency did not significantly affect shoot growth, it severely depressed nodule mass and particularly leghemoglobin content, number of bacteroids and nitrogenase activity, compared with those plants five days after a foliar spray of iron. In contrast to peanut, in lupin *(Lupinus angustifolius)* iron is not retranslocated into the nodules after a foliar spray, and direct iron supply at the infection sites at the roots is required for effective nodulation (Tang *et al.*, 1990).. In lupin and peanut, nodule development is much more susceptible to a shortage of Fe than are other parameters such as plant shoot and root weights (O'Hara *et al.*, 1988).

2.7.3 Boron

Boron (B) is one of the eight essential micronutrients, also called trace elements, required for the normal growth of most plants. Yamagishi and Yamamoto (1994) reported strong alterations in N_2 fixation in soybean plants with a low B supply. Bolanos *et al.* (1996) made a study of the effect boron on *Rhizobium*-legume cell-surface interaction and nodule development in pea. In boron-deficient plants, the number of *Rhizobia* infecting the host cells and the number of infection threads were reduced and the infection threads developed morphological aberrations. The cell walls of root nodules of boron-deficient plants showing structural aberrations are reported to lack the covalently bound hydroxyproline/proline rich

proteins (Bonilla *et al.,* 1997), which contribute to an O_2 barrier preventing inactivation of nitrogenase and associated decrease in N_2 fixation.

2.7.4 Copper

Apart from its role in respiratory proteins that are required for N_2 fixation in *Rhizobia* (Delgado *et al.,* 1998), copper also plays a role in a protein that is expressed co-ordinately with the nifgenes and may affect the efficacy of bacteroid function. Several *Rhizobial* strains, particularly *R. leguminosarum*, make the pigment melanin. The meIA gene, specifying the copper-containing enzyme tyrosinase is expressed at high levels in bacteroids, this being under the control of the regulatory *R. leguminosarum* nifA gene (Hawkins and Johnston, 1988). There is increasing interest in the phenomenon whereby bacteria enter a state that is 'viable but non-culturable'. For reasons that are not clear, adding Cu to Agrobacterium or *R. leguminosarum* cells sends them to this state (Alexander *et al.,* 1999). However, Cu deficiency decreased nitrogen fixation in subterranean clover (Snowball *et al.,* 1980).

2.7.5 Zinc

Zinc is a micronutrient needed in small amounts by crop plants, but its importance in crop production has increased in recent years. Weisany *et al.* (2011) reported that zinc application on plants exposed to salinity stress caused a noticeable enhancement of photosynthesis, water use efficiency, mesophyll efficiency and quantum yield compared with plants exposed to salinity stress alone. Also Weisany *et al.* (2012) reported that lipid peroxidation and hydrogen peroxide concentration under salinity treatments significantly reduced as a result of zinc application. In addition to the possible role of zinc in the function of the Ros/MucR transcriptional regulators, there is a description of protein engineering by Chauhan and O'Brian (1995), which relates to Zn and *B. japonicum*. In this bacterium, the enzyme S-aminolaevulinic acid dehydratase (the product of hemB) normally has Mg^{2+} as a cofactor.

In contrast, the corresponding enzyme in plants contains Zn^{2+}. By site-directed mutagenesis of *B. japonicum* hemB, they substituted the N-terminal amino acids of the *B. japonicum* enzyme, and showed that this caused the engineered protein to bind Zn^{2+} and not Mg^{2+}. This did not affect symbiotic N_2 fixation, despite the known requirement for a functional hemB for N_2 fixation to occur (Chauhan and O'Brian, 1995). Thus, the plant can supply the extra load of Zn^{2+} that would be required by inoculant strains.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Sites

Two field trials were conducted at the research farm of the Institute for Agricultural Research (IAR) Samaru in 2015. Samaru is located in the northern Guinea savanna of Nigeria which lies between latitudes 9º04′ to 11º50′ N and longitudes 6º09′ to 10º41′ E. The mean annual temperature is 25ºC while the long-term annual rainfall was calculated at 1011±161mm distributed almost entirely in the five months (May/June to September/October) of the cropping season (Oluwasemire and Alabi, 2004). The soil is classified as leached tropical

ferruginous, Typic Haplustalf in Soil Taxonomy, Acrisol in the FAO system or Alfisol in the USDA system (Uyovbisere *et al.*, 2000). The first trial was conducted on an acidic soil located at Latitude N11º10.958′ and Longitude E7º36.927′ while the second trial was conducted on a non-acidic soil located at Latitude N11º10.651′ and Longitude E7º36.793′. The experimental fields were labelled S13 and S7 for the acidic and non-acidic soils respectively.

3.1.1 Soil sampling and analyses

Eijelkamp soil auger was used to collect soil samples from the experimental fields at a depth of 0-15cm prior to land preparation. Twenty soil samples were collected systematically in a zigzag manner from each fields and bulked to form two separate composite samples, one per field. A subsample of each of the composite samples was air dried, crushed and sieved using a 2mm and 0.5mm sieves depending on size requirement for the various parameters to be analysed. Routine analysis was carried out in the Department of Soil Science laboratories at IAR Samaru. Soil pH was measured in a 1:2.5 soil/water as well as 1:2.5 soil/$CaCl_2$ ratio using a glass electrode pH meter as described by Hendershot *et al.*, (1993) where 10 g of soil was mixed with 25 g of distilled water and $CaCl_2$ respectively, stirred and allowed to equilibrate for 30 minutes before measurement. Soil organic carbon was determined by the Walkley and Black method as described by Nelson and Sommers (1982), particle size distribution was determined by the hydrometer method as described by Gee and Orr (2002) using calgon as dispersing agent and total nitrogen of the soil was determined by the Kjeldahl method as described by Bremner and Mulvaney (1982) by the digestion of 1 g of soil sample in 10 ml of H_2SO_4 along with K_2SO_4 and Se catalyst. The digest was distilled with NaOH into boric acid containing methyl orange indicator and subsequently titrated with Na_2CO_3. Total P was determined by the molybdate blue method of He and Honeycutt (2005) after

digestion with a mixture H_2SO_4 and H_2O_2, while available phosphorus was determined by the Bray 1 method (Olsen and Sommers, 1982). Exchangeable Acidity was determined by titration method after extraction with 1N KCl (Anderson and Ingram, 1993). Exchangeable bases (Ca, Mg, K and Na) were extracted in 1 N NH_4OAC solution as described by Rhoades (1982). Calcium and Mg in the extract were read using atomic absorption spectrophotometer (AAS), while K and Na were determined using flame photometry. Cationic micronutrients (Fe, Mn, Cu and Zn) were extracted simultaneously with a 0.001 M DTPA (diethylenetriaminepentaacetic acid) solution that was prepared in combination with triethanolamine (TEA) and $CaCl_2$, buffered at pH 7.3 (Lidsday and Norvell, 1978). The cations in solution were determined using the AAS.

3.1.2 Chemical analysis of manure

The manure (cow dung) used in this study was collected from the National Animal Production Research Institute (NAPRI) Shika. It was air-dried and packed in a bag and then sampled randomly from various regions in the bag, bulked, crushed and sieved using a 0.5 mm sieve. The pH in water, total N, total P and organic carbon in the manure sample were determined using standard procedures as used for the soil samples analysis given in section 3.1.1. The exchangeable bases were read from the digest used for total P determination using the AAS for Ca and Mg while Na and K were read using the flame photometry.

3.1.3 Determination of Most Probable Number of *Rhizobia*

Most probable number (MPN) of *Rhizobia* in the two soils was determined using the plant infection method (Somasegaran and Hoben, 1985). The groundnut seeds were surface sterilized by immersing them in 70% ethanol for 30 seconds, drained and submerged in 3% sodium hypochlorite for 3-5 minutes, drained and rinsed six times with sterile water as described by Vincent (1970), transferred onto 1% agar water and pre-germinated inside an

incubator at 25ºC for 72 hours. Upon emergence of the radicle, the pre-germinated seeds were transferred aseptically into growth pouches (two plants per pouch) containing 100ml of sterilized N-free nutrient solution (Woomer *et al.,* 1988) and were placed in a growth chamber. The pouches were put into rackets for support and a six step five-fold serial dilution of a subsample of the experimental soil was carried out, replicated four times and 1 ml of aliquot was inoculated onto the root zone of the cultured plants 6 days after transplanting. After four weeks, the plants were observed for nodulation and nodulated units recorded. This was done for all the four replicates.

The MPN was calculated using the following formula:

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

Where,

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

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

v = volume of aliquot applied to plant.

3.2 Colony Forming Units of Inoculant

In order to determine the viable bacterial concentration of the inoculant (NC 92), colony forming units (CFU) was determined using the pour plate method as described by Somasegaran and Hobben (1985). Seven ten-fold serial dilution of the inoculant using one gram of the inoculant to 10 ml of sterile water was carried out designated as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. Using the dilution levels of 10^{-5}, 10^{-6} and 10^{-7}, 1 ml aliquot of each of the dilution levels was inoculated aseptically into a plate and molten Yeast Manitol Agar medium amended with Congo red dye and maintained in a water bath at 50ºC was poured, swirled gently and allowed to solidify in a laminar flow. Each dilution level was replicated twice and incubated at 25ºC for 5 days. The plates with 30-300 colonies were selected per

dilution and counted on a digital colony counter and the average on selected dilution was taken using its replicates. Numbers of colony forming units per gram (CFU/g) of the inoculant was determined using;

$$N = \frac{n}{C \times V}$$

Where: N = number of cells per gram

n = number of isolated colonies counted on the plate

C = Dilution

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

3.3 Field Preparation and Layout

The experimental fields were harrowed, ridged and marked out. The fields were laid out using a randomised complete block design (RCBD) replicated three times. Each block had plots with a dimension of 3x3 m, with inter row spacing of 75cm and intra-row plant spacing of 10cm. Each plot was separated by a distance of 0.75 m and 1.5 m between the blocks. The net plot had a size of 12x63 m.

3.3.1 Treatment and experimental design

Treatments included lime ($Ca(OH)_2$), P as single super phosphate (SSP), K as muriate of potash (MOP) and micronutrients (Agrolyzer, containing Fe, Mn, Bo, Mo, Cu and Zn) on the acidic field while the non-acidic field had the following treatements: Organic manure, P as single super phosphate (SSP), K as muriate of potash (MOP) and micronutrients (Agrolyzer) in a nutrient omission trial using factorial combinations on both fields. The test crop was groundnut variety SAMNUT 24 and a reference crop ICGL 5 (non-nodulating groundnut genotype) was used for the estimation of BNF in each location. The groundnut seeds were inoculated with the groundnut inoculant, NC 92 and planted on all the plots excluding the reference plot. The treatments for the acidic soil (S13) were two levels each

for lime, phosphorus, potassium and micronutrients. The two levels for Lime were zero (0 kg/ha) and 250 kg/ha (based on the soil's lime requirement determined by the single addition of $Ca(OH)_2$ titration method described by Kissel *et al.*, (2007)) applied as $Ca(OH)_2$ in planting furrows for plots requiring lime application two weeks before planting. At planting, phosphorus was applied at 0 kg P_2O_5/ha and 54 kg P_2O_5 /ha as SSP while potassium was applied at 0 kg K_2O/ha and 25 kg K_2O/ha as MOP using basal application method. Micronutrients (formulated with the trade name, Agrolyzer) were applied as foliar application three weeks after planting at 0 g/L and 2 g/L of water. On the non-acidic soil (S7), manure was applied at the rate 0 tons/ha and 1.7 tons/ha (equivalent to 10 kg N/ha) (Table 1) two weeks before planting. Phosphorus, potassium and micronutrients were applied at two levels as described above for the acidic field. A factorial combination of these nutrient levels gave a total of 16 treatments per block on each location.

The treatment combinations on the acidic soil were: Control, L, P, K, M, L+P, L+K, L+M, P+K, P+M, K+M, L+P+K, L+P+M, L+K+M, P+K+M and L+P+K+M. Where L=lime, P=phosphorus, K=potassium and M=micronutrients while that for the non-acidic soil were: Control, CD, P, K, M, CD+P, CD+K, CD+M, P+K, P+M, K+M, CD+P+K, CD+P+M, CD+K+M, P+K+M and CD+P+K+M. Where CD=Cow dung, P=phosphorus, K=potassium and M=micronutrients.

Table 1. Chemical Properties of Cow dung Used in the Study

Parameter	
pH (H_2O)	6.0
Organic C (%)	12.8

Total N (%)	0.59
Total P (%)	0.57
Ca (mg/kg)	20.0
Mg (mg/kg)	69.03
Na (mg/kg)	1225.0
K (mg/kg)	800.0

3.4 Inoculation, Planting and Other Cultural Practices

The inoculant was applied at 5 g per kg of seeds with 16% gum Arabic as an adhesive using the two-step method (Woomer, 2010). In the first step, the pre-weighed seeds in a container were uniformly coated with 16% (w/v) gum arabic (sticker), then the container was closed and swirled until all the seeds were uniformly wet. In the second step, the inoculant was added to the sticky seeds and the container closed and swirled gently until seeds were uniformly covered. The seeds were air dried to enhance adhesion before planting. One seed per hole was planted at a depth of 5 cm with an intra-row spacing of 10 cm. Three weeding sessions were conducted to make sure that the plots were kept weed free throughout the growing season.

3.5 Plant Sampling and Measurement

Plant sampling at 50% flowering was confined to the two border (outer) rows in each plot. During sampling, four groundnut plants were carefully dug out from each plot (two per

border ridge) after loosening the soil around the plant using a hand fork to avoid damage to the roots. The plant samples were processed for nodule number, fresh and dry weight of nodules, shoot and root dry weight as well as measurement of N_2-fixation.

3.5.1 Nodulation

The sampled plants were put in labelled polythene bags from the field. The roots were separated from the shoot and were put in a 1 mm mesh sieve and washed under running tap water to remove adhered soil particles. The nodules were then gently removed with hands, counted and weighed on a mettler balance.

3.5.2 Shoot, root and nodule dry weight

The shoots and roots of harvested plant samples were put in well labelled envelopes and oven-dried at 65^{o}C for 72 hours to a constant weight and their dry weights were recorded and then ground and passed through 0.5mm sieve for tissue analysis. Similarly, the nodules were oven dried to a constant weight at 65 °C and the dry weights were recorded.

3.5.3 Plant tissue analysis

Total N in the shoots and roots of the ground plant tissues as well as the non nodulating reference crop was determined by the Kjeldahl procedure as described by Bremner and Mulvaney (1982).

3.5.4 Determination of atmospheric nitrogen fixed by groundnut

The amount of N_2 fixed by groundnut in each treatment was estimated using the Total Nitrogen Difference (TND) method. This was done by comparing total N of the legume with that of a reference crop (non-nodulating genotype) (Murray *et al*., 2008). The amount of N fixed was calculated by subtracting total N of the reference crop from that of the legume (groundnut) and the difference was assumed to be N derived by BNF (N_2 fixed).

Thus, N_2 fixed = Total N in legume -Total N in reference crop ……....(i)

$$\text{Where, Total N in plants} = \frac{(\text{Dry matter weight (kg/ha) X \% N in plants})}{100} \quad \ldots\ldots\ldots\text{(ii)}$$

$$\%\ \text{Ndfa} = \frac{[\text{Total N in legume} - \text{Total N in reference crop}]}{\text{Total N in legume}} \times 100 \quad \ldots\ldots\ldots\text{(iii)}$$

Where % Ndfa is the percentage of N_2 derived from the atmosphere.

3.5.5 Soil Nitrogen Balance

The net contribution of N_2 fixation to the soil N balance was estimated using: Net N balance = Nf-Ns (Peoples and Craswell, 1992). Where Nf is the amount of N_2 fixed and Ns represents total N in the grains.

3.6 Harvesting and Yield Assessment

Harvesting was carried out when the crop was physiologically mature, observed when leaves senescence became manifest. Number of plant in the net plot (two inner rows) were counted and all the plants were uprooted with a hand hoe and assessed for yield and yield components including grain and haulm yield among others.

