GROWTH AND YIELD OF COWPEA (Vigna unguiculata) FOLLOWING NITROGEN FERTILIZER APPLICATION AND INOCULATION

KNUST

BY

DANSO JACOB

NYANSAPƆ WƆSANE NO BADWENMA

NOVEMBER, 2016

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI, GHANA

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CROP AND SOIL SCIENCES

KNUST

GROWTH AND YIELD OF COWPEA (Vigna unguiculata) FOLLOWING NITROGEN FERTILIZER APPLICATION AND INOCULATION

BY

DANSO JACOB

NOVEMBER, 2016

GROWTH AND YIELD OF COWPEA (Vigna unguiculata) FOLLOWING NITROGEN FERTILIZER APPLICATION AND INOCULATION

KNUST

BY

DANSO JACOB

BSc. AGRICULTURE SCIENCE (HONS)

A Thesis submitted to the Department of Crop and Soil Sciences, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology, Kumasi, in partial fulfillment of the requirements for the degree of

MASTER OF PHILOSOPHY

IN

AGRONOMY (CROP PHYSIOLOGY)

NOVEMBER, 2016

DECLARATION

I hereby declare that, this work is the result of my own original research and that this thesis has neither in whole nor part been presented anywhere for a degree except for the references cited in relation to other works.

KNUST

Danso Jacob …… ……..

PG 1629414 Signature Date

Student‟s Name & ID

Certified By

Prof. J. Sarkodie-Addo …… ……..

Principal Supervisor Signature Date

Certified By

Mrs. Duku S. …… ……..

Co-supervisor Signature Date

Certified By

Dr. E. Osekre …… ……..

Head of Department Signature Date

DEDICATION

This work is dedicated to, Miss Nana Adwoa Danso , Mr. Kwadwo Asare Danso and Ms. Owusu Vera Tandoh for their love, support , prayers and encouragement.

KNUST

ACKNOWLEDGEMENTS

I take this opportunity to express my gratitude to my supervisors Prof. Joseph Sarkodie-Addo and Mrs. Stephanie Duku of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi Ghana for their constant expert guidance, advice and support through all the stages of this research work. My thanks also goes to Dr. Amoah, Director of CSIR, Dr. Obuor Agyemang and Dr. George Opoku, Deputy Director of CRIG for their support offered my throughout this study. I also wish to thank Mr. Ayamah Azumah and Nortey of the KNUST Microbiology and Soil Science Laboratory respectively for their assistance in the analysis of the products and soil used for the study. My sincerest appreciation also goes to my wife, Owusu Vera Tandoh for funding my course and research work. Finally, I would like to thank my parents (Mr. and Mrs. Asare Badu), family and friends especially Christopher Appiagyei and Caleb Melenya for their unfailing support and encouragement in all my endeavors.

I say God bless you all.

ABSTRACT

A field study was conducted to assess the growth and yield of cowpea (Vigna unguiculata) following N fertilizer application and inoculation at the Plantation Section, Faculty of Agriculture, KNUST. Two inoculants (Eco-Rhiz-Soya and BR 3262) and nitrogen fertilizer at four levels which (0 kg N/ha, 15 kg N/ha, 30 kg N/ha and 45 kg N/ha) were the two main factors. The initial soil analysis was conducted at the Soil Science Laboratory at KNUST to determine microbial population, pH, phosphorus content, exchangeable cations, organic carbon and organic matter. Planting was done manually at three seeds per hill and later thinned to two seedlings per hill at a spacing of 60 x 20 cm. The N fertilizer was applied two weeks after planting the seeds. Growth parameters measured were number of days to emergence, plant height, number of leaves, leaf area, leaf area index, number of days to 50 % flowering, number of branches, percent effective nodules, number of nodules and nodule dry weight. The yield parameters measured were number of pods, number of seeds per pod, weight of seeds per pod, 100 seed weight, grain yield, residue N and seed N content. The results showed that at 25 and 35 DAP, plants that received 45 kg N/ha were the tallest, and this was significantly higher than all other treatment means and both inoculants produced similar effects, and either effect was significantly higher than the control treatment. The 45 kg N/ha produced the greatest number of branches, and this was significantly higher than the other treatments where as 30 kg N /ha treatment recorded the greatest nodule number plant per plant (2.98) which was significantly higher than all other treatment effects. The 30 kg N /ha treatment produced the greatest number of effective nodules on both sampling periods but the Eco Rhiz soya inoculation produced significantly higher effective nodules than all other treatments at 25 DAP only. The grain yield did differ significantly among the nitrogen fertilizer levels. The 45 kg N/ha treatment recorded the greatest grain yield which was significantly higher than all other treatment effects. Grain yield following inoculation was greatest in the BR 3262 inoculant, which was significantly

higher than those of the control and Eco Rhiz soya. The N fixed and N derived from the atmosphere, from the 45 kg N/ha treatment were significantly higher than all other treatments. Inoculation significantly affects mean of N fixed, as the treatment effect of the BR 3262 inoculant was greater than those of the control and the Eco Rhiz soya but N derived from the atmosphere was not significantly different among the inoculation treatments. The results indicated that for greater growth, N fixation and grain yield of cowpea, inoculation or starter N application cannot be overlooked.

KNUST

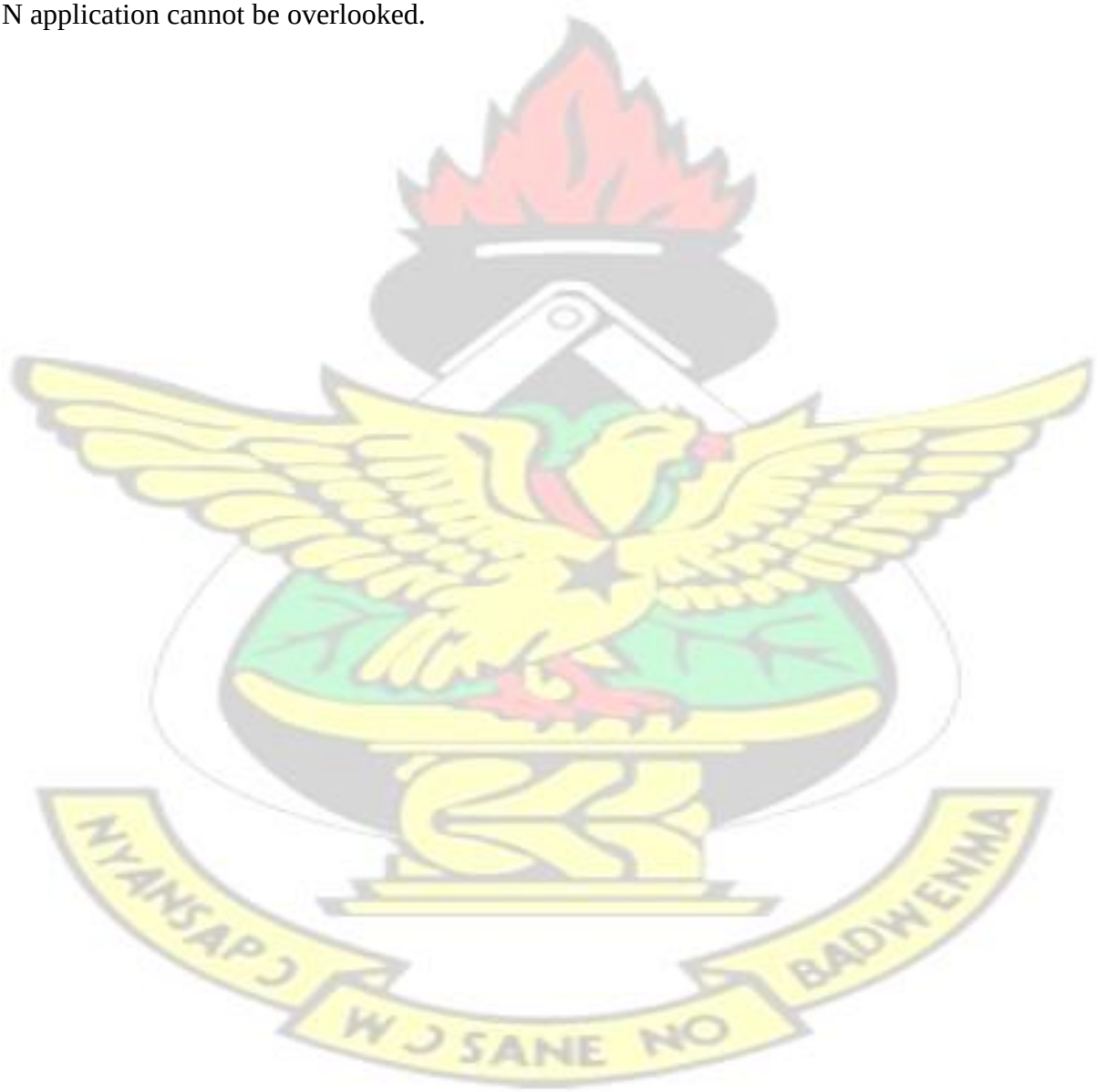

TABLE OF CONTENT

DECLARATION..... i

DEDICATION..... ii

ACKNOWLEDGEMENTS iii

ABSTRACT iv

TABLE OF CONTENT vi

LIST OF TABLES ix

CHAPTER ONE 1

1.0 INTRODUCTION 1

CHAPTER TWO 5

LITERATURE REVIEW 5

2.0 Cowpea taxanomy 5

2.1 Origin, domestication and distribution 5

2.2 Morphology and biology..... 7

2.3 Uses of cowpea 8

2.4 Cowpea production systems 10

2.5 Cowpea production in Ghana..... 10

2.6 Constraints to cowpea production in Ghana 11

2.7 Fertilizer use..... 11

2.8 Constraints to mineral fertilizer use 12

2.9 Fertilizer use in cowpea production 13

2.10 The contribution of N fertilizers to growth and yield of cowpea 14

2.11 Biological nitrogen fixation in legumes 15

2.12 Factors affecting BNF 16

2.13 Effect of applied nitrogen in cowpea production 18

2.14 The need for inoculation in legume production 18

2.15 Inoculant strains and carriers 19

2.16 Importance of inoculant quality 21

2.17 Factors limiting the quality of inoculants 23

CHAPTER THREE 24

MATERIALS AND METHODS 24

3.1 Experimental site 24

3.2 Field work 24

3.2.1 Source of planting materials 24

3.2.2 Biofertilizer products used 24

3.2.3 Land preparation, inoculant application and planting..... 25

3.2.4 Treatments and experimental design 25

3.3 Soil sampling and sample preparation 26

3.4 Laboratory analysis 26

3.4.1 Determination of soil chemical properties 26

3.4.1.1 Soil pH 26

3.4.1.2 Soil organic carbon 26

3.4.1.3 Organic matter 27

3.4.1.4 Soil total nitrogen 28

3.4.1.5 Available phosphorus..... 28