3.6.1 Yield assessment

At harvest, all mature pods from plants uprooted from the net plots on an area of 1.5 m^2 per plot were left on the haulm to sun-dry in the field for three days before they were picked and weighed. 200 g of the fresh pods per plot was weighed and oven dried at 65^{o}C for 72 hours to a constant weight and re-weighed and was then used to calculate the pod and grain yields. Pod yield was calculated as:

$$\text{Pod Yield (kg ha}^{-1}) = \frac{\text{Total pod FW (g) x Sub-sample pod DW (g) x 10}}{\text{Sub-sample pod FW (g) x Net area harvested (m}^2)}$$

Shelling percentage as a yield component was calculated per plot. The oven dried pod sub samples taken from the entire harvest of each experimental unit were weighed (pod dry weight) and then shelled. The shelled grains and the husks were weighed separately and the shelling percentage was calculated using the formula below:

$$\text{Shelling percentage (\%)} = \frac{\text{weight of shelled groundnut}}{\text{Weight of unshelled groundnut}} \times 100$$

Grain yield per plot was calculated using the formula:

Grain Yield (kg ha^{-1}) = Shelling percentage/100 x Pod Yield.

One hundred seeds were randomly picked from each plot and weighed on a mettler balance to give the 100 seed weight.

The haulm yield was also determined from the net plot at harvest. This followed the removal of all the pods from the plants. The haulm and the fallen leaves were collected and weighed. A 200 g weight sub-sample was taken and oven dried at $65^{O}C$ for 72 hours until constant weight was achieved. The sub-samples were re-weighed to determine the dry weight. From the sub-sample values of fresh weight (FW) and dry weight (DW) obtained above, the haulm yield was calculated using the formula below:

$$\text{Haulm Yield (kg ha}^{-1}) = \frac{\text{Total haulm FW (g) x Sub-sample haulm DW (g)}}{\text{Sub-sample haulm FW (g) x Net area harvested (m}^2)} \times 10$$

Haarvest index as a yield component was determined using the relationship:

$$\text{Harvest index} = \frac{\text{Grain Yield}}{\text{Total Biomass}} \times 100$$

3.7 Statistical Analysis

All data collected were subjected to analysis of variance (ANOVA). Where the F ratios were found to be significant, the means of measured parameters were separated using Duncan

Multiple Range Test (DMRT) at 5% level of probability. The relationship between observed parameters was determined using stepwise multiple regression and simple correlation. The SAS software (version 9.2. 2008) was used for all statistical analysis.

CHAPTER FOUR

4.0 RESULTS

4.1 Physical, Chemical and Microbiological Properties of the Experimental Soils

The results of the physical and chemical properties of the experimental soils determined before the establishment of the trials are shown in Table 4.1.

4.1.1 Physical and chemical properties of the experimental soils

The results obtained are shown on Table 4.1. Interpretation of the results was based on the crtical values for nutrients as given by FMNAR (1990). The analytical values show that on the acidic soil (S13), the soil texture was sandy loam. The pH of the soil in water was rated as strongly acidic. Organic carbon, total N and available P contents were interpreted to be low. Exchangeable bases (Ca^{2+}, Mg^{2+} and Na^{+}) were all of the low class while K^{+} was moderate. The values for the available micronutrients (Zn, Cu, Mn and Fe) were all high.

For the non-acidic soil (S7), the textural class was sandy loam. The pH in water indicated moderately acidic. The organic carbon content and total N of the soil were low. The value

for the available P shows that it is of moderate concentration. The exchangeable bases Ca^{2+}, Na^{+} and K^{+} were moderate while Mg^{2+} was low. The available micronutrients were interpreted to be high.

4.1.2 Most probable number of indigenous *Rhizobia* in experimental soils

The most probable number (MPN) counts of indigenous groundnut *Rhizobia* using the plant infection method indicates a population of 9×10^{1} cells/g on the acidic soil. The non-acidic soil had a population of 1.0×10^{3} cells/g of native groundnut *Rhizobia.*

4.2 Colony Forming Units of *Rhizobial* Cells in Inoculant

The results obtained for the colony forming units (CFUs) of cells per gram of the *Rhizobial* inoculant (NC 92) shows that the inoculant had a CFU of 1.9×10^{6} cells/g of the inoculant.

Table 4.1. Physical and Chemical Properties of Experimental Soils

Parameter	**Acidic soil**	**Non-acidic soil**
Particle size distribution (%)		
Sand	56	58
Silt	28	28
Clay	16	14
Textural class	Sandy Loam	Sandy Loam
Soil pH (H_20) 1:2.5	5.3	5.9
Soil pH ($CaCl_2$) 1:2.5	4.6	5.1
Total N (g kg^{-1})	0.56	0.35
Organic C (g kg^{-1})	6.3	3.2
Total P (mg/kg)	600	510
Available P (mg/kg)	7.00	14.53
Exchangeable bases (cmol kg^{-1})		
Calcium (Ca)	4.6	6.0

Magnesium (Mg)	0.36	0.3
Potassium (K)	0.28	0.24
Sodium (Na)	0.09	0.15
Exchangeable acidity (H+Al) (cmol kg^{-1})	1.4	1.0
ECEC (cmol kg^{-1})	6.73	7.69
Micronutrients (mg kg^{-1})		
Copper (Cu)	3.6	1.6
Manganese (Mn)	15.67	10.52
Iron (Fe)	57.73	71.36
Zinc (Zn)	8.52	4.61

4.3 Effects of Lime, Phosphorus, Potassium and Micronutrients on Nodulation, Biomass and Nitrogen Fixation of Groundnut on the Acidic Soil

4.3.1 Effects of lime, phosphorus, potassium and micronutrients on nodulation of groundnut

Results obtained from the statistical analysis of the effects of lime, phosphorus, potassium and micronutrients on nodulation of groundnut as presented in Table 4.2 shows that the sole application of lime and phosphorus had statistical significance on nodule number at $P \leq 0.01$. However, the application of potassium and micronutrients was not significant on nodulation. A similar trend was observed on nodule dry weight where the application of lime and phosphorus had significant effect on nodule dry weight while there was no significant effect of the application of potassium and micronutrients.

The interaction effects of lime, phosphorus, potassium and micronutrients on number of nodules as presented in Table 4.3 shows that the four way interaction of lime, P, K and micronutrients was significant ($P \leq 0.01$). This interaction shows that the combination of lime, P, K and micronutrients gave the highest nodule number and is observed to be of most

relevance in improving nodule number on the experimental soil. For the nodule dry weight as shown on Table 4.4, the interaction of P, K and micronutrients shows that the combination of these three factors gave the highest nodule dry weight. The addition of lime in the interaction of lime and K despite being significant had a lower value for nodule dry weight compared to the combined application of P, K and micronutrients.

4.3.2 Effects of lime, phosphorus, potassium and micronutrients on biomass of groundnut

The three way interaction of lime, potassium and micronutrients was the only significant interaction on root dry weight at $P \leq 0.05$. As shown on Figure 4.1, this interaction shows that the combination of potassium and micronutrients without lime gave the highest root dry weight. Also, the combination of lime and K without micronutrients gave a statistically similar root dry weight.

As observed in the results presented in Table 4.5, the interaction effects of lime and phosphorus ($P \leq 0.01$) gave significantly highest shoot dry weight. In this interaction, the combination of lime and phosphorus was statistically highest compared to other combinations. The third order interaction of lime, K and micronutrients further shows the importance of lime to the shoot dry weight. The addition of lime with and without K and micronutrients gave the highest shoot dry weight. However, the addition of K without lime and micronutrients gave a similar result.

The results in Table 4.2 indicate that there was no significant difference on root dry weight by sole application of lime and micronutrients, but the application of phosphorus as well as potassium gave significant higher root dry weight. Application of lime and phosphorus resulted in a significant increase in shoot dry weight, while no significant difference was recorded by the application of potassium and micronutrients.

Table 4.2 Effects of Lime, Phosphorus, Potassium and Micronutrients on Nodulation, Biomass and Nitrogen Fixation of Groundnut on the Acidic Soil

Treatment	Number of nodules per plant	Nodule dry weight (g/plant)	Root dry weight (g/plant)	Shoot dry weight (g/plant)	N_2-fixed (kg ha^{-1})	Ndfa (%)	N balance (kg ha^{-1})
Lime (L)							
Plus Lime	249.26[a]	0.22[a]	2.83[a]	28.77[a]	98.71[a]	72.68[a]	58.29[a]
Minus Lime	218.60[b]	0.20[b]	2.82[a]	27.23[b]	85.59[b]	69.73[b]	38.34[b]
Phosphorus (P)							
Plus Phosphorus	251.65[a]	0.24[a]	2.98[a]	29.55[a]	101.62[a]	73.38[a]	56.28[a]
Minus Phosphorus	216.22[b]	0.18[b]	2.68[b]	26.45[b]	82.68[b]	69.03[b]	40.35[b]
Potassium (K)							
Plus Potassium	237.01[a]	0.21[a]	3.01[a]	28.23[a]	94.70[a]	71.90[a]	51.21[a]
Minus Potassium	230.85[a]	0.21[a]	2.64[b]	27.77[a]	89.60[b]	70.51[b]	45.42[b]
Micronutrients (M)							
Plus micronutrients	233.95[a]	0.21[a]	2.90[a]	28.05[a]	95.18[a]	71.92[a]	48.72[a]
Minus micronutrients	233.92[a]	0.21[a]	2.75[a]	27.95[a]	89.12[b]	70.49[b]	47.91[b]
SE	**3.47**	**0.005**	**0.07**	**0.34**	**1.66**	**0.36**	**0.24**
SEM	**4.91**	**0.007**	**0.10**	**0.48**	**2.34**	**0.51**	**0.34**
Interactions							
L x P	*	NS	NS	**	*	*	**

L x K	*	*	NS	NS	NS	NS	**
L x M	NS	NS	NS	NS	**	**	**
P x K	*	*	NS	NS	NS	NS	**
P x M	NS	NS	NS	**	*	*	*
K x M	NS	*	NS	NS	*	*	**
L x P x K	NS	NS	NS	NS	*	*	**
L x P x M	*	NS	NS	NS	NS	NS	**
L x K x M	*	NS	*	*	NS	NS	**
P x K x M	*	*	NS	NS	NS	NS	**
L x P x K x M	**	NS	NS	NS	NS	NS	**

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01 and NS- Not Significant at P≤0.05.
Ndfa=Amount of nitrogen derived from the atmosphere; SE = Standard Error; SEM = Standard Error of Mean

Table 4.3 Interaction Effect of Lime, Phosphorus, Potassium and Micronutrients on Number of Nodules per Plant of Groundnut

LxP*		
	+P	**-P**
+L	260.1[a]	238.42[b]
-L	243.19[b]	194.03[c]
LxK*		
	+K	**-K**
+L	263.15[a]	235.38[b]
-L	210.88[c]	226.33[b]
PxK*		
	+K	**-K**
+P	261.13[a]	242.17[b]
-P	212.3[c]	219.54[c]
SEM	**±6.93**	

LxKxM*				
	+K+M	**+K-M**	**-K+M**	**-K-M**
+L	272.88[a]	253.42[ab]	221.25[b]	249.5[ab]
-L	208.58[bc]	213.17[bc]	233.08[b]	219.58[bc]
PxKxM*				
	+K+M	**+K-M**	**-K+M**	**-K-M**
+P	270.08[a]	252.17[a]	230.42[b]	253.92[a]
-P	211.38[b]	214.42[b]	223.92[b]	215.17[b]

LxPxM*	+P+M	+P-M	-P+M	-P-M
+L	246.63^{ab}	273.58^{a}	247.5^{ab}	229.33^{ab}
-L	253.88^{a}	232.5^{ab}	187.79^{c}	200.25^{c}
SEM	**±9.80**			

LxPxKxM**	+P+K+M	+P+K-M	+P-K+M	+P-K-M	-P+K+M	-P+K-M	-P-K+M	-P-K-M
+L	296.58^{a}	261.25^{b}	196.67^{cd}	285.92^{a}	249.17^{b}	245.58^{b}	245.83^{b}	213.08^{c}
-L	243.58^{bc}	243.08^{bc}	264.17^{b}	221.92^{c}	173.58^{e}	183.25^{cd}	202^{c}	217.25^{c}
SEM	**±13.86**							

L=Lime; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean
Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

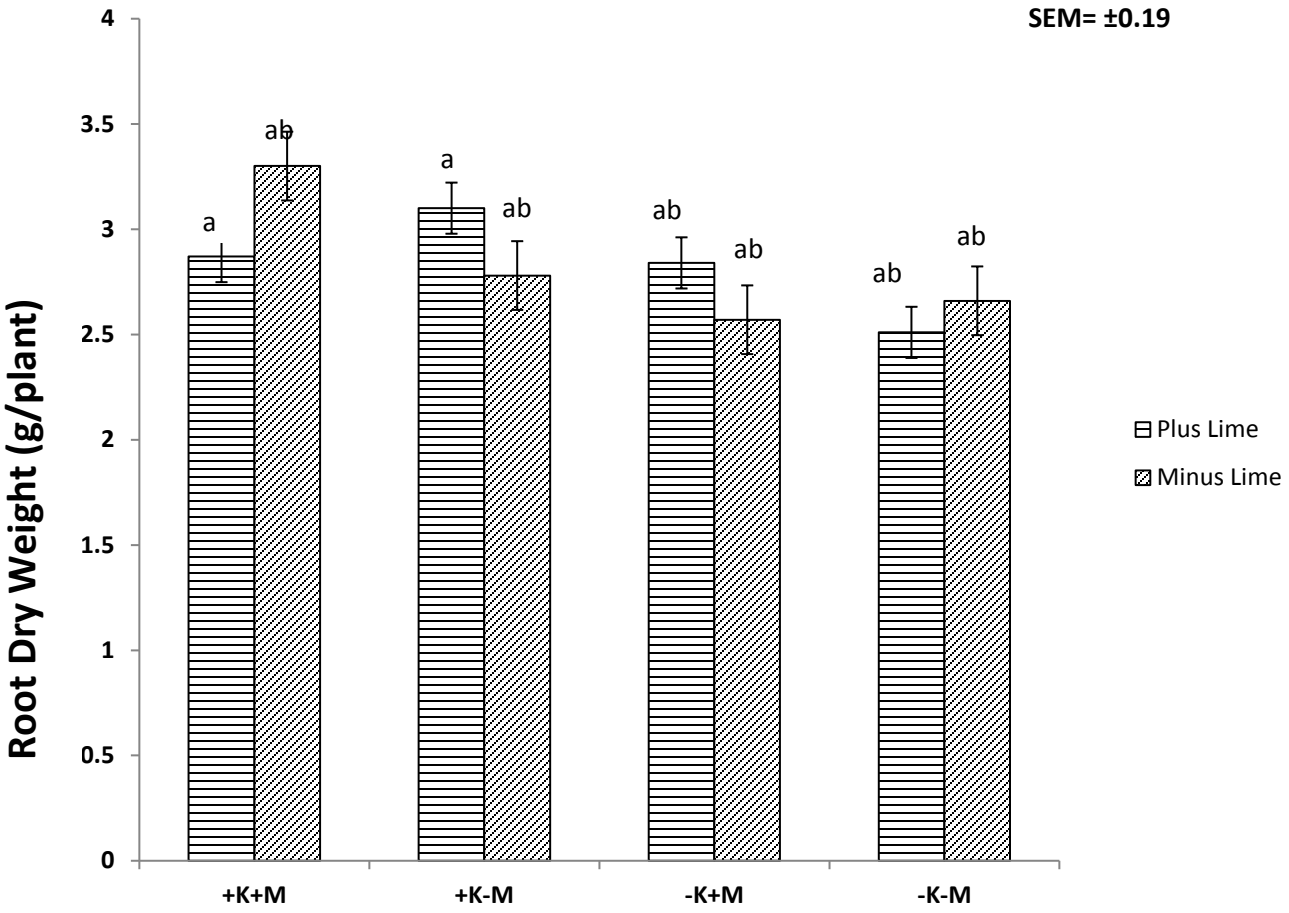
Table 4.4 Interaction Effect of Lime, Phosphorus, Potassium and Micronutrients on Nodule Dry weight (g/plant) of Groundnut

LxK*	+K	-K
+L	0.23^{a}	0.21^{ab}
-L	0.19^{b}	0.20^{b}
PxK*	**+K**	**-K**
+P	0.26^{a}	0.22^{b}
-P	0.17^{cd}	0.19^{c}
KxM*	**+M**	**-M**
+K	0.22^{a}	0.20^{ab}
-K	0.19^{b}	0.22^{a}
SEM	**±0.01**	

PxKxM*	+K+M	+K-M	-K+M	-K-M
+P	0.29^{a}	0.23^{b}	0.20^{bc}	0.23^{b}
-P	0.16^{cd}	0.18^{b}	0.19^{c}	0.20^{bc}
SEM	**±0.02**			

L=Lime; M=Micronutrients; K=Potassium; P=Phosphorus. SEM=Standard Error of Mean.