3.4.1.6 Extraction of exchangeable cations 30

3.4.1.6.1 Determination of exchangeable potassium 30

3.4.1.6.2 Determination of exchangeable calcium and magnesium 31

3.4.2 Plant total nitrogen 31

3.5 Number of days to emergence 32

3.6 Plant height 32

3.7 Plant leaf area 33

3.8 Nodule count 33

3.9 Effective nodules and nodule dry weight..... 33

3.10 BNF determination..... 34

3.11 Number of pods per plant, seeds per pod and 100 seed weight 34

3.12 Grain yield 34

3.13 Crop residue dry weight 35

3.14 Quality assessment of inoculants used in the study 35

3.14.1 Viable cell count 35

3.14.2 pH 35

3.14.3 Moisture content 36

3.15 Statistical analysis 37

CHAPTER FOUR 38

RESULTS 38

4.0 Growth parameters 38

4.1 Plant height 38

4.2 Number of branches 39

KNUST

4.3 Number of leaves 40

4.4 Number of nodules and nodule dry weight 41

4.5 Nodule effectiveness, leaf area and leaf area index 42

4.6 Days to emergence and number of days to 50 % flowering 44

4.7 Yield and yield components..... 45

4.7.1 Number of pods per plant and number of seeds per pod 45

4.7.2 Hundred seed weight and grain yield..... 46

4.7.3 Residue and seed N content 46

4.7.4 Nitrogen fixed and N derived from the atmosphere 48

CHAPTER FIVE 49

DISCUSSION 49

5.1 Starter N and inoculation on growth of cowpea 49

5.2 Starter N application and inoculation on nodulation and N-fixation of cowpea ... 51

5.2.1 Nodulation..... 51

5.2.2 Nitrogen fixed and N derived from the atmosphere 52

5.4 Starter N and inoculation on grain yield and some yield components 53

CHAPTER SIX 56

CONCLUSIONS AND RECOMMENDATIONS 56

6.1 Conclusions 56

6.2 Recommendations 57

REFERENCES 58

LIST OF TABLES

Table 4.1 Effect of N fertilizer application and inoculation on plant height 39

Table 4.2 Effect of N fertilizer application and inoculation on number of branches 40

Table 4.3 Effect of N fertilizer application and inoculation types on number of leaves 42

Table 4.4 Effect of N fertilizer application and inoculation on number of nodules per plant and nodule dry weight 42

Table 4.5 Effect of N fertilizer application and inoculation on percent effective nodules, leaf area and leaf area index 43