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.



K = Potassium

M = Mironutrients

SEM=Standard Error of Mean

Figure 4.1 Interaction Effects of Lime, Potassium and Micronutrients on Root Dry Weight (g/plant) of Groundnut

Table 4.5 Interaction Effect of Lime, Phosphorus, Potassium and Micronutrients on Shoot Dry Weight (g/plant) of Groundnut

LxP**	**+P**	**-P**		
+L	31.76^{a}	25.76^{b}		
-L	27.34^{b}	27.12^{bc}		
PxM**	**+M**	**-M**		
+P	27.5^{b}	28.39^{b}		
-P	31.6^{a}	24.5^{c}		
SEM	**±0.68**			
LxKxM*	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+L	29.58^{a}	26.81^{ab}	28.73^{a}	29.95^{a}
-L	26.57^{ab}	28.1^{a}	26.91^{ab}	27.32^{ab}
SEM	**±0.96**			

L=Lime; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean. Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

4.3.3 Effects of lime, phosphorus, potassium and micronutrients on soil nitrogen balance by groundnut

The results in Table 4.6 indicates that in the fourth order interaction of lime, P, K and micronutrients, the addition of lime had a negative influence on the soil N balance. The highest soil N balance was obtained with the combination of P and micronutrients. Similarly, the addition of K and micronutrients without P and lime gave a statistically similar value. As observed in other interactions presented on Table 4.6, addition of P tends to favour the soil N balance. In addition, micronutrients addition is observed to be of major influence when not in combination with lime. Therefore it can be deduced that the application of P and micronutrients is important to obtaining higher soil N on the experimental soil.

The result on Table 4.2 shows that sole application of lime, P, K and micronutrients significantly influenced the soil nitrogen balance on the experimental soil.

4.3.4 Effects of lime, phosphorus, potassium and micronutrients on amount of nitrogen fixed by groundnut

The results in Table 4.8 on the interaction effects of lime, P, K and micronutrients on the amount of N_2 fixed shows that the third order interaction of lime, P and K was significant and further reveals that the addition of P without lime and K gave the highest amount of N_2 fixed. The importance of P to N_2 fixation was also observed in the two way interactions of lime and P as well as that of P and micronutrients. It is therefore inferred that the addition of P is of greater importance for N_2 fixation in groundnut on the experimental soil. The results presented in Table 4.2 shows that the sole application of lime and phosphorus was significant at $P \leq 0.01$ while potassium and micronutrients addition was significant at $P \leq 0.05$ on the amount of N_2 fixed by groundnut.

Table 4.6 Interaction Effect of Lime, Phosphorus, Potassium and Micronutrients on Soil Nitrogen Balance (kg N/ha)

LxP**	+P	-P
+L	48[c]	28.7[d]
-L	64.6[a]	52.0[b]
LxK**	**+K**	**-K**
+L	40.2[c]	36.5[d]
-L	62.3[a]	54.3[b]
LxM**	**+M**	**-M**
+L	30.1[d]	46.6[c]
-L	67.3[a]	49.2[b]
SEM	**±0.48**	

LxPxK**	+P+K	+P-K	-P+K	-P-K
+L	50.2[c]	45.8[d]	30.1[f]	27.3[g]
-L	61.6[b]	67.5[a]	63.0[b]	41.4[e]
LxPxM*	**+P+M**	**+P-M**	**-P+M**	**-P-M**
+L	31.4[f]	64.6[c]	28.8[g]	28.6[g]
-L	68.5[a]	60.7[d]	66.2[b]	37.8[e]
LxKxM**	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+L	30.3[f]	50.0[d]	29.9[f]	43.2[e]
-L	68.3[a]	56.3[c]	66.4[b]	42.2[e]
PxKxM**	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+P	49.6[b]	62.2[a]	50.2[b]	63.1[a]
-P	49.0[b]	44.0[d]	46.0[c]	22.4[e]
SEM	**±0.68**			

LxPxKxM**	+P+K+M	+P+K-M	+P-K+M	+P-K-M	-P+K+M	-P+K-M	-P-K+M	-P-K-M
+L	38.1[e]	62.4[c]	24.7[g]	66.8[b]	22.6[h]	37.6[e]	35.1[f]	19.6[i]
-L	61.2[c]	62[c]	75.8[a]	59.3[bc]	75.4[a]	50.5[d]	57[c]	25.2[g]
SEM	**±0.96**							

L=Lime; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean. Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

Table 4.7 Interaction Effect of Lime, Phosphorus, Potassium and Micronutrients on Amount of Nitrogen Fixed (kg N ha^{-1}) by Groundnut

LxP*				
	+P	**-P**		
+L	99.04[a]	72.14[b]		
-L	104.2[a]	93.22[ab]		
LxM**				
	+M	**-M**		
+L	79.78[c]	91.4[b]		
-L	103.58[a]	86.83[b]		
KxM*				
	+M	**-M**		
+K	94.58[a]	94.82[a]		
-K	95.78[a]	83.43[b]		
PxM*				
	+M	**-M**		
+P	100.17[a]	103.08[a]		
-P	90.19[b]	75.17[c]		
SEM	**±3.31**			
LxPxK*				
	+P+K	**+P-K**	**-P+K**	**-P-K**
+L	103.8[a]	94.28[ab]	70.28[bc]	74.0[b]
-L	101.27[ab]	107.13[a]	103.43[a]	83.0[b]
SEM	**±4.69**			

L=Lime; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean. Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

4.3.5 Effects of lime, phosphorus, potassium and micronutrients on amount of nitrogen derived from the atmosphere by groundnut

The results in Table 4.7 on the interaction effects of lime, P, K and micronutrients on the amount of N derived from the atmosphere (Ndfa) show that the third order interaction of lime, P and K was significant. This interaction further showed that the addition of P without lime and K gave the highest Ndfa. Statistically similar to this was the addition of P and K with and without lime. In addition, the two way interaction between lime and P revealed that the addition of P with or without lime gave similar amount of Ndfa. This explains that addition of lime have no effect on the Ndfa while addition of P is most important for N_2 fixation. Micronutrients addition without lime showed significantly higher Ndfa in the two way interaction of lime and micronutrients. The importance of micronutrients to the Ndfa was however not consistent in most interactions observed.

From the result presented in Table 4.2, it was observed that the application of lime and phosphorus showed significant ($P \leq 0.01$) effects on the Ndfa by groundnut, while the application of potassium and micronutrients was significant at $P \leq 0.05$.

Table 4.8 Interaction Effect of Lime, Phosphorus, Potassium and Micronutrients on Amount of Nitrogen (%) Derived from the Atmosphere by Groundnut

LxP*	**+P**	**-P**		
+L	72.83[a]	66.63[b]		
-L	73.93[a]	71.43[ab]		
LxM**	**+M**	**-M**		
+L	68.56[bc]	70.91[b]		
-L	73.28[a]	70.07[b]		
KxM*	**+M**	**-M**		
+K	71.78[a]	72.03[a]		
-K	72.07[a]	68.97[b]		
PxM*	**+M**	**-M**		
+P	73.06[a]	73.69[a]		
-P	70.76[b]	67.28[c]		
SEM	**±0.73**			
LxPxK*	**+P+K**	**+P-K**	**-P+K**	**-P-K**
+L	73.95[a]	71.72[b]	66.02[cd]	67.25[c]
-L	73.67[a]	74.18[a]	73.97[a]	68.88[c]
SEM	**±1.01**			

L=Lime; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean. Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

4.4 Effects of Lime, Phosphorus, Potassium and Micronutrients on Yield and Yield Components of Groundnut on the Acidic Soil

4.4.1 Effects of lime, phosphorus, potassium and micronutrients on 100 seed weight of groundnut

The results in Table 4.9 indicate that the sole addition of lime and potassium had no statistical significance on one hundred seed weight of groundnut. However, the application of phosphorus and micronutrients was significant at $P \leq 0.05$.

The result of the interaction of lime, phosphorus, potassium and micronutrients on the 100 seed weight of groundnut is presented in Table 4.10. From the result obtained, the third order interaction of lime, phosphorus and micronutrients on 100 seed weight was significant ($P \leq 0.01$). Phosphorus in the absence of lime and micronutrients gave the highest seed weight but was statistically similar to the effect observed with the combination of lime and phosphorus without micronutrients. From this interaction, the effect of phosphorus on the 100 seed weight was more pronounced as the highest value was obtained with +P while with or without lime had no effect while the addition of micronutrients reduced the seed weight. This was also evident in the main effects and other significant interactions that the addition of P improved the 100 seed weight as compared to the addition of micronutrients which is of less importance and not consistent.

Table 4.9 Effects of Lime, Phosphorus, Potassium and Micronutrients on Yield and Yield Components of Groundnut on the Acidic Soil

Treatments	100 seed weight (g)	Shelling %	Pod yield (kg ha^{-1})	Grain Yield (kg ha^{-1})	Haulm Yield (kg ha^{-1})	Harvest Index (%)
Lime (L)						
Plus Lime	35.54[a]	67.96[a]	2993.7[a]	2035.3[a]	4910.5[a]	68[a]
Minus Lime	33.5[a]	66.7[b]	2703.1[a]	1763.8[b]	4420.6[b]	56[b]
Phosphorus (P)						
Plus Phosphorus	34.08[a]	67.67[a]	2926.7[a]	1983.0[a]	5038.4[a]	65[a]
Minus Phosphorus	32.96[b]	66.46[b]	2770.2[b]	1816.1[b]	4292.7[b]	59[b]
Potassium (K)						
Plus Potassium	33.71[a]	67.17[a]	2919.3[a]	1935.0[a]	4724.8[a]	63[a]
Minus Potassium	33.33[a]	66.96[a]	2777.5[b]	1864.1[b]	4606.3[a]	61[a]
Micronutrients (M)						
Plus micronutrients	33.92[a]	68.13[a]	2997.0[a]	1956.6[a]	4808.8[a]	63[a]
Minus micronutrients	33.13[b]	66.0[b]	2699.8[b]	1842.4[b]	4522.4[b]	61[a]
SE	**0.18**	**0.22**	**24.81**	**11.03**	**59.78**	**1.0**
SEM	**0.25**	**0.31**	**35.08**	**15.60**	**84.54**	**1.4**
Interactions						
L x P	NS	NS	**	**	*	**
L x K	**	*	NS	NS	NS	*
L x M	*	**	**	**	*	**
P x K	NS	NS	**	**	*	**
P x M	*	*	NS	NS	*	NS
K x M	NS	NS	*	**	NS	NS
L x P x K	NS	*	*	**	**	*
L x P x M	**	NS	NS	*	*	*
L x K x M	NS	*	*	*	NS	*
P x K x M	*	NS	NS	NS	NS	NS
L x P x K x M	NS	NS	**	**	NS	**

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01 and NS- Not Significant at P≤0.05. SE=Standard Error; SEM=Standard Error of Mean.

Table 4.10 Interaction Effect of Lime, Phosphorus, Potassium and Micronutrients on 100 Seed Weight (g) of Groundnut

LxK**					
	+K	**-K**			
+L	32.75^{b}	34.33^{a}			
-L	33.92^{a}	33.08^{b}			
LxM*					
	+M	**-M**			
+L	32.83^{bc}	34.25^{a}			
-L	33.41^{b}	33.58^{b}			
PxM*					
	+M	**-M**			
+P	33.25^{b}	34.92^{a}			
-P	33.0^{b}	32.92^{bc}			
SEM	**±0.35**				
PxKxM*					
	+K+M	**+K-M**	**-K+M**	**-K-M**	
+P	32.5^{b}	35.33^{a}	34.0^{ab}	34.5^{a}	
-P	33.0^{ab}	32.5^{b}	33.0^{ab}	33.33^{ab}	
LxPxM**					
	+P+M	**+P-M**	**-P+M**	**-P-M**	
+L	33.8^{b}	34.5^{a}	31.83^{c}	34.0^{b}	
-L	32.67^{c}	35.33^{a}	34.17^{b}	31.8^{c}	
SEM	**±0.5**				

L=Lime; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean. Means with the same letter(s) within a treatment are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

4.4.2 Effects of lime, phosphorus, potassium and micronutrients on shelling percentage of groundnut

The result on the interaction of lime, phosphorus, potassium and micronutrients on the shelling percentage of groundnut is presented in Table 4.11. From the third order interaction between lime, potassium, and micronutrients, the addition of lime together with micronutrients appears to be the most important interaction. This points out that the combined application of lime and micronutrients in this experimental soil is paramount to obtaining higher shelling percentage. However, this effect was counteracted because a statistically similar result was obtained in the absence of potassium, lime and micronutrients. Therefore, it can be concluded that the application of lime, K and micronutrients had no influence on shelling percentage. Also, the interaction of lime, P and K showed that application of lime with and without P and K had the statistically highest values. In addition, the application of lime did not show a consistent trend. It can therefore be said that aside lime to a little extent, none of the combinations of the inputs had a consistent influence on the shelling percentage of groundnut on the experimental soil.

The result in Table 4.9 shows that the application of lime and micronutrients was significant on the shelling percentage at $P \leq 0.01$. Phosphorus application was significant. However, sole application of potassium had no significant effect on the shelling percentage of groundnut.

Table 4.11 Interaction Effect of Lime, Phosphorus, Potassium and Micronutrients on Shelling Percentage (%) of Groundnut

LxK*				
	+K	**-K**		
+L	67.42[b]	68.5[a]		
-L	66.92[b]	65.42[c]		
LxM**				
	+M	**-M**		
+L	67.83[a]	68.08[a]		
-L	64.17[b]	68.17[a]		
PxM*				
	+M	**-M**		
+P	67.17[ab]	68.17[a]		
-P	64.83[b]	68.08[a]		
SEM	**±0.44**			
LxPxK*				
	+P+K	**+P-K**	**-P+K**	**-P-K**
+L	68.17[a]	68.5[a]	66.67[ab]	68.5[a]
-L	67[ab]	67.0[ab]	66.83[b]	63.83[c]
LxKxM*				
	+K+M	**+K-M**	**-K+M**	**-K-M**
+L	66.83[ab]	68.0[a]	68.83[a]	68.17[a]
-L	65.17[c]	68.67[a]	63.17[d]	67.67[a]
SEM	**±0.63**			

L=Lime; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean. Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

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4.4.3 Effects of lime, phosphorus, potassium and micronutrients on pod yield of groundnut

The result on the interaction of lime, phosphorus, potassium and micronutrients on pod yield of groundnut is presented in Table 4.12. The four way interaction between lime, P, K and micronutrients was highly significant. This interaction shows that the addition of lime, P and K without micronutrients had the statistically highest pod yield. It was observed that the addition of micronutrients generally reduced pod yield across all the interactions found to be significant. It is therefore important to note that the addition of micronutrients had negative effect on the pod yield as the yields tend to reduce where it was applied. This indicates that the addition of lime, phosphorus and potassium are important for improving the pod yield of groundnut on the experimental soil.