Table 4.6 Effect of N fertilizer application and inoculation on days to emergence and number of days to 50 % flowering 44

Table 4.7 Effect of N fertilizer application and inoculation on number of pods per plant and number of seeds per pod 45

Table 4.8 Effect of N fertilizer application and inoculation on 100 seed weight and grain yield 47

Table 4.9 Effect of N fertilizer application and inoculation on total nitrogen in residue and seed 47

Table 4.10 Effect of N fertilizer application and inoculation on nitrogen fixed and percent nitrogen (N) derived from the atmosphere 48

KNUST

CHAPTER ONE

1.0 **INTRODUCTION**

Cowpea (Vigna unguiculata (L) Walp), as a grain legume crop is an important source of food, income and livestock feed and forms a major component of tropical farming systems because of its ability to improve marginal lands through nitrogen fixation and as cover crop. It is a valuable and reliable asset that brings income for many smallholder farmers and traders in sub-Saharan Africa (Langyintuo et al., 2003). The grain is also a good source of human protein, while the haulm is an important source of livestock protein (Fatokun, 2002). Cowpea is extensively cultivated in Ghana under rain fed conditions, mainly in the savanna and transition zones (CRI, 2006) and about 80% of the cowpea produced in Ghana is grain largely in the savannah zone of the country (FAO, 1999). Even though a wide range of seed yield have been recorded for cowpea, yet yields are the least in the world, an average of 310 kg/ha (Ofosu-Budu et al., 2007). Among the factors responsible for low yields are the use of local varieties and low soil fertility, as most tropical soils are deficient in essential nutrients particularly N and P (Jones and Wild, 1975). Thus, efforts have been made to improve cowpea production in Ghana through various means, including the introduction of new varieties (Addo-Quaye et al., 2011) and more productive agricultural technologies. None of these improved varieties could achieve optimal performance without recommendations of appropriate and specific fertilizers. On the other hand, fertilizers are limited in use in agriculture in Ghana (less than 8 kg/ ha) which is among the highest in Africa (FAO, 2005a). Traditionally, soil fertility in West Africa has been maintained through fallow. However, in Ghana intensive cropping is gradually replacing the traditional shifting cultivation that was associated with long fallow, and hence low soil nutrient and yield levels of crop plants are rampant. The steady decline in food production due to reduced length of fallow on land has prompted farmers to amend soil with different materials (organic and inorganic) in order to enhance plant growth and increase yield (Adepetu, 1997).

It has been suggested that organic manure should be used in place of chemical fertilizers to avoid long-term negative effect of chemical fertilizer on the soil (Parr et al., 1990). However, organic manure is usually required in large quantities to sustain crop production and may not be available to smallholder farmers (Nyathi and Campbell, 1995), hence, the need for inorganic fertilizer. The positive effect of the application of inorganic fertilizers on crop yields have been reported (Carsky and Iwuafor, 1999). Although cowpea symbiotically fixes nitrogen, plants depending on symbiotically fixed N may well suffer from temporary N deficiency during seedling growth once the cotyledon reserves have been exhausted. Usually prior to the onset of symbiotic N fixation, cotyledonary reserves are mobilized during hypocotyl elongation in cowpea and cotyledons are usually shed one or two days from emergence. It has thus been recognised and demonstrated that application of small nitrogen fertilizer enhances early vegetative growth (Darth et al., 1977). Burries (1959) and Martin et al. (2003) stated that nitrogen has a stimulating effect on root activity and rooting pattern of the crops. It has also been reported that available nitrogenous compounds allow seedlings to make a good start before nitrogen fixation occur. Other workers have shown that plants given an inorganic N during vegetative period were much larger by the onset of flowering than those dependant on symbiotic N fixation (Minchin et al., 1981). Such plants also had more branches and produced many peduncles resulting in greater number of pods and seeds and significantly larger yields. There is the need to optimize Biological Nitrogen Fixation (BNF) which provides a cheaper source of nitrogen for resource poor farmers to maximize yield. Even though there are many reported studies about the effect of P and rhizobia inoculant application on growth and yield of cowpea (Owolade et al., 2006), there is little information on the effects of N fertilizer and rhizobia inoculants on growth and yield of cowpea in Ghana. This study was therefore designed to evaluate the growth and yield responses of cowpea to different levels of N fertilizer and two rhizobia inoculants under field conditions in Ghana.