On the main effect, the result as shown in Table 4.9 indicates that the sole application of lime, phosphorus, potassium and micronutrients had significant effect on the pod yield.

4.4.4 Effects of lime, phosphorus, potassium and micronutrients on grain yield of groundnut

The result in Table 4.13 shows that the fourth order interaction of lime, phosphorus, potassium and micronutrients on grain yield was observed to be significant ($P \leq 0.01$). As observed, the combination of lime, P and K minus micronutrients gave the statistically highest grain yield. The addition of micronutrients tends to decrease the grain yield in some interactions. Similarly, in the third order interaction of lime P and K, the combined application of these three factors gave the highest grain yield. It can therefore be said that the combination of lime, phosphorus and potassium is of more importance on grain yield.

The result on the main effects as shown in Table 4.9 shows that the sole application of lime, phosphorus, potassium and micronutrients showed significant response ($P \leq 0.01$) on grain yield of groundnut.

Table 4.12 Interaction Effect of Lime, Phosphorus, Potassium and Micronutrients on Pod Yield (kg ha^{-1}) of Groundnut

LxP**	**+P**	**-P**
+L	3233.8^{a}	2753.5^{b}
-L	2619.4^{c}	2786.8^{b}
LxM**	**+M**	**-M**
+L	2935.4^{b}	3051.9^{a}
-L	3058.0^{a}	2347.7^{c}
PxK**	**+K**	**-K**
+P	2985.1^{a}	2868.2^{ab}
-P	2570.0^{b}	2970.3^{a}
KxM*	**+M**	**-M**
+K	2966.6^{a}	2588.5^{c}
-K	3027.4^{a}	2811.1^{b}
SEM	**±49.62**	

LxPxK*	**+P+K**	**+P-K**	**-P+K**	**-P-K**
+L	3356.5^{a}	3111.2^{b}	2533.3^{c}	2973.7^{b}
-L	2613.7^{c}	2625.2^{c}	2606.7^{c}	2967.0^{b}
LxKxM*	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+L	2986.3^{b}	2903.5^{b}	2884.5^{b}	3200.3^{a}
-L	2946.8^{b}	2273.5^{c}	3270.3^{a}	2421.8^{c}
SEM	**±70.17**			

LxPxKxM**	**+P+K+M**	**+P+K-M**	**+P-K+M**	**+P-K-M**	**-P+K+M**	**-P+K-M**	**-P-K+M**	**-P-K-M**
+L	3188.3^{b}	3524.7^{a}	3072.0^{b}	3150.3^{b}	2784.3^{c}	2282.3^{d}	2697.0^{c}	3250.3^{b}
-L	3106.0^{b}	2121.3^{e}	2814.7^{c}	2435.7^{d}	2787.7^{c}	2425.7^{d}	3026.0^{b}	2408^{d}
SEM	**±99.24**							

L=Lime; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean. Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

Table 4.13 Interaction Effects of Lime, Phosphorus, Potassium and Micronutrients on Grain Yield (kg ha^{-1}) of Groundnut

LxP**	**+P**	**-P**
+L	2209.1^{a}	1861.5^{b}
-L	1756.9^{c}	1770.6^{c}

LxM**	**+M**	**-M**
+L	1992.7^{b}	2077.9^{a}
-L	1920.5^{c}	1607.0^{d}

PxK**	**+K**	**-K**
+P	2016.1^{a}	1949.9^{b}
-P	1712.0^{c}	1920.1^{b}

KxM**	**+M**	**-M**
+K	1960.1^{a}	1768.1^{b}
-K	1953.2^{a}	1916.8^{a}

SEM **±22.06**

LxPxK**	**+P+K**	**+P-K**	**-P+K**	**-P-K**
+L	2287.8^{a}	2130.4^{b}	1685.5^{e}	2039.5^{c}
-L	1744.5^{d}	1769.3^{d}	1738.5^{d}	1802.7^{d}

LxKxM*	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+L	1999.1^{b}	1974.1^{b}	1986.3^{b}	2181.6^{a}
-L	1921.0^{bc}	1562.0^{d}	1920.0^{bc}	1652.0^{c}

LxPxM*	**+P+M**	**+P-M**	**-P+M**	**-P-M**
+L	2154.2^{b}	2264.0^{a}	1831.3^{cd}	1891.7^{c}
-L	1939.3^{c}	1574.5^{d}	1901.7^{c}	1639.5^{d}

SEM **±31.19**

LxPxKxM**	**+P+K+M**	**+P+K-M**	**+P-K+M**	**+P-K-M**	**-P+K+M**	**-P+K-M**	**-P-K+M**	**-P-K-M**
+L	2178.7^{b}	2396.8^{a}	2129.6^{bc}	2131.2^{bc}	1819.5^{c}	1551.5^{de}	1843.0^{c}	2232.0^{b}
-L	2039.4^{bc}	1449.7^{e}	1839.3^{c}	1699.3^{d}	1802.7^{c}	1674.3^{d}	2000.7^{bc}	1604.7^{d}
SEM	**±44.12**							

L=Lime; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean. Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

4.4.5 Effects of lime, phosphorus, potassium and micronutrients on haulm yield of groundnut

The result in Table 4.14 shows that the interaction of lime, P and K on haulm yield gave a highly significant relationship. In this interaction, it was observed that the addition of P without K and lime gave the highest haulm yield. Also, in the third order interaction of lime, P and micronutrients, the addition of P with or without micronutrients minus lime gave the highest haulm yield. In addition, the presence of P was observed to favour the haulm yield of groundnut in all interactions where there was its addition with or without micronutrients. It can therefore be inferred that the addition of P is the major factor that improved the haulm yield of groundnut in the experimental soil.

As observed on the main effect in Table 4.9, the sole application of lime and P had highly significant effect on the haulm yield of groundnut. The application of micronutrients showed significant effect while the application of potassium however, showed no significance on the haulm yield.

4.4.6 Effects of lime, phosphorus, potassium and micronutrients on harvest index of groundnut

The result in Table 4.15 shows that in the four way interaction of lime, P, K and micronutrients, the highest value for harvest index was obtained by the application of micronutrients without lime, P and K. However, as observed in other interactions, the gains attributed to micronutrients application was not consisitent. In most cases, statistically similar values were obtained with or without micronutrients. Also in the fourth order interaction, the statistically highest value for harvest index was obtained by the addition of lime without P, K and micronutrients. This indicates the importance of lime application in determining harvest index on the experimental soil.

Table 4.14 Interaction Effect of Lime, Phosphorus, Potassium and Micronutrients on Haulm Yield (kg ha^{-1}) of Groundnut

LxP*				
	+P	**-P**		
+L	4702.8^{b}	4138.4^{d}		
-L	5374.0^{a}	4447.0^{c}		
LxM*				
	+M	**-M**		
+L	4463.1^{b}	4378.2^{ab}		
-L	5154.4^{a}	4666.6^{b}		
PxK*				
	+K	**-K**		
+P	4857.3^{b}	5219.5^{a}		
-P	4355.3^{c}	4230.2^{c}		
PxM**				
	+M	**-M**		
+P	5093.8^{a}	4983.0^{a}		
-P	4523.7^{b}	4061.8^{c}		
SEM	**±119.55**			
LxPxK**	**+P+K**	**+P-K**	**-P+K**	**-P-K**
+L	4705.5^{ab}	4700.2^{ab}	3872.7^{bc}	4404.2^{ab}
-L	5009.2^{a}	5138.8^{a}	4837.8^{a}	4056.2^{bc}
LxPxM*	**+P+M**	**+P-M**	**-P+M**	**-P-M**
+L	4476.8^{b}	4928.8^{a}	4449.3^{b}	3827.5^{c}
-L	5110.8^{a}	5037.2^{a}	4598.0^{b}	4296.0^{b}
SEM	**±169.07**			

L=Lime; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean. Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

Table 4.15 Interaction Effect of Lime, Phosphorus, Potassium and Micronutrients on Harvest Index (%) of Groundnut

LxP**	**+P**	**-P**
+L	69[a]	67[a]
-L	49[b]	63[ab]
LxK*	**+K**	**-K**
+L	69[a]	68[a]
-L	53[c]	59[b]
LxM**	**+M**	**-M**
+L	66[b]	71[a]
-L	61[c]	51[d]
PxK**	**+K**	**-K**
+P	62[b]	56[bc]
-P	60[b]	71[a]
SEM	**±2.0**	

LxPxK*	**+P+K**	**+P-K**	**-P+K**	**-P-K**
+L	72[a]	67[ab]	65[ab]	68[a]
-L	52[b]	46[bc]	54[b]	73[a]
LxPxM*	**+P+M**	**+P-M**	**-P+M**	**-P-M**
+L	70[a]	69[a]	62[b]	72[a]
-L	52[bc]	45[c]	70[a]	57[b]
LxKxM*	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+L	69[a]	69[a]	62[ab]	73[a]
-L	56[b]	50[bc]	67[ab]	52[b]
SEM	**±3.2**			

LxPxKxM**	**+P+K+M**	**+P+K-M**	**+P-K+M**	**+P-K-M**	**-P+K+M**	**-P+K-M**	**-P-K+M**	**-P-K-M**
+L	71[b]	74[b]	68[b]	65[bc]	67[b]	64[bc]	57[bc]	80[a]
-L	56[bc]	47[bc]	48[bc]	44[bc]	55[bc]	53[bc]	86[a]	60[bc]
SEM	**±4.1**							

L=Lime; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean. Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

4.5 Effects of Organic Manure, Phosphorus, Potassium and Micronutrients on Nodulation, Biomass and Nitrogen Fixation of Groundnut on the Non-Acidic Soil

4.5.1 Effects of cow dung, phosphorus, potassium and micronutrients on nodulation of groundnut

The results in Table 4.17 reveal that the third order interaction of cow dung, K and micronutrients on nodule number shows that the combined application of cow dung, K and micronutrients gave the highest nodulation. It can be inferred that the combined application of cow dung, K and micronutrients is more important for obtaining higher nodule number on the experimental soil.

The three way interaction of cow dung, K and micronutrients on nodule dry weight as shown in Table 4.18 was significant and indicates that the combined application of cow dung, K and micronutrients gave the highest nodule dry weight relative to other combinations. Therefore this combination favoured the nodule dry weight most on the experimental soil.

The results on the statistical analysis on effects of cow dung, phosphorus, potassium and micronutrients on nodulation, biomass and nitrogen fixation in groundnut on the non-acidic soil as presented in Table 4.16 indicates that the sole application of cow dung and phosphorus were highly significant on nodulation (nodule number and dry weight). Micronutrients application was significant. Addition of potassium did not show significant effect on nodulation.

Table 4.16 Effects of Organic Manure, Phosphorus, Potassium and Micronutrients on Nodulation and Biomass and Nitrogen Fixation of Groundnut on the Non-acidic Soil

Treatments	Number of nodules per plant	Nodule dry weight (g/plant)	Root dry weight (g/plant)	Shoot dry weight (g/plant)	Ndfa (%)	N_2-fixed (kg ha^{-1})	Nbalance (kg ha^{-1})
Cow dung (CD)							
Plus cow dung	234.27[a]	0.19[a]	2.02[a]	22.32[a]	78.72[a]	81.9[a]	46.19[a]
Minus cow dung	186.74[b]	0.15[b]	1.90[a]	20.71[a]	74.99[b]	67.97[b]	38.2[b]
Phosphorus (P)							
Plus Phosphorus	228.13[a]	0.18[a]	1.96[a]	22.56[a]	77.75[a]	78.51[a]	45.91[a]
Minus Phosphorus	192.89[b]	0.16[b]	1.95[a]	20.47[b]	75.96[b]	71.35[b]	38.48[b]
Potassium (K)							
Plus Potassium	214.17[a]	0.17[a]	1.96[a]	21.55[a]	77.38[a]	77.25[a]	45.97[a]
Minus Potassium	206.84[a]	0.17[a]	1.94[a]	21.48[a]	76.33[b]	72.62[b]	38.43[b]
Micronutrients (M)							
Plus micronutrients	217.33[a]	0.18[a]	1.97[a]	22.07[a]	78.33[a]	80.32[a]	47.90[a]
Minus micronutrients	203.68[b]	0.16[b]	1.94[a]	20.96[a]	75.38[b]	69.55[b]	36.50[b]
SE	**3.34**	**0.004**	**0.071**	**0.67**	**1.10**	**0.45**	**0.27**
SEM	**4.73**	**0.006**	**0.10**	**0.95**	**1.55**	**0.63**	**0.32**
Interactions							
CD x P	*	*	NS	NS	**	**	**
CD x K	NS	NS	NS	NS	NS	NS	*
CD x M	NS	NS	NS	NS	**	NS	*
P x K	NS	NS	NS	NS	**	**	**
P x M	*	NS	*	NS	*	**	*
K x M	*	*	*	NS	**	**	**
CD x P x K	*	**	NS	NS	**	**	**
CD x P x M	NS	NS	NS	NS	*	*	**
CD x K x M	*	*	NS	NS	**	**	**
P x K x M	NS	NS	NS	NS	**	**	**
CD x P x K x M	NS	NS	NS	NS	**	**	**

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01 and NS- Not Significant at P≤0.05. SE=Standard Error; SEM=Standard Error of Mean.

Ndfa=Amount of nitrogen derived from the atmosphere.

Table 4.17 Interaction Effects of Cow Dung, Phosphorus, Potassium and Micronutrients on Number of Nodules per Plant of Groundnut

CDxP*	**+P**	**-P**		
+CD	256.71[a]	211.83[b]		
-CD	199.54[b]	173.94[c]		
KxM*	**+M**	**-M**		
+K	231.19[a]	197.15[d]		
-K	203.48[c]	210.21[b]		
PxM*	**+M**	**-M**		
+P	240.63[a]	215.63[b]		
-P	194.04[c]	191.73[c]		
SEM	**±6.69**			
CDxPxK*	**+P+K**	**+P-K**	**-P+K**	**-P-K**
+CD	257.42[a]	256.0[a]	224.96[b]	198.71[b]
-CD	211.42[b]	187.67[bc]	162.88[c]	185.0[bc]
CDxKxM*	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+CD	267.79[a]	214.58[b]	221.92[b]	232.79[b]
-CD	194.58[bc]	179.71[bc]	185.04[bc]	187.63[bc]
SEM	**±9.45**			

CD=Cow dung; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean.

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

Table 4.18 Interaction Effects of Cow Dung, Phosphorus, Potassium and Micronutrients on Nodule Dry Weight (g/plant) of Groundnut

CDxP*	**+P**	**-P**
+CD	0.19[a]	0.19[a]
-CD	0.17[b]	0.13[c]
KxM*	**+M**	**-M**
+K	0.19[a]	0.16[bc]
-K	0.17[ab]	0.18[a]
SEM	**±0.009**	

CDxPxK**	**+P+K**	**+P-K**	**-P+K**	**-P-K**
+CD	0.19[a]	0.2[a]	0.21[a]	0.17[ab]
-CD	0.18[ab]	0.16[bc]	0.11[c]	0.15[bc]
CDxKxM*	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+CD	0.22[a]	0.17[b]	0.16[bc]	0.19[b]
-CD	0.15[bc]	0.14[bc]	0.17[b]	0.14[cd]
SEM	**±0.01**			

CD=Cow dung; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean.

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

4.5.2 Effects of cow dung, phosphorus, potassium and micronutrients on biomass of groundnut

The result on interaction effect of cow dung, Phosphorus, Potassium and micronutrients on the root dry weight per plant as observed in Table 4.19 shows that the interaction of P and micronutrients was significant and further revealed that the combination of P and micronutrients gave highest root dry weight. In the interaction of K and micronutrient, the addition of K without micronutrients as well as the addition of micronutrients without K gave statistically similar values It can therefore be concluded that the combination of P and micronutrients is best for obtaining a higher root dry weight on the experimental soil.

The results on the statistical analysis on effects of cow dung, Phosphorus, Potassium and micronutrients on the root dry weight of groundnut as presented in Table 4.16 indicates that the sole application of cow dung, Phosphorus, Potassium and micronutrients had no significant effect on the root dry weight per plant.

The interaction effects of cow dung, phosphorus, potassium and micronutrients showed no significant effect on the shoot dry weight of groundnut in all the possible interactions analysed.

As shown in Table 4.16, the application of phosphorus showed a significant increase in the shoot dry weight per plant. However, sole applications of cow dung, potassium and micronutrients had no significant effect on the shoot dry weight per plant.

Table 4.19 Interaction Effects of Phosphorus, Potassium and Micronutrients on Root Dry weight (g/plant) of Groundnut

KxM*	+M	-M
+K	1.8[ab]	2.09[a]
-K	2.08[a]	1.84[a]
PxM*	**+M**	**-M**
+P	2.09[a]	1.83[a]
-P	1.79[ab]	2.1[a]
SEM	**±0.14**	

M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean.
Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

4.5.3 Effects of cow dung, phosphorus, potassium and micronutrients on amount of nitrogen fixed by groundnut

The result of the four way interaction of cow dung, phosphorus, potassium and micronutrients on amount of nitrogen fixed by groundnut is presented in Table 4.20. The result shows that the combination of cow dung, P, K and micronutrients gave the highest amount of N_2 fixed among all other combinations, and is therefore of paramount importance for improving the amount of N_2 fixed on the experimental soil.

As observed in Table 4.16, the application of cow dung, P, K and micronutrients all had significant effect ($P \leq 0.01$) on the amount of nitrogen fixed by groundnut.

4.5.4 Effects of cow dung, phosphorus, potassium and micronutrients on amount of nitrogen derived from the atmosphere by groundnut

The interaction effects as obtained in Table 4.21 reveals that the four way interaction of cow dung, P, K and micronutrients was highly significant and further shows that the combined application of cow dung, P, K and micronutrients gave the highest Ndfa. However, subsequent interactions shows that the presence or absence of P to some extent does not influence the Ndfa but it still remains important in obtaining higher Ndfa. Therefore, the combined application of cow dung, P, K and micronutrients is of immense importance in achieving higher Ndfa.

The result of the analysis of the effect of cow dung, phosphorus, potassium and micronutrients on amount of nitrogen derived from the atmosphere (NDFA) by groundnut as presented in Table 4.16 shows that the sole application of cow dung, P, K and micronutrients had significant effect ($P \leq 0.01$) on Ndfa.

Table 4.20 Interaction Effects of Cow Dung, Phosphorus, Potassium and Micronutrients on Amount of Nitrogen Fixed (kg N ha^{-1}) by Groundnut

CDxP**	**+P**	**-P**
+CD	81.42[a]	82.38[a]
-CD	75.61[b]	60.33[c]

CDxM**	**+M**	**-M**
+CD	87.22[a]	76.58[b]
-CD	73.42[c]	62.52[d]

PxK**	**+K**	**-K**
+P	73.53[b]	83.49[a]
-P	71.7[b]	71b[c]

KxM**	**+M**	**-M**
+K	82.23[a]	63.0[d]
-K	78.4[b]	76.1[c]
SEM	**±0.89**	

CDxPxK**	**+P+K**	**+P-K**	**-P+K**	**-P-K**
+CD	80.25[ab]	82.58[a]	79.83[ab]	84.93[a]
-CD	66.82[b]	84.4[a]	63.57[c]	57.08[d]

CDxKxM**	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+CD	92.3[a]	67.78[e]	82.13[c]	85.38[b]
-CD	72.17[d]	58.22[f]	74.67[d]	66.82[e]

PxKxM**	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+P	88.52[a]	58.55[e]	82.35[b]	84.63[b]
-P	75.95[c]	67.45[d]	74.45[c]	67.57[d]

CDxPxM*	**+P+M**	**+P-M**	**-P+M**	**-P-M**
+CD	89.1[a]	73.7[d]	85.3[b]	79.5[c]
-CD	81.8[c]	69.5[e]	65.1[f]	55.6[g]
SEM	**±1.26**			

CDxPxKxM**	**+P+K+M**	**+P+K-M**	**+P-K+M**	**+P-K-M**	**-P+K+M**	**-P+K-M**	**-P-K+M**	**-P-K-M**
+CD	94.9[a]	65.6d[e]	83.3[c]	81.9[c]	89.7[b]	70.0[d]	81.0[c]	88.9[b]
-CD	82.1[c]	51.5[f]	81.4[c]	87.4[b]	62.2[e]	64.9[de]	67.9[d]	46.2[g]
SEM	**±1.78**							

CD=Cow dung; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard error of Mean. Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

Table 4.21 Interaction Effects of Cow Dung, Phosphorus, Potassium and Micronutrients on Amount of Nitrogen (%) Derived from the Atmosphere

CDxP**	**+P**	**-P**
+CD	78.53[a]	78.9[a]
-CD	76.96[b]	73.03[c]
CDxM**	**+M**	**-M**
+CD	79.83[a]	77.6[b]
-CD	76.82[c]	73.17[d]
PxK**	**+K**	**-K**
+P	76.34[b]	79.15[a]
-P	76.33[b]	75.6[c]
PxM*	**+M**	**-M**
+P	79.52[a]	75.98[c]
-P	77.13[b]	74.79[d]
SEM	**±0.19**	

CDxPxK**	**+P+K**	**+P-K**	**-P+K**	**-P-K**
+CD	78.13[b]	78.93[a]	78.35[b]	79.45[a]
-CD	74.55[c]	79.37[a]	74.3[c]	71.75[d]
CDxKxM**	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+CD	80.78[a]	75.7[f]	78.88[c]	79.5[b]
-CD	76.47[e]	72.38[h]	77.17[d]	73.95[g]
PxKxM**	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+P	80.1[a]	72.58[f]	78.93[b]	79.37[b]
-P	77.15[c]	75.5[d]	77.12[c]	74.08[e]
CDxPxM*	**+P+M**	**+P-M**	**-P+M**	**-P-M**
+CD	80.17[a]	76.9[d]	79.5[b]	78.3[c]
-CD	78.87[c]	75.05[e]	74.77[e]	71.28[f]
SEM	**±0.27**			

CDxPxKxM**	**+P+K+M**	**+P+K-M**	**+P-K+M**	**+P-K-M**	**-P+K+M**	**-P+K-M**	**-P-K+M**	**-P-K-M**
+CD	81.27[a]	75.0[d]	79.07[c]	78.8[c]	80.3[b]	76.4[d]	78.7[c]	80.2[b]
-CD	78.9[c]	70.17[f]	78.8[c]	79.9[b]	74.0[ef]	74.6[d]	75.53[d]	67.97[g]
SEM	**±0.38**							

CD=Cow dung; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean.

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

4.5.5 Effects of cow dung, phosphorus, potassium and micronutrients on soil nitrogen balance

The result shown in Table 4.22 reveals that in the fourth order interaction of cow dung, P, K and micronutrients, the application of P without cow dung, K and micronutrients gave the highest value for soil N balance. Similar observation was made in the third order interaction of P, K and micronutrients where the highest value was obtained with the addition of P without K and micronutrients. In addition, the third order interaction of cow dung, P and K also shows that the highest soil N balance was obtained where P was applied without cow dung and K. It is therefore deduced that P is the major factor that controls soil N balance in the experimental soil.

The result obtained in Table 4.16 on the main effects shows that the sole applications of cow dung, P, K and micronutrients were significant on the soil N balance.

Table 4.22 Interaction Effects of Cow Dung, Phosphorus, Potassium and Micronutrients on Soil Nitrogen Balance (kg N/ha)

CDxP**	**+P**	**-P**
+CD	45.8[a]	46.6[a]
-CD	46.0[a]	30.4[b]
PxK**	**+K**	**-K**
+P	39.2[b]	52.6[a]
-P	37.6[c]	39.4[b]
PxM*	**+M**	**-M**
+P	52.0[a]	39.8[c]
-P	43.8[b]	33.2[d]
KxM**	**+M**	**-M**
+K	46.9[b]	29.9[d]
-K	48.9[a]	43.1[c]
SEM	**±0.45**	

CDxPxK**	**+P+K**	**+P-K**	**-P+K**	**-P-K**
+CD	43.6[d]	47.9[c]	40.9[e]	52.3[b]
-CD	34.9[e]	57.2[a]	34.3[e]	26.4[f]
CDxPxM**	**+P+M**	**+P-M**	**-P+M**	**-P-M**
+CD	54.4[a]	37.1[d]	49.6[b]	43.6[c]
-CD	49.6[b]	42.5[c]	38.0[d]	22.8[e]
CDxKxM**	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+CD	54.0[a]	30.6[f]	50.1[b]	50.2[b]
-CD	39.9[d]	29.3[f]	47.7[c]	36.0[e]
PxKxM**	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+P	51.6[a]	26.9[e]	52.4[a]	52.7[a]
-P	42.3[c]	33.0[d]	45.3[b]	33.4[d]
SEM	**±0.64**			

CDxPxKxM**	**+P+K+M**	**+P+K-M**	**+P-K+M**	**+P-K-M**	**-P+K+M**	**-P+K-M**	**-P-K+M**	**-P-K-M**
+CD	57.0[b]	30.2[i]	51.8[c]	44.1[g]	50.9[d]	30.9[i]	48.3[e]	56.2[b]
-CD	46.1[f]	23.6[j]	53.0[c]	61.4[a]	33.6[h]	35.0[h]	42.3[g]	10.5[k]
SEM	**±0.91**							

CD=Cow dung; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean.

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

4.6 Effects of Organic Manure, Phosphorus, Potassium and Micronutrients on Yield and Yield Components of Groundnut on the Non-Acidic Soil

4.6.1 Effects of cow dung, phosphorus, potassium and micronutrients on 100 seed weight of groundnut

The result on the interaction effects of cow dung, phosphorus, potassium and micronutrients on the 100 seed weight of groundnut as shown in Table 4.24 reveals that P, K and micronutrients interaction was significant and shows that the combined application of P, K and micronutrients gave the highest 100 seed weight relative to other combinations. It was observed that the presence of micronutrients and K had positive influence on the 100 seed weight in all interactions involving their addition. The addition of cow dung, K and micronutrients was also observed to be influential on the 100 seed weight. The sole application of P and K which showed no significance (Table 4.23) was observed to be more effective when in combination.

The results of the analysis on the effects of cow dung, phosphorus, potassium and micronutrients on 100 seed weight of groundnut as presented in Table 4.23 shows that the application of cow dung as well as micronutrients significantly ($P \leq 0.01$) increased the 100 seed weight of groundnut. The application of P and K had no significant effect on the 100 seed weight of groundnut.

Table 4.23 Effects of Cow Dung, Phosphorus, Potassium and Micronutrients on Yield and Yield Components of Groundnut

Treatment	100 seed weight (g)	Shelling %	Pod yield (kg ha^{-1})	Grain Yield (kg ha^{-1})	Haulm Yield (kg ha^{-1})	Harvest Index (%)
Cow dung (CD)						
Plus cow dung	38.12[a]	71.21[a]	2600.8[a]	1852.6[a]	4698.7[a]	56[a]
Minus cow dung	36.58[b]	68.88[b]	2239.1[b]	1541.7[b]	4238.6[b]	53[b]
Phosphorus (P)						
Plus Phosphorus	37.54[a]	70.42[a]	2449.1[a]	1712.7[a]	4667.2[a]	56[a]
Minus Phosphorus	37.17[a]	69.67[b]	2390.8[b]	1681.6[b]	4270.2[b]	53[b]
Potassium (K)						
Plus Potassium	37.58[a]	70.33[a]	2504.1[a]	1761.4[a]	4477.4[a]	56[a]
Minus Potassium	37.13[a]	69.75[a]	2335.9[b]	1632.9[b]	4459.9[a]	53[b]
Micronutrients (M)						
Plus micronutrients	38.08[a]	70.54[a]	2458.0[a]	1712.3[a]	4582.8[a]	55[a]
Minus micronutrients	36.63[b]	69.54[b]	2382.0[b]	1682.0[b]	4354.5[b]	53[b]
SE	**0.21**	**0.24**	**11.89**	**10.71**	**23.84**	**0.2**
SEM	**0.29**	**0.34**	**16.81**	**15.15**	**33.71**	**0.3**
Interactions						
CD x P	NS	**	NS	*	NS	NS
CD x K	NS	*	NS	NS	*	NS
CD x M	NS	NS	NS	NS	*	*
P x K	NS	NS	NS	NS	*	**
P x M	**	**	**	**	*	**
K x M	**	*	**	**	**	NS
CD x P x K	*	NS	**	**	*	**
CD x P x M	NS	NS	*	*	**	**
CD x K x M	*	NS	**	**	NS	**
P x K x M	*	**	**	NS	**	**
CD x P x K x M	NS	*	**	**	**	**

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01 and NS- Not Significant at P≤0.05. SE=Standard Error; SEM=Standard Error of Mean.

Table 4.24 Interaction Effects of Cow Dung, Phosphorus, Potassium and Micronutrients on 100 Seed Weight (g) of Groundnut

KxM**		
	+M	**-M**
+K	39.0[a]	36.17[c]
-K	37.17[b]	37.08[b]
PxM**		
	+M	**-M**
+P	39.42[a]	35.67[c]
-P	36.75[b]	37.58[b]
SEM	**±0.41**	

CDxPxK*				
	+P+K	**+P-K**	**-P+K**	**-P-K**
+CD	39.0[a]	38.17[a]	37.5[b]	37.83[a]
-CD	36.0[bc]	37.0[b]	37.83[a]	35.5[cd]
CDxKxM*				
	+K+M	**+K-M**	**-K+M**	**-K-M**
+CD	39.83[a]	36.67[bc]	37.33[bc]	38.67[a]
-CD	38.17[ab]	35.67[cd]	37.0[bc]	35.5[cd]
PxKxM*				
	+K+M	**+K-M**	**-K+M**	**-K-M**
+P	40.5[a]	34.5[e]	38.33[b]	36.83[bc]
-P	37.5[b]	37.83[b]	36.0[cd]	37.33[b]
SEM	**±0.58**			

CD=Cow dung; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean.

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

4.6.2 Effects of cow dung, phosphorus, potassium and micronutrients on shelling percentage of groundnut

Results on the interaction effect of cow dung, phosphorus, potassium and micronutrients on the shelling percentage of groundnut as presented in Table 4.25 shows that the four way interaction of cow dung, P, K and micronutrients was significant. In this interaction, the combined application of cow dung, P, K and micronutrients gave the highest value but was however similar to the value obtained where there was addition of K without cow dung, P and micronutrients. Although, the trend of shelling percentage increase with K application was not consistent. Also within the fourth order interaction, the combination of cow dung and P was among the statistically highest values. This was further confirmed in the second order interaction involving cow dung and P where the highest value for the shelling percentage was obtained. Therefore, the combination of cow dung and P is most important for the shelling percentage of groundnut on the experimental soil.

The results presented in Table 4.23 shows that the application of cow dung was significant ($P \leq 0.01$). Phosphorus and micronutrients application also had significant effects on the shelling percentage. The application of K however, had no significant effect on the shelling percentage.