The specific objectives were to:

- evaluate the growth and yield of cowpea after inoculant and fertilizer N application;
- estimate nodulation and N fixation potential of two different cowpea inoculants;
- assess the influence of starter N on nodulation and N fixation in cowpea;
- estimate the relative efficiencies of N fixation following inoculant application.

KNUST

HYPOTHESIS

- There is no significant difference between the growth and yield of cowpea after inoculants and combined N application
- There is no significant difference between nodulation and N fixation potential of two different cowpea inoculants

CHAPTER TWO

LITERATURE REVIEW

2.0 Cowpea taxanomy

Cowpea [Vigna unguiculata (L) Walp.] is a dicotyledonous crop in the order Fabaceae, subfamily Faboideae (Syn. Papillionoideae), tribe Phaseoleae, subtribe Phaseolinae, genus Vigna and section Catiang. It is a diploid plant containing 22 chromosomes and its nuclear genome size is estimated to cover 620 million base pairs (Timko and Singh, 2008). The genus was divided into sub-genera based upon morphological characteristics, the extent of genetic hybridization and geographical distribution of the species. The major groups consist of the African sub-genera Vigna and Haydonia, the Asian sub-genus Ceratotropis, and the American sub-genera Sigmoidotropis and Lasiopron (Timko and Singh, 2008). V. unguiculata sub-species unguiculata includes four cultivated groups: unguiculata, biflora (or cylindrical), sesquipedalis, and textilis (Ng and Maréchal, 1985). Vigna unguiculata subspecies dekindiana, stenophylla, and tenuis are intermediat wild progenitors of cultivated cowpea and form the major portion of the primary gene pool of cowpea. Fatokun and Singh (1987) pointed out that wild subspecies like pubescence that do not readily hybridize and show some degree of pollen sterility form a secondary gene pool.

2.1 Origin, domestication and distribution

Cowpea (Vigna unguiculata) is one of the most ancient human food sources and has probably been used as a crop plant since Neolithic times (Summerfield et al., 1974). A lack of archaeological evidence has resulted in contradicting views supporting Africa, Asia, and South America as origin (Coetzee, 1995; Tindall, 1983; Summerfield et al., 1974). One view is that cowpea was introduced from Africa to the Indian sub- continent approximately 2000 to 3500 years ago (Allen, 1983). Before 300 BC, cowpeas had reached Europe and possibly North Africa from Asia. In the 17th century AD, the Spanish took the crop to West India. The slave

trade from West Africa resulted in the crop reaching the southern USA early in the 18th century. Another view was that the Transvaal region of the Republic of South Africa was the centre of speciation of V. unguiculata, due to the presence of most primitive wild varieties (Padulosi and Ng, 1997). Presently, cowpea is grown throughout the tropic and sub tropic areas of the world. Ng (1995) postulated that during the process of evolution of V. unguiculata, there was change of growth habit, from perennial to annual breeding and from predominantly out breeding to inbreeding, while cultivated cowpea (subsp. unguiculata) evolved through domestication and selection of the annual wild cowpea (var. dekindtiana). During the process of domestication and after the species was brought under cultivation through selection, there was a loss in seed dormancy and pod dehiscence, corresponding with an increase in seed and pod size. The precise location of origin of where cowpea was first domesticated is also still under speculation. The wide geographical distribution of var. dekindtiana throughout sub-Saharan Africa suggests that the species could have been brought under cultivation in any part of the region. However, the centre of maximum diversity of cultivated cowpea is found in West Africa, in an area encompassing the savannah region of Nigeria, southern Niger, part of Burkina Faso, northern Benin, Togo, and the northwestern part of Cameroon (Ng and Marechal, 1985).

2.2 Morphology and biology

Based on the investigation conducted by Padulosi and Ng (1997) and supported by Baudouin and Merechal (1985); and Padulosi (1987) about the range of variation and number of varieties found in wild cowpeas as well as their primitive characteristics, such as hairiness, small size of pods and seeds, pod shattering with pronounced exine on the surface of the pollen, out-breeding and bearded stigma.The highest genetic diversity and most primitive forms of wild V.unguiculata occur in southern Africa. Variability in morphology of different cowpea accession is very high. There are three types according to their uses: for grain, forage or dual

purpose. Vigna unguiculata is a herbaceous trailing, prostrate, climbing, bushy, or sub erect annual plant, growing 15-80 cm high. Leaves are alternating trifoliate with petiole 5-25 cm long. The lateral leaflet is opposite and asymmetrical, while the central leaflet is symmetrical and ovate. Leaves exhibit considerable variation in size (6-16 x 4-11 cm) and shape (linear, haste, lanceolate to ovate) and they are usually dark green. The stems are striate, smooth or slightly hairy and sometimes tinged with purple. The inflorescences are racemose or intermediate at the distal ends of 5-60 cm long peduncles. The flowers are borne in alternate pairs, with usually only two flowers per inflorescence. These are conspicuous, self-pollinating, borne on short pedicels and the corollas may be white, cream, pink, pale, blue, yellow or purple. Flowers open in the early day and close at approximately midday. After blooding (opening once) they wilt and collapse. Growth pattern is either determinate or usually indeterminate under favourable conditions. Fruit are pods that vary in size, shape, colour and texture. They may be erect, crescent-shaped or coiled. They are usually slightly yellow when ripe, but may also be brown or purple in colour. Seeds are relatively large (0.2-1.2 cm long) and weigh 5-30 g/100 seeds. They are variable in size and shape: kidney, ovoid, crowder, globose and rhomboid (IBPGR, 1983). The testa may be smooth or wrinkled, white, green, buff, red, brown, black, speckled, blotched, eyed (hilum white surrounded by a dark ring) or mottled in colour. Seed shape is correlated with that of the pod. Pod length ranges from 8-22 cm with 10-20 seeds per pod.

2.3 Uses of cowpea

Cowpea is a multipurpose crop, providing food for human and feed for livestock and it is a cash generating commodity for farmers, small and medium-size entrepreneurs. It has a wide variety of uses namely as a nutritious component in the human diet as well as nutritious livestock feed. Cowpea can be used at all stages of growth as a vegetable crop. The tender green leaves are an important food source in Africa and are prepared as a pot herb, like spinach.

Immature snapped pods are used in the same way as snap-beans, often being mixed with other foods. Green cowpea seeds are boiled as a fresh vegetable, or may be canned or frozen. Dry mature seeds are also suitable for cooking and canning. Cowpea can also be used as cover crop (Timko and Singh, 2008; Langyintuo et al., 2003; Singh, 2002). The very early maturity characteristics of some cowpea varieties provide the first harvest earlier than most other crops during production period. This is an important component in hunger fighting strategy, especially in Sub-Saharan Africa where the peasant farmers can experience food shortage a few months before the maturity of the new crop. Its drought tolerance, relatively early maturity and nitrogen fixation characteristics fit very well to the tropical soils where moisture, erosion and low soil fertility is the major limiting factor in crop production (Hall, 2004; Hall et al., 2002). In many areas of the world, cowpea is the only available high quality legume hay for livestock feed. Cowpea may be used green or as dry fodder. It can also be used for intercropping with other main crops like pearl millet (Pennisetum glaucum), maize or sorghum, cassava (Manihot esculenta L.). Cowpeas are sacred to Hausa and Yoruba tribes, and are prescribed for sacrifices to abate evil and to pacify the spirits of sickly children. Hausa and Edo tribes use cowpea medicinally; one or two seeds are ground and mixed with soil or oil to treat stubborn bowels (Nkouannessi, 2005). The protein found in cowpea is similar to the one from other legumes, rich in the essential amino acids lysine and tryptophan (Timko and Singh, 2008). However, the protein nutritive value of cowpea and other legumes is lower than that of animal proteins because they are deficient of sulfur amino acids and contain a non-nutritional factors (phytates and polyphenols), enzymes inhibitors (against trypsin, chymotrypsin and R-amylase) and hemagglutinins (Jackson, 2009). Minerals and vitamins are the other nutritional important constituents of the cowpea seeds. It has been reported that folic acid, a vitamin B necessary during pregnancy to prevent birth defect in the brain and spine content is found in higher quantity in cowpea compared to other plants (Timko and Singh, 2008; Hall et al., 2003). The

total crude protein in foliage ranges from 14-21% and in crop residues; it is 6-8%. The high protein content in all cowpea parts consumable by human and animal (leaves, stems, pods and seeds), is the key factor in alleviating the malnutrition among women and children and improvement of healthy status of the livestock in resource-limited households where regular access to animal protein is limited due to low economic status. Different dishes can be prepared from cowpea. The young tender leaves can be cooked and eaten as vegetable, the green pods can be cooked and eaten just like gree beans, the seeds can be cooked when fresh (semi-ripe) and, when fully matured and dry, eaten as pulses. Several legumes are produced in Ghana, but cowpea is preferred on account of its short life cycle, fodder use and quality. The dry seeds may be boiled and eaten with "Gari" (a cassava product). It is also boiled together with rice and a colouring agent to give "Waakye". The boiled seeds could also be served with fried ripe plantain (Quaye et al., 2009). It is also used in preparation of weaning foods. In Ghana and other African countries like Tanzania and Niger, cowpea is used for preparation of stew that is either used together with cereal dishes or directly mixed with the cereals as maize, wheat, sorghum and rice. In Ghana, cowpea is boiled and also prepared in traditional dishes called "Gari" and "beans". The young leaves are used to prepare green sauce for different dishes. During the raining season, farmers can use immature pods to resolve their food problems before other crops are harvested.