Table 4.25 Interaction Effects of Cow Dung, Phosphorus, Potassium and Micronutrients on Shelling Percentage (%) of Groundnut

CDxP**	**+P**	**-P**
+CD	75.2[a]	69.92[c]
-CD	66.8[d]	70.92[b]
CDxK*	**+K**	**-K**
+CD	70.92[a]	71.5[a]
-CD	69.75[b]	68.0[c]
KxM*	**+M**	**-M**
+K	70.5[a]	70.17[a]
-K	68.58[b]	70.92[a]
PxM**	**+M**	**-M**
+P	70.33[b]	69.0[c]
-P	68.75[c]	72.08[a]
SEM	**±0.49**	

PxKxM**	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+P	72.33[a]	67.67[c]	68.33[c]	70.33[b]
-P	68.67[c]	72.67[a]	68.83[c]	71.5[a]
SEM	**±0.69**			

CDxPxKxM*	**+P+K+M**	**+P+K-M**	**+P-K+M**	**+P-K-M**	**-P+K+M**	**-P+K-M**	**-P-K+M**	**-P-K-M**
+CD	74.0[a]	70.33[ab]	72.33[a]	73.33[a]	68.0[bc]	71.33[ab]	68.0[bc]	72.33[a]
-CD	70.67[ab]	65.0[e]	64.33[e]	67.33[cd]	69.33[bc]	74.0[a]	69.67[bc]	70.0[bc]
SEM	**±1.78**							

CD=Cow dung; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean.

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

4.6.3 Effects of cow dung, phosphorus, potassium and micronutrients on pod yield of groundnut

The interaction effect of cow dung, phosphorus, potassium and micronutrients on the pod yield of groundnut is shown in Table 4.26. The fourth order interaction of cow dung, P, K and micronutrients shows that the combination of cow dung, P, K and micronutrients gave the highest pod yield. Statistically similar to this are the combinations of: cow dung, K and micronutrients without P as well as the combination of cow dung and K without P and micronutrients. Therefore, combined application of cow dung, K and micronutrients can give similar yield benefit with or without P.

The result obtained from the statistical analysis of the effect of cow dung, phosphorus, potassium and micronutrients on pod yield of groundnut is presented on table 4.23. The result shows that the application of cow dung, K and micronutrients were significant ($P \leq 0.01$) on pod yield whereas, P application was significant at $P \leq 0.05$.

4.6.4 Effects of cow dung, phosphorus, potassium and micronutrients on grain yield of groundnut

The results in Table 4.27 shows that in the fourth order interaction of cow dung, P, K and micronutrients, the combination of all these factors gave the highest grain yield. This is of importance as all other interactions gave a lower grain yield. In addition, all the factors increased grain yield when considered individually along with cow dung within the interactions. This shows the relevance of cow dung in enhancing the efficiency of other factors on the grain yield in the experimental soil.

The results presented in table 4.23 shows that sole application of cow dung and K was significant ($P \leq 0.01$) on grain yield. The application of P was significant while the application of micronutrients showed no significance on grain yield.

Table 4.26 Interaction Effects of Cow Dung, Phosphorus, Potassium and Micronutrients on Pod Yield (kg ha^{-1}) of Groundnut

KxM**	**+M**	**-M**
+K	2634.5^{a}	2373.7^{b}
-K	2281.4^{c}	2390.3^{b}
PxM**	**+M**	**-M**
+P	2550.1^{a}	2348.2^{c}
-P	2365.8^{c}	2415.8^{b}
SEM	**±23.77**	

CDxPxK**	**+P+K**	**+P-K**	**-P+K**	**-P-K**
+CD	2666.7^{a}	2563.0^{b}	2735.0^{a}	2438.7^{c}
-CD	2428.5^{c}	2138.33^{d}	2186.2^{d}	2203.5^{d}
CDxKxM**	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+CD	2775.0^{a}	2626.7^{b}	2476.2^{c}	2525.5^{c}
-CD	2494.0^{c}	2120.7^{e}	2086.7^{e}	2255.2^{d}
PxKxM**	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+P	2684.7^{a}	2410.5^{d}	2415.5^{d}	2285.8^{e}
-P	2584.3^{b}	2336.8^{e}	2147.3^{f}	2494.8^{c}
CDxPxM*	**+P+M**	**+P-M**	**-P+M**	**-P-M**
+CD	2669.3^{a}	2560.3^{b}	2581.8^{b}	2591.8^{b}
-CD	2430.8^{c}	2136.0^{e}	2149.8^{e}	2239.8^{d}
SEM	**±33.62**			

CDxPxKxM**	**+P+K+M**	**+P+K-M**	**+P-K+M**	**+P-K-M**	**-P+K+M**	**-P+K-M**	**-P-K+M**	**-P-K-M**
+CD	2786.3^{a}	2547.0^{b}	2552.3^{b}	2573.7^{b}	2763.7^{a}	2706.3^{a}	2400.0^{cd}	2477.3^{bc}
-CD	2583.0^{b}	2274.0^{e}	2278.7^{e}	1998.0^{f}	2405.0^{cd}	1967.3^{f}	1894.7^{fg}	2512.3^{b}
SEM	**±47.54**							

CD=Cow dung; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean.

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

Table 4.27 Interaction Effects of Cow dung, Phosphorus, Potassium and Micronutrients on Grain Yield (kg ha^{-1}) of Groundnut

CDxP*	**+P**	**-P**
+CD	1899.6^{a}	1808.7^{b}
-CD	1528.8^{c}	1554.6^{c}

KxM**	**+M**	**-M**
+K	1858.7^{a}	1664.1^{b}
-K	1565.9^{c}	1699.9^{b}

PxM**	**+M**	**-M**
+P	1799.8^{a}	1625.5^{c}
-P	1624.8^{c}	1738.5^{b}

SEM **±21.43**

CDxPxK**	**+P+K**	**+P-K**	**-P+K**	**-P-K**
+CD	1926.8^{a}	1866.3^{a}	1905.2^{a}	1712.2^{b}
-CD	1651.8^{b}	1405.7^{d}	1561.7^{c}	1547.5^{c}

CDxKxM**	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+CD	1971.0^{a}	1861.0^{b}	1739.0^{c}	1839.5^{b}
-CD	1746.3^{c}	1467.2^{e}	1392.8^{f}	1560.3^{d}

CDxPxM*	**+P+M**	**+P-M**	**-P+M**	**-P-M**
+CD	1954.2^{a}	1839.0^{b}	1755.8^{c}	1861.5^{b}
-CD	1645.5^{d}	1412.0^{f}	1493.7^{e}	1615.5^{d}

SEM **±30.31**

CDxPxKxM**	**+P+K+M**	**+P+K-M**	**+P-K+M**	**+P-K-M**	**-P+K+M**	**-P+K-M**	**-P-K+M**	**-P-K-M**
+CD	2062.3^{a}	1791.3^{bc}	1846.0^{b}	1886.7^{b}	1879.7^{b}	1930.7^{b}	1632.0^{c}	1792.3^{bc}
-CD	1825.3^{bc}	1478.3^{d}	1465.7^{d}	1345.7^{e}	1667.3^{c}	1456.0^{d}	1320.0^{e}	1755.0^{bc}

SEM **±42.85**

CD=Cow dung; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean.

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

4.6.5 Effects of cow dung, phosphorus, potassium and micronutrients on haulm yield of groundnut.

The result as obtained and presented in Table 4.28 shows that the fourth order interaction of cow dung, P, K and micronutrients was highly significant. This interaction reveals that the combined application of cow dung, P, K and micronutrients gave the highest haulm yield. However, this was countered by a statistically similar value obtained by the addition of P without cow dung, K and micronutrients. Elsewhere in the third order interaction involving cow dung, P and micronutrients, the combination of these three factors gave the highest haulm yield. Therefore, to attain higher haulm yields, the addition of cow dung, P, K and micronutrients is the most important be considered on the experimental soil.

The results presented in table 4.23 shows that sole application of cow dung, phosphorus and micronutrients had significant effect ($P \leq 0.01$) on the haulm yield of groundnut. However, application of K did not show any significant contribution to haulm yield.

4.6.6 Effects of cow dung, phosphorus, potassium and micronutrients on harvest index of groundnut

The results in Table 4.29 indicate that in the fourth order interaction of cow dung, P, K and micronutrients, the absence of all four factors gave the highest Harvest index. Similarly, in the third order interaction of P, K and micronutrients, the highest value was obtained with the absence of all three factors. This same trend cuts across all other interactions except in the third order interaction of cow dung, P and K as well as cow dung, K and micronutrients where the combination of cow dung and K in the respective interactions gave the highest value for the harvest index. However, application of cow dung was observed to improve the harvest index along all interactions and can be useful in improving the harvest index of groundnut on the soil. Sole application of cow dung, P, K and micronutrients were significant on the harvest index (Table 4.23).

Table 4.28 Interaction Effects of Cow dung, Phosphorus, Potassium and Micronutrients on Haulm Yield (kg ha^{-1}) of Groundnut

CDxK*	**+K**	**-K**
+CD	4778.3^{a}	4619.1^{b}
-CD	4176.6^{d}	4300.7^{c}

CDxM*	**+M**	**-M**
+CD	4849.4^{a}	4547.9^{b}
-CD	4316.2^{c}	4161.1^{d}

PxK*	**+K**	**-K**
+P	4603.6^{b}	4730.7^{a}
-P	4351.3^{c}	4189.1^{d}

KxM**	**+M**	**-M**
+K	4771.9^{a}	4182.9^{d}
-K	4393.7^{c}	4526.1^{b}

PxM*	**+M**	**-M**
+P	4726.6^{a}	4607.7^{b}
-P	4439.0^{c}	4101.3^{d}
SEM	**±47.66**	

CDxPxK*	**+P+K**	**+P-K**	**-P+K**	**-P-K**
+CD	4943.8^{a}	4844.0^{a}	4612.7^{b}	4394.2^{c}
-CD	4263.3^{d}	4617.3^{b}	4089.8^{e}	3984.0^{e}

PxKxM**	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+P	4945.8^{a}	4261.3^{c}	4507.3^{b}	4954.0^{a}
-P	4598.0^{b}	4104.5^{d}	4280.0^{c}	4098.0^{d}

CDxPxM**	**+P+M**	**+P-M**	**-P+M**	**-P-M**
+CD	5096.3^{a}	4691.5^{b}	4602.5^{b}	4404.3^{bc}
-CD	4356.8^{bc}	4523.8^{b}	4275.5^{bc}	3798.3^{c}
SEM	**±67.4**			

CDxPxKxM**	**+P+K+M**	**+P+K-M**	**+P-K+M**	**+P-K-M**	**-P+K+M**	**-P+K-M**	**-P-K+M**	**-P-K-M**
+CD	5291.7^{a}	4596.0^{bc}	4901.0^{b}	4787.0^{b}	4898.7^{b}	4326.7^{cd}	4306.3^{cd}	4482.0^{bc}
-CD	4600.0^{bc}	3926.7^{de}	4113.7^{cde}	5121.0^{a}	4297.3^{cd}	3882.3^{de}	4253.7^{cd}	3714.0^{e}
SEM	**±95.32**							

CD=Cow dung; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean.

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

Table 4.29 Interaction Effects of Cow dung, Phosphorus, Potassium and Micronutrients on Harvest Index (%) of Groundnut

CDxM*	**+M**	**-M**
+CD	54[b]	57[a]
-CD	53[b]	54[b]
PxK**	**+K**	**-K**
+P	56[a]	50[b]
-P	56[a]	56[a]
PxM**	**+M**	**-M**
+P	54[b]	52[bc]
-P	53[b]	59[a]
SEM	**±0.6**	

CDxPxK**	**+P+K**	**+P-K**	**-P+K**	**-P-K**
+CD	54[c]	53[c]	59[a]	56[b]
-CD	57[b]	47[d]	53[c]	56[b]

CDxPxM**	**+P+M**	**+P-M**	**-P+M**	**-P-M**
+CD	52[c]	55[b]	56[b]	60[a]
-CD	56[b]	48[e]	50[d]	59[a]

CDxKxM**	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+CD	55b	59[a]	53[bc]	55[b]
-CD	56b	54[bc]	50[c]	53[bc]

PxKxM**	**+K+M**	**+K-M**	**-K+M**	**-K-M**
+P	54[c]	57[b]	53[c]	47[e]
-P	56[b]	57[b]	50[d]	61[a]
SEM	**±0.8**			

CDxPxKxM**	**+P+K+M**	**+P+K-M**	**+P-K+M**	**+P-K-M**	**-P+K+M**	**-P+K-M**	**-P-K+M**	**-P-K-M**
+CD	53[d]	55[c]	51[e]	55[c]	56[c]	63[b]	56[c]	55[c]
-CD	56[c]	58[b]	55[c]	39[g]	56[c]	51[e]	45[f]	68[a]
SEM	**±1.1**							

CD=Cow dung; M=Micronutrients; K=Potassium; P=Phosphorus; SEM=Standard Error of Mean.

Means with the same letter(s) within a treatment group are not significantly different (P≤0.05) using DMRT (Duncan Multiple Range Test). *- Significant at P≤0.05, **- Highly Significant at P≤0.01.

4.7 Stepwise Regression Analysis of Nodulation and Biomass Parameters on Nitrogen Fixation, Yield and Yield Components of Groundnut.

The results of the stepwise multiple regression of the independent variables: nodulation and biomass parameters (nodule number, nodule dry weight, root dry weight and shoot dry weight) on the dependent variables: nitrogen fixation (Ndfa, N_2 fixed and soil N balance) and yield components (Pod yield, grain yield, haulm yield and harvest index) are presented in the tables below. From the results, any independent variable that did not significantly influence the dependent variable being considered is eliminated in steps while those with significant contributions were retained based on the prediction equation. For the acidic soil, Table 4.30 shows that for pod yield and Ndfa, only shoot dry weight was retained in the first step. The amount of N_2 fixed was predicted by the root dry weight while the for grain yield, in step 1, nodule dry weight was retained while both nodule dry weight and the shoot dry weight were retained in the second step. The result on Table 4.31 shows that the amount of N_2 fixed influenced pod yield in the first step of the regression while it was also retained alongside soil N balance in the second step. N_2 fixed was retained for haulm yield in the first step while soil N balance was retained for the harvest index.

For the non-acidic soil, the result on Table 4.32 shows that the nodule number was the predictor for pod yield, grain yield, soil N balance, Ndfa and the amount of N_2 fixed. The nodule number was retained for haulm yield in the first step while the nodule number and root dry weight was retained in the second step. The root dry weight was retained in the first step for harvest index prediction. As shown on Table 4.33, for pod and grain yields, N_2 fixed was retained in the first step while both amount of N_2 fixed and soil N balance were retained in the step 2. The amount of N_2 fixed was retained for the haulm yield while or harvest index,

soil N balance was retained in step 1 and both soil N balance and amount of N_2 fixed were retained in step 2.

Table 4.30 Stepwise Regression for Yield Components and Nitrogen Fixation (Dependent Variables) versus Nodulation and Plant Biomass (Independent variables) for the Acidic Soil

	Pod Yield	**Grain Yield**		**Ndfa**	**N_2 fixed**
Independent variables	**Step 1**	**Step 1**	**Step 2**	**Step 1**	**Step 1**
Intercept	1807	1462	877.9	60.28	52.6
Nodule number (x)					
Nodule Dry weight (x_2)		2096	1865		
Root dry weight (x_3)					14.0
Shoot dry weight (x_4)	37		23	0.39	
R-square (R^2)	8.85	13.76	22.22	10.08	10.13

Ndfa=Amount of nitrogen derived from the atmosphere.

Table 4.31 Stepwise Regression for Yield Parameters (Dependent Variables) and Nitrogen Fixation (Independent variables) for the Acidic Soil

	Pod Yield		**Haulm Yield**	**Harvest Index**
Independent variables	**Step 1**	**Step 2**	**Step 1**	**Step 1**
Intercept	2130	1884	2144	0.7461
Ndfa (x)				
N_2 fixed (x_2)	7.8	16.9	27.4	
N balance (x_3)		-12.3		0.00259
R-square (R^2)	11.33	24.15	60.14	15.78

Ndfa=Amount of nitrogen derived from the atmosphere.

Table 4.32 Stepwise Regression for Yield Components and Nitrogen Fixation (Dependent Variables) versus Nodulation and Plant Biomass (Independent Variables) for the Non-acidic Soil

Independent variables	Pod Yield	Grain Yield	Haulm Yield		N balance	Ndfa	N2 fixed
	Step 1	Step 1	Step 1	Step 2	Step 1	Step 1	Step 1
Intercept	1445	848.7	2762	3299	4.689	66.63	32.79
Nodule number (x)	4.63	4.03	8.1	8.7	0.131	0.049	0.2
Nodule Dry weight (x_2)							
Root dry weight (x_3)				-343			
Shoot dry weight (x_4)							
R-square (R^2)	41.98	47.6	46.77	55.24	13.55	24.85	29.92

Ndfa=Amount of nitrogen derived from the atmosphere.

Table 4.33. Stepwise regression for yield parameters (dependent variables) and nitrogen fixation (Independent variables) for the non-acidic soil

Independent variables	Pod Yield		Grain Yield		Haulm Yield	Harvest Index	
	Step 1	Step 2	Step 1	Step 2	Step 1	Step 1	Step 2
Intercept	2001.29	70.86	1340.4	-251	2495	0.643	0.3228
Ndfa (x)							
N_2 fixed (x_2)	5.6	54	4.8	44.7	26.3		0.0074
N balance (x_3)		-52.7		-43.4		0.00305	-0.01025
R-square (R^2)	8.19	78.45	8.9	80.38	66.17	41.26	67.47

Ndfa=Amount of nitrogen derived from the atmosphere.

4.8 Correlation Studies between Nodulation, Biomass, Nitrogen Fixation, Yield and Yield Components

The result of the correlation studies for the acidic soil as shown in Table 4.34 indicates that nodule number had positive correlation with nodule dry weight. Also grain yield increased significantly (0.01 level) with nodule dry weight. The root dry weight was significant with Ndfa and N_2 fixed (0.05 level). Shoot dry weight influenced pod yield, grain yield, Ndfa and N_2 fixed (0.05 level). The soil N balance was significant (0.01 level) with haulm yield, Ndfa, N_2 fixed and negatively with harvest index. Pod yield was significant (0.01 level) with grain yield and harvest index and also with Ndfa and N_2 fixed (0.05 level). Harvest index significantly influenced grain yield (0.01 level). There was significant (0.01 level) relationship between haulm yield and Ndfa as well as N_2 fixed with a negative relationship with harvest index. The amount of N_2 fixed increased with the Ndfa.

For the non-acidic soil as shown in Table 4.35, the correlation analysis shows that nodule number correlates significantly with other variables except the root dry weight and harvest index. A similar trend was observed for the nodule dry weight with these variables. The soil N balance correlates significantly (0.01 level) with haulm yield, Ndfa, N_2 fixed and negatively with harvest index. Pod yield had a significant (0.01 level) relationship with grain yield, haulm yield and harvest index as well as N_2 fixed. Haulm yield showed significance (0.01 level) with Ndfa, N_2 fixed and negatively with Harvest index. The amount of N_2 fixed increased significantly with Ndfa. Both N_2 fixed and Ndfa has a negative relationship with the harvest index.

Table 4.34 Correlation between Nodulation, Biomass, Nitrogen Fixation, Yield and Yield Components of Groundnut on the Acidic Soil

Variables	Nodno	NodDW	RootDW	ShootDW	Nbal	100seedwt	Pod Yield	Grain Yield	Haulm Yield	Ndfa	N_2fixed	HI
Nodno												
NodDW	.646**											
RootDW	-.127	.178										
ShootDW	.258	.139	.140									
Nbal	.012	-.067	.177	.243								
100seedweight	-.130	.051	.133	.318*	.334*							
PodYield	.138	.259	.114	.297*	.008	.076						
GrainYield	.225	.371**	.192	.340*	-.080	.140	.916**					
HaulmYield	.152	.109	.219	.198	.605**	.054	.093	.107				
Ndfa	.052	.080	.306*	.318*	.748**	.279	.334*	.252	.748**			
N_2fixed	.050	.063	.318*	.307*	.740**	.252	.337*	.255	.775**	.991**		
HI	-.017	.118	-.077	.076	-.397**	.038	.723**	.637**	-.612**	-.267	-.283	

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed); Degree of freedom (DF) = N-2; N = 12; r = 0.735 at 1%; r = 0.602 at 5%

Nodno=Number of nodules; NodDW=Nodule dry weight; ShootDW=Shoot dry weight; Nbal=Nitrogen balance; Ndfa=Amount of nitrogen derived from the atmosphere; HI=Harvest Index.

Table 4.35 Correlation between Nodulation, Biomass, Nitrogen Fixation, Yield and Yield Components of Groundnut on the Non-acidic Soil

Variables	Nodno	NodDW	RootDW	ShootDW	Nbal	100seedwt	Pod Yield	Grain Yield	Haulm Yield	Ndfa	N_2fixed	HI
Nodno												
NodDW	.744**											
RootDW	.181	.312*										
ShootDW	.447**	.470**	.556**									
Nbal	.368*	.334*	-.121	.134								
100 seedweight	.495**	.244	.079	.212	.463**							
Pod Yield	.648**	.542**	.167	.278	.003	.437**						
Grain Yield	.690**	.474**	.134	.275	.012	.568**	.950**					
Haulm Yield	.684**	.487**	-.162	.240	.732**	.366*	.383**	.405**				
Ndfa	.498**	.457**	-.049	.208	.944**	.576**	.249	.268	.789**			
N_2fixed	.547**	.481**	-.054	.216	.947**	.582**	.286*	.298*	.813**	.987**		
HI	.018	.071	.289*	.049	-.642**	.062	.615**	.543**	-.484**	-.470**	-.445**	

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed); Degree of freedom (DF) = N-2; N = 12; r = 0.576 at 1%; r = 0.708 at 5%

Nodno=Number of nodules; NodDW=Nodule dry weight; ShootDW=Shoot dry weight; Nbal=Nitrogen balance; Ndfa=Amount of nitrogen derived from the atmosphere; HI=Harvest Index.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physical, Chemical and Microbiological Properties of the Experimental Soils

5.1.1 Physical and chemical properties of the experimental soils

The results obtained showed that the texture of the soils is sandy loam and is suitable for groundnut production (Raemaekers, 2001). The pH in water of the acidic soil indicated strongly acidic reaction (FMNAR, 1990). This could be a constraint to crop production. According to Chude *et al.* (2005), soils with pH values of less than 5.5 are considered acidic and as such might require liming. The non-acidic soil had a moderate acidity which is suitable for crop production. The organic carbon contents of both soils were low (FMNAR, 1990). Low organic carbon of savanna soils have been largely attributed to the rapid decomposition and mineralization of organic materials under high temperatures (Sharu *et al.,* 2013). Total N content of both soils were low (FMNAR, 1990). This suggests the need to supply additional N to optimize crop production through fertilization or BNF. Available P was low on the acidic soil and moderate on the non-acidic soil (FMNAR, 1990). However, total P which was higher in the acidic soil translated to a lower available P. The low available P of the acidic soil is a common occurrence in acidic conditions as P is readily fixed as insoluble and unavailable compounds of Al and Fe. This is because acidic soils are highly weathered and contain large quantities of Al and Fe hydrous oxides that have the ability to adsorb major elements onto their surfaces such that much of added nutrients are fixed instead of being made available for crop use (Akinrinade *et al.,* 2006). Kwari (2005) reported that low P content of soils may restrict *Rhizobia* population and legume root development, which in turn can affect their N_2 fixing potential.

Exchangeable bases were low in both soils with the exception of K which was moderate in both soils and Na which was also moderate on the non-acidic field. Low Ca content of the acidic field

can be attributed to the low pH of the soil, hence the need to supply it through liming. Calcium deficiency is known to restrict the amount of N_2 fixed in legumes, resulting in reduced plant growth due to inadequate nitrogen which is required as building blocks of proteins. The micronutrients contents for the cationic elements analysed were high (FMNAR, 1990). The high Fe content of the soils is in agreement with the observation of Uyovbisere *et al.* (2000) that the soils in the study area are tropical ferruginous in nature.

5.1.2 Most probable number of indigenous *Rhizobia* in the experimental soils

Results obtained showed that the population of indigenous *Rhizobia* in the acidic soil was low. Amarger (2001) reported that *Rhizobia* are widespread in tropical soils as a result of natural distribution and cultivation of legumes, however there are soils where specific strains of *Rhizobia* for legumes are absent or in low numbers. Their low number could also be attributed to the low pH status of the soil which can affect *Rhizobium* strain survival (Wood *et al*., 1984). Published data suggests that a *Rhizobial* population of >1000 cells/g of soil is required for optimum nodulation and N_2 fixation (Singleton *et al.,* 1992; Nazih and Weaver 1994). Therefore, inoculation was necessary on this soil. The primary aim of inoculation is to increase the number of desirable strains of *Rhizobia* in the rhizosphere (Lupwayi *et al*., 2000) and subsequently increase biological nitrogen fixation and yield. The non-acid soil had optimum number of *Rhizobia* but no history of groundnut cultivation in recent time, thus necessitating the need for inoculation (Deaker *et al*., 2006).

5.2 Colony Forming Units of *Rhizobial* Cells in Inoculant

The number of cells found in the inoculant (NC 92) was lower than the recommended number of viable cells expected in high quality inoculant which was estimated at 1.0×10^9 ml^{-1} (Woomer *et al*., 2010). This may affect maximum benefit from inoculation.

5.3 Effects of Lime, Phosphorus, Potassium and Micronutrients on Nodulation, Biomass, Nitrogen Fixation, Yield and Yield Parameters of Groundnut on the Acidic Soil

5.3.1 Effects of lime, phosphorus, potassium and micronutrients on nodulation and biomass of groundnut

The combined application of lime, P, K and micronutrients produced the highest number of nodules and nodule dry weights. Also, the combination of P, K and micronutrients gave a similar result for the nodule dry weight. Combined application of lime with P fertilizer has been shown to improve soil environmental conditions for the development of *Rhizobial* population, root colonization and nodulation (Fatima *et al*., 2006). In addition, the presence of K which is required by certain *Rhizobia* for nodulaton (Vincent, 1977) as well as micronutrients which favour nodulation (Harold *et al.,* 1992) influenced this combination. In contrast, Lynd and Ansman (1989) found reduction in nodule number of groundnut when K was applied alone, but not when P and Ca were added. This is in line with the present study as sole application of K was not significant on nodulation.

On the main effects, the increase in nodule number and dry weight in groundnut due to the application of lime was in agreement with the study of Bekere *et al*. (2013), where it was reported that lime application significantly increased number of nodules. Phosphorus has been reported to promote nodule number and nodule mass in different legumes (Kamara *et al.,* 2011; Amba *et al.,* 2013).

The combination of K and micronutrients was the only significant interaction found on root dry weight and confirms that potassium influences root growth (Singh and Kataria, 2012) coupled

with the influence of micronutrients on plant development (Ashraf *et al.*, 2012). The combination of lime and P which significantly favoured shoot growth consolidates the views of GART (2001) and Mitchell *et al.* (2005) that the yield benefits of liming have also been shown to be evident especially when in combination with fertilizers. According to Ranjit *et al.* (2007), liming in acid soils increase P uptake and subsequently N uptake thus, increasing biomass yield. Plant biomass increase due to P supply supports the report of Rabia *et al.* (2015) who observed that P application significantly improved total dry weight of groundnut as well as root dry weight as observed in this study. The effect of P on biomass production has been previously reported by Kamara *et al.* (2011), Kasaule *et al.* (2007) and Tarawali and Quee (2014). The primary role of phosphorus compounds in plants is to store and transfer energy produced by photosynthesis to be used for growth and reproduction (Leidi *et al*., 2000). Studies by Ndakidemi *et al*. (2006) and Shahid *et al*. (2009) also provided proofs that increased phosphorus application enhances plant growth significantly.

5.3.2 Effects of lime, phosphorus, potassium and micronutrients on nitrogen fixation of groundnut

Application of P was observed to be the major determinant of higher nitrogen fixation (N_2 fixed and Ndfa). This current study agrees with the report of Saxena and Rewari, (1991) who stated that N_2 fixation is strongly influenced by P availability. The values obtained are within the values reported by Woomer (2010) who observed that nitrogen fixation in groundnut is about 150 kg N/ha, and also, Yusuf *et al.* (2011) who reported 76.6 kg N/ha and 55.4 kg N/ha as the highest amount of N_2 fixed by groundnut genotypes in the Sudan and northern Guinea savannas of Nigeria respectively. Similar to the present observation, Hayat *et al.* (2008) reported an increase of up to 32% in % Ndfa due to application of P (80 kg P_20_5 ha^{-1}) using mung bean and mash bean

as test crops. Fatima *et al.* (2007) observed that there was an increase in nodule number and nitrogenase activity with P application which resulted in an increase in % Ndfa.

The benefit of groundnut in terms of soil N enrichment largely depends on the amount of N harvested in the grains relative to the amount of N_2 fixed. The soil N balance was most influenced by the supply of P and micronutrients. Higher nitrogen fixation influences the soil N balance. Phosphorus contributes in improving the activity of bacteroids inside the root nodules and often results in large amounts of N_2 fixed, in most of tropical grain legumes (Dakora and Keya, 1997) which translates to higher soil N balance. Micronutrients have important roles in nitrogen fixation, complimenting the roles of P in increasing the soil N balance through improved nitrogen fixation. The range of values observed for soil N balance is in conformity with the ranges of -8 to 89 kg N ha^{-1} given by Bado *et al.* (2006).

Sole application of lime increased the amount of N_2 fixed by groundnut. According to Nekesa *et al*. (2005), indirect effects of lime include increased availability of P, Mo and B, and more favourable conditions for microbially mediated reactions such as nitrogen fixation and nitrification, and in some cases improved soil structure. In addition, the favourable pH created in the soil for microbial activities by the application of lime might have contributed to stimulating the activities of the inoculated *Rhizobia*, thereby significantly influencing N_2 fixation. Similarly, Lapinskas and Piaulokaitė-Motuzienė, (2006) working under acid soil in Lithuania found that lime applied to inoculated seed fixed 106 kg N ha^{-1} compared to other treatments where lower values were obtained without lime.

5.3.3 Effects of lime, phosphorus, potassium and micronutrients on yield components of groundnut

Lime, P and micronutrients interaction projects P as the major nutrient required for higher 100 seed weight. It was observed by Nekesa *et al.* (2005) that indirect effect of lime include increased

availability of P. This effect contributed in increasing the efficiency of the P fertilizer added. Iman and Zahran (2014) observed an increase in groundnut seed weight due to P fertilization by doubling its quantity applied. Similarly, Kabir *et al.* (2013) reported that 50 kg P/ ha led to significant increases in 100 pods weight compared to control (0 kg/ ha).

Nutrients interaction on shelling percentage did not follow a consistent trend, thereby not showing clearly, which nutrient interaction favoured it except lime, to smaller extent. In addition, sole application of lime was significant. The positive response of groundnut to liming with respect to shelling percentage is an indication of the positive contribution of Ca on pod filling and reduction of 'pops' or empty pods (Kabir et *al.,* 2013). Similar results were reported by Kamara *et al.* (2011) on positive effects of gypsum (which also supplies Ca) application on shelling percentage.

Sole phosphorus application showed positive response to shelling percentage. Studies by Ndakidemi and Semoka (2006) reported that, for farmers who can afford P fertilizers, their combined use with *Rhizobial* inoculants can further increase grain yield and enhance symbiotic establishment for increased N_2 fixation. The increase in grain yield will result to bigger seeds, thereby increasing the shelling percentage.