2.4 Cowpea production systems

Traditionally in West and Central Africa, and Asia, cowpeas are grown on small farms often intercropped with cereals by the small scale farmers. Fertilizers and pesticides are generally not used, because they are too expensive or not available for the small farmers. In Western Africa; Ghana, Mali, Niger and Nigeria both fodder and grain type varieties are grown sometimes as a pure crop and its commercial production is mostly done in these states. The cultivation of cowpea is mechanized in developed countries (Fery, 1985).

2.5 Cowpea production in Ghana

Cowpea is an important component of sustainable cropping system in Ghana. It is cultivated for the leaves, green pods, grain and haulm for livestock feed. Cowpea is an important source of vegetable protein and minerals for over 70% of Ghana population and it is the second most important grain legume. It is currently a food security crop (MOFA, 2010). Thus, rotating or intercropping cowpea with crops such as maize, sorghum, millet and cassava contributes to the improve soil fertility. Sources of cowpea seeds for planting include market/traders, stored seed from own farm and from other farmers who preserve seeds for sale (ash is used to preserve seeds) (MOFA, 2005).

2.6 Constraints to cowpea production in Ghana

The major constraints to cowpea production in Ghana are insect pests, diseases, drought and low soil fertility (ICRISAT, 2013). Chiezey et al. (1990) and Kan‟ankuk‟a (1999) also identified absence of right strains of rhizobia in the soil as one of the constraints to cowpea production. Lack of inputs such as fertilizer, insecticides and improved seeds, poor cultural practices and lack of appropriate machinery for expanding planted area are other constraints experienced. Most cowpea crops are rain fed and although it is drought tolerant, cowpea farmers in the dry areas of sub-Saharan Africa obtain low yields, estimated at about 350 kg per hectare.

2.7 Fertilizer use

Approaches for soil-fertility management range from recurring fertilizer applications to low external input agriculture based on organic sources of nutrients (Sanchez and Leakey, 1997). Although both extremes work well in specific circumstances, they pose major limitations for most smallholder farmers in Africa. Fertilizer application in tropical agriculture has the

potential to dramatically increase production due to the highly weathered soils and the limited reserves of nutrients (Stewart et al., 2005) and therefore should be at the core of strategies to restore soil fertility and raise crop productivity. Over the years, there has been a rapid increase in fertilizer application worldwide as a result of the favourable policies which were created by introducing fertilizer subsidies and crop price-support programme and investment in distribution systems (Bumb, 1989). However, there has been a reduction in the use of fertilizer especially in sub-Saharan Africa (Sanchez and Leakey, 1997) due to several reasons ranging from high cost to availability. In Africa approximately 1.38 million tons of fertilizer per year is applied to cultivated lands resulting in an average fertilizer consumption of 8.3 kg /ha. This consumption represents only 2% of worldwide demand and the lowest in the world (Morris et al., 2007).

2.8 Constraints to mineral fertilizer use

Although mineral fertilizers can improve crop nutrition, they are sparingly used by farmers in Ghana, as in many regions in sub-Saharan Africa, partly due to the prohibitive cost as a result of removal of government subsidies (Gerner et al., 1995). Sanginga and Woomer (2009) stated that fertilizer consumption pattern within nations in Africa are often sketchy and inconsistent. Most smallholder farmers in Africa use fertilizers, but they are seldom able to apply them at the recommended rates and at the appropriate time because of high cost, lack of credit, delivery delays, and low variable returns (Heisey and Mwangi, 1996; Larson and Frisvold, 1996). Such constraints are largely due to the lack of an enabling policy environment in rural areas caused by the poor road and market infrastructure typical in most African countries. The price of fertilizers in rural areas of Africa is usually at least twice the international price (Bumb and Baanante, 1996). African countries subsidized fertilizers; however, the removal of fertilizer subsidies by most African governments has increased fertilizer prices in relation to crop prices