5.3.4 Effects of lime, phosphorus, potassium and micronutrients on yield of groundnut

The combined application of lime, phosphorus and potassium was observed to give the highest pod and grain yields. Liming contributes to Ca availability which has influence on pod filling and reduction of 'pops' or empty pods (Kabir et *al.,* 2013), thereby increasing yields. In addition to the supply of Ca and Mg, liming also makes phosphorus that is added to the soil to be more available for plant growth and increases the availability of nitrogen by facilitating the decomposition of organic matter (Donald, 2011**)**, resulting to an increase in the efficiency of

added P. Khurana and Sharma (2000) stated that K has important roles in major plant processes such as photosynthesis, respiration, osmoregulation, growth and yield of plants. Therefore, when plants are deficient in K, proteins are not synthesized despite an abundance of available nitrogen. The enzyme nitrate reductase catalyzes the formation of proteins and K is likely responsible for its activation and synthesis (Chandok *et al.*, 2003) thereby influencing N availability for increased yields. The yield benefits of lime have also been shown to be evident especially when in combination with fertilizer (GART, 2001; Mitchell *et al.*, 2005). Similarly, Chude *et al*. (2012) reported that application of lime improved the efficiency of applied inorganic fertilizer, hence better growth and yield.

The haulm yield was most influenced with the combination of lime and P, with higher yield observed with the addition of P without lime. Phosphorus application was reported to influence haulm yield of groundnut significantly (Ranjit *et al.*, 2007). Jahangir *et al*. (2009) and Sharma *et al*. (2011) reported increased N uptake under P fertilizer application, and this is likely to improve vegetative growth. The presence of P resulting to higher yields might be attributed to its role in activating metabolic processes and contributing in building phospholipids. In addition, the presence of phosphorus is known to help in developing extensive root system that helps the plant in absorbing water and nutrients efficiently (Gobarah *et al.*, 2006), which in turn enhances the plant to produce more assimilates which was reflected in higher biomass.

Harvest index was most favoured by lime addition. Liming which contributes to the supply of Ca^{2+} significantly increased pod yield in groundnut (Dosani *et al*., 2003; Dutta *et al*., 2004). Since harvest index is the ratio of grain yield to total biomass yield, the higher grain yield as influenced by liming will no doubt increase the harvest index of groundnut. The range of values

observed for the harvest index is in agreement with the ranges of 30-70 % given by Bado *et al.* (2006).

Some antagonistic interaction effects were observed between K and lime as observed on pod, grain and haulm yields, Ndfa and N_2 fixation. This may have been associated with excessive cations in the soil as a result of liming, thereby creating competition and imbalances between K and other nutrients especially Ca since these are strong competitors of K (Lin, 2010).

5.4 Effects of Cow dung, Phosphorus, Potassium and micronutrients on Nodulation, Biomass, Nitrogen Fixation, Yield and Yield Components of Groundnut on the Non-Acidic Soil

5.4.1 Effects of cow dung, phosphorus, potassium and micronutrients on nodulation and biomass of groundnut

The combination of cow dung, K and micronutrients was observed to favour nodulation. The increase in nodulation due to amendment with cow dung corroborates the findings of Tagoe *et al*. (2008), where 39% significant increase was recorded in number of nodules as a result of manure application. This may be as a result of increase in biological activities in the soil and consequently increasing the activities of symbiotic *Rhizobia* for nitrogen fixation. The complimentary effects of cow dung, K and micronutrients to improve nodulation confirms documented evidences of increase in soil P, K, Ca, Mg and micronutrients due to organic manure application (Walker and Bernal, 2004; Rodriguez *et al.,* 2005). Interestingly, K which was hitherto not significant during sole application combines well with other nutrients to improve the nodule dry weight thereby showing a complimentary effect of cow dung and micronutrients in improving the effectiveness of K. Analysis of the manure shows that the concentration of K was high, thereby significantly contributing to the observed effects. Cow dung has been shown to increase soil K content (Yogananda *et al.,* 2004), thereby increasing the effectiveness of applied K. In addition, Kobraee *et al.* (2011) reported that application of different levels of micronutrients

had significant effect on nodule number and nodule dry weight. Foliar applications of Mo to grain legumes in field conditions increased levels of N_2 fixation and nodule mass, resulting in higher N content and seed yield (Vieira *et al.*, 1998).

Root dry weight was influenced most by the combination of P and micronutrients and their effects could be attributed to the importance of P on roots formation as well as the provision of micronutrients needed for root development as correlation between plant growth and micronutrients availability has been reported (Ashraf *et al.*, 2012). The increase in root biomass due to P fertilization is in line with the observation of Olivera *et al*. (2004) that adequate P improves root growth.

5.4.2 Effects of cow dung, phosphorus, potassium and micronutrients on nitrogen fixation of groundnut

The combined application of cow dung, P, K and micronutrients was observed to improve nitrogen fixation (N_2 fixed and Ndfa). The various combinations of cow dung with other nutrients significantly influenced N_2 fixation. Organic amendments are known to increase the abundance of various components of the soil food web, including the bacterial communities (Forge *et al.*, 2008), in addition to supplying other nutrients which might be due to increased release of macro as well as micronutrients (Dosani *et al.*, 1999) most of which contributes to N_2 fixation. The amount of water retained in organically amended soils is greater than that retained in un-amended soils as soil-water tension increases (Elsharawy *et al.*, 2003). This could result to increased microbial activities as well as dissolution of nutrients for increased uptake, thereby enhancing their functions in nitrogen fixation. However, sole application of K influenced Ndfa, but the interaction effect of the combined application of cow dung, P, K and micronutrients showed that the presence of K in this interaction was not influential. This could be attributed to the moderate K levels in the soil which must have met the crop's requirement. The presence of P has been

shown to increase nodule number and nitrogenase activity which resulted in an increase in % Ndfa (Fatima *et al.,* 2007).

Nitrogen fixation was observed to be enhanced by the sole application of micronutrients. Harold *et al*. (1992) observed that various microelements like Cu, Mo, Co and B are necessary for N_2 fixation.

The soil N balance was best influenced by the presence of P. Soil N balance is mainly influenced by the amount of N_2 fixed and grain yield. The range of values observed for soil N balance is in conformity with the ranges of -8 to 89 kg N ha^{-1} given by Bado *et al.* (2006). The roles of P in influencing it stems from the established roles of P in N_2 fixation. Chiezey and Odunze (2009) and Amba *et al*. (2011) have shown that P fertilization increases N_2 fixation thereby influencing the soil N balance positively.

5.4.3 Effects of cow dung, phosphorus, potassium and micronutrients on yield components of groundnut

The combined application of P, K and micronutrients which produced the highest 100 seed weight can be due to the complementary effects of these nutrients. Higher seed weight was also observed by El-Far and Ramadan (2000) and Hossain *et al.* (2007) as a result of P fertilization. Iman and Zahran (2014) reported increased seed weight of groundnut by the application of K and further increment was recorded by doubling the quantity applied. The presence of micronutrients may have created an enabling environment for the increased uptake of P and K, resulting to their increased effectiveness in the combination as its role in crop productivity has been observed (Ashraf *et al.,* 2012).

The combination of cow dung and P was observed to favour the shelling percentage of groundnut the most. Cow dung has been reported to contribute to the release of P in the soil (Eghball *et al.*, 2004), therefore improving its contribution to yield and yield parameters. In groundnut,

application of FYM at 10 to 15 tons ha^{-1} increased the shelling percentage and mature kernel compared to the recommended dose of fertilizers (Subrahmaniyan *et al.,* 2000). Jagdev and Singh (2000) observed that the application FYM increased the shelling percentage of groundnut. Organic manures have been observed to contain essential nutrients which are released into the soil for plants uptake, thereby influencing yield and yield parameters. Organic amendment has been shown to increase uptake of major nutrients including P, thereby increasing its efficiency. The influence of P on the shelling percentage could also be related to the role of P in pod and grain yield and subsequently shelling percentage. This is so because P treated plots have been reported to produce significantly higher grain yield than plots without phosphorus (Tran Thi, 2003; Anil *et al.*, 2008) thereby significantly influencing the shelling percentage.

5.4.4 Effects of cow dung, phosphorus, potassium and micronutrients on yield of groundnut

Pod, grain and haulm yields were highest with the combined application of cow dung, P, K and micronutrients. However, the presence or absence of P did not show statistical difference on the pod yield in this interaction. This could be partly attributed to the moderate available P content of the soil as the cultivar may be adapted to the inherent P content of the soil (Yusuf *et al.,* 2011). The increase in pod, grain and haulm yields due to this combination is in line with observations that the application of organic manure either separately or in combination with inorganic fertilizer gave higher yields in groundnut (Sriramachandrasekaran, 2001; Manikandan, 2003). Higher N content of organically soils have also been reported (Sullivan *et al.*, 2003; Rodriguez *et al.,* 2005) thereby significantly influencing yield. Deshmukh *et al.* (2005) reported that the beneficial effect of FYM in conjunction with recommended dose of fertilizers may be due to the effect of organic matter in improving physical, chemical and biological environment of soil conducive to better plant growth while ensuring optimum uptake of the added fertilizers. In support of this, Zerihun *et al.* (2013) reported that combined application of manure and mineral

fertilizers resulted in 50% increase in the yield of grain legumes compared to the sole application of manure. Chandrasekaran *et al.* (2007) also reported that the application of FYM increased the number of pods and pod yield per plant in groundnut. In addition to the supply of N and P, researchers have documented increases in soil K (Khatik and Dikshit, 2001; Rodriguez *et al.*, 2005), calcium and magnesium (Eghball *et al.*, 2002) and micronutrients (Yogananda *et al.*, 2004) due to addition of organic manures. This will, to a larger extent improve yield and other yield parameters.

Harvest index was favoured by the combination of cow dung and K. This combination also favoured grain yield. Since harvest index is the ratio of grain yield to total biomass yield, the higher grain yield as influenced by application of cow dung and K will lead to an increase in the harvest index of groundnut in the study.

5.5 Relationship between nodulation, biomass, nitrogen fixation, yield and yield parameters on the acidic and non-acidic soils

Observations from the stepwise regression and correlation studies of nodulation, biomass, nitrogen fixation, yield and yield parameters revealed similar trends. The independent factors that predicted the dependent variables in the stepwise regression were also observed to have significant relationships with such variables in the correlation studies.

As observed on the acidic soil, nodule number did not influence N_2 fixation and other yield attributes. This is similar to the observation of Van Rossum (1993) where nodulation hardly led to significant effect in biomass yield and N_2 fixation in groundnut. The nodule dry weight was however significant with the grain yield similar to the report that nodulation, N_2 fixation and crop yield were significantly correlated in mungbean (Bushby and Lawn, 1992). Also, grain yield was positively correlated with shoot dry matter which is at par with the findings of Herridge *et al.* (2005). In agreement to the observations in this study where N_2 fixation had positive

relationship with yield, Meghvansi *et al.* (2010) reported the importance of symbiotic N_2 fixation in yield of soybean. Soil N balance which shows negative relationship with the grain yield could be ascribed to higher N removed by grains, thereby reducing the residual N content of the soil. Varieties with low N harvest index will be suited to enhance positive soil N balance.

On the non-acidic soil, nodulation, shoot biomass and N_2 fixation were positively and significantly correlated similar to the observation of Herridge *et al.* (2005) in mungbean. Similarly, Ngakou *et al.* (2012) reported that nodulation positively stimulated biological nitrogen fixation, and was correlated with the pod and seed yield.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

Soils of the Nigerian savanna are inherently low in fertility resulting from low organic matter, N and P contents as well as low pH. This has contributed to low groundnut productivity over the years. Current fertilizer recommendations for groundnut are based on single nutrient trials which do not take into account the effect of lime, organic manures or micronutrients, in single or combined application. The study was designed to identify the major factors that influence biological nitrogen fixation and yield of groundnut in a savanna Alfisol through fertilizer application and soil amendment on an acidic and non-acidic soil so as to optimize productivity. A nutrient omission trial using factorial combinations was used to achieve this aim. The study was carried out in field trials at two locations at the Institute for Agricultural Research (IAR) farm, Samaru, on an acidic (S13) and non-acidic soil (S7) using groundnut genotype, SAMNUT

24 from July to October, 2015. The treatments used on the acidic soil were two levels each of lime, phosphorus, potassium and micronutrients. On the non-acidic soil, there were also two levels each of organic manure, phosphorus, potassium and micronutrients. These levels were zero (0) and the recommended rate for each nutrient. Phosphorus was applied at 54 kg P_2O_5/ha as SSP, K at 25 kg K_2O/ha as MOP, micronutrients with the trade name Agrolyzer at 2 g/L, lime at 250 kg/ha and cow dung at 1.7 tons/ha (equivalent to 10kg N ha^{-1}). The treatments were arranged in a factorial combination and laid out in a randomized complete block design replicated three times on plots of $9m^2$ for each location. The SAMNUT 24 seeds were inoculated with *Rhizobial* inoculant, NC 92 to enhance biological nitrogen fixation (BNF) and were planted on all plots. A non-nodulating groundnut genotype, ICGL 5 was included for estimation of BNF. The effects of the main treatments and their interactions were observed on nodule number, nodule dry weight, root and shoot dry weights, nitrogen fixation, 100 seed weight, shelling percentage, pod, grain and haulm yields, as well as harvest index. On the acidic soil, the amount of N_2 fixed and nitrogen derived from the atmosphere (Ndfa) were highest with the application of P while soil N balance was most favoured by the application of P and micronutrients. The combination of lime, P and K was observed to favour pod and grain yields the most. Haulm yield however was most favoured by the application of P only while harvest index was improved mostly by liming. On the non-acidic field, the highest Ndfa, N_2 fixed, grain and haulm yields were obtained by the combination of cow dung, P, K and micronutrients, while the soil N balance was most influenced by addition of P. The combined application of cow dung, K and micronutrients was best for pod yield. Harvest index was highest under the combination of cow dung and K. Stepwise regression and correlation studies showed that the nodule dry weight was important in predicting the grain yield on the acidic soil. On the non-acidic soil, nodule number influenced

BNF and yield of groundnut. Similarly, N_2 fixation significantly influenced yield parameters on both soils. These indicate that *Rhizobium* inoculation with NC 92 was effective in enhancing BNF and yield in the soils that were characterized with low indigenous *Rhizobial* population.

Most of the soil properties determined in the experimental soils fell within the low range. Therefore, responses observed in this study can be attributed to the inputs applied. However, the response to micronutrients applied on the non-acidic soil could be connected to their lower values compared to the acidic soil. Generally, response to lime and P was observed in the acidic soil while K was only effective when applied in combination with other nutrients, signifying the importance of their combination to groundnut production. On the non-acidic soil, response was observed mainly with the application of cow dung, K and micronutrients. Some antagonistic interaction effects were observed where only K and lime were applied together as observed on the pod yield, grain yield, haulm yield, Ndfa and amount of N_2 fixed on the acidic soil.

This study showed that groundnut yield was significantly increased by liming and fertilization on the acidic soil. Liming of the acidic soil gave 21% higher pod yield than the non-acidic soil. In addition, *Rhizobium* inoculation was sufficient to meet the N requirement of groundnut, especially on the acidic soil where initial *Rhizobial* population was low. The positive N balance in both locations indicates improved soil quality and can be beneficial for non-fixing crops in rotation. Micronutrients addition showed no significant difference on pod yield on both locations, indicating sufficiency of inherent micronutrients levels in the soils but may need to be added especially on the non-acid soil to maintain yields while preserving that which is in the soil. This has further shown that poor nutrient management strategies are among the key factors that has affected groundnut productivity over the years in Nigeria. This trend can be reversed through application of fertilizer nutrients and soil amendment.

6.1 Recommendations

Adequate liming of soils with pH of less than 5.5 in combination with recommended rates of fertilizer should be carried out to improve groundnut yields. Practices aimed at increasing the organic matter content of soils is highly encouraged to improve the efficiency of added mineral fertilizers. Also, the use of the *Rhizobial* inoculant NC 92 could be adopted as a source of N for groundnut production in the northern Guinea savanna especially on soils with low *Rhizobial* population.

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Appendix 1

Broughton and Dilworth N-free Plant Nutrient Solution

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

Appendix II

Yeast-Mannitol Agar (YMA)

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