in many of these countries (Bumb and Baanante, 1996). Fertilizer recommendations disregard variations in crop demand and soil properties and farmers‟ access to inputs and commodity markets with scales that are too large to capture soil heterogeneity (Smaling et al., 2002). Farmers on their part lack information about the best fertilizer to use for their particular fields and cropping practices, making the crop response to fertilizers more erratic and less profitable. Even within more localized recommendation domains, households operate at different stages of economic development leading to misuse and associated economic (Chase et al., 1991) and environmental risks (Bundy et al., 2001). The decline in the use of mineral fertilizer in Ghana can be attributed to policy changes by the Government of Ghana since 1988. CSIR – NARP (1998) identified privatization of the importation and distribution of fertilizers and the removal of subsidies as one of the causes of low fertilizer use. Obeng et al. (1990) also showed that economic response to fertilizer use by farmers varies with the type of farming systems and level of fertilizer application. Fertilizer supply and availability at the right time also affects its usage. Moreover, the little fertilizer available is often not the correct type required for various crops and farmers are unfamiliar with its correct usage.

2.9 Fertilizer use in cowpea production

In many African countries including Ghana, the main use of fertilizer is on maize, sorghum/millet and rice (Camara and Heinemann, 2006) with cowpea receiving little attention from farmers in terms of fertilizer application. As farmers lack adequate nutrient resources to fertilize all crops, they prefer to apply fertilizers to cereals and rarely target fertilizers directly to grain legumes which are mostly grown on residual fertility (Zingore et al., 2008). It is generally believed by most cowpea growers that the production of legumes do not require inorganic fertilizer application (Kan‟ankuk‟a, 1999). This is due to the excessive vegetation at expense of grain production of this crop under fertilized fields. Cowpea can fix about 40 kg

N/ha fromnodules in the presence of right rhizobia strain which can satisfy the crop nitrogen requirements (Singh et al., 1997). There are some reports indicating that in poor soils, cowpea hardly satisfies N requirements but the crop performance is improved by fertilization (Chiezey et al., 1990; Kan‟ankuk‟a, 1999; FAO, 2005b).

KNUST

2.10 The contribution of N fertilizers to growth and yield of cowpea

In the Southern Guinea Savanna zone of Nigeria, Abayomi et al. (2008) reported that the application of nitrogenous based fertilizer in a mixed fertilizer (30-15-15 kg NPK /ha) which gave a yield of 1.29 tons/ha is beneficial for cowpea production in the area. There was no significant increase in yield when the application rate of nitrogenous based in a mixed fertilizer was doubled (60-30-30 kg NPK /ha) which gave a yield of 1.23 tons /ha. Hasan et al. (2010) conducted a study to investigate the effect of nitrogen on the biomass yield of cowpea forage and found that application of N at the rate of 25 kg/ ha gave a biomass yield of 5.47 ton /ha and increasing the application to 30 kg /ha did not differ significantly with a yield of 5.49 tons/ ha. Ayodele and Oso (2014) working in Ekiti, sub-humid Nigeria, found that application of P at 20 kg /ha in the presence of basal nitrogen (20 kg N) and 30 kg K2O /ha is optimum P rate for cowpea production with a grain yield of 1.26 tons/ha compared to 0.78 tons /ha for the control. Similarly, Magani and Kuchinda (2009) reported that grain yields realized with 35.5 kg P /ha did not differ significantly from that of 75 kg P /ha giving a yield of 1.85 tons /ha and 1.91 ton /ha respectively.

2.11 Biological nitrogen fixation in legumes

N2, which occurs in the atmosphere and released through decomposition of organic material, is converted to ammonia by the process of BNF. This process is done through rhizobial fixation in legumes by free-living diazotrophs. Ammonia is further converted by oxidation or reduction

to the forms NO3-N and NH4+-N respectively, which are available to plants (Zahran, 1999). The plant furnishes the necessary energy that enables the bacteria to fix gaseous N2 from the atmosphere and pass it on to the plant for use in producing protein. During nodulation, host plants excrete flavonoids and bacteria Nod-protein recognize proper flavonoids, and initiate synthesis of Nod factor by a series of nod genes products (Date and Halliday, 1987). Nod factor, in return initiate early processes of nodulation. The first nodules form within one week after seedling emergence and become visible as they increase in size. Ten to fourteen days later, the nodule bacteria are able to supply most of the plant‟s nitrogen requirements. The nodules allow fixation of atmospheric nitrogen but are energetically expensive to develop and maintain (Shantharam and Mattoo, 1997). Hence the host suppresses the growth of most potential root nodules soon after the initial bacterial invasion of root hairs (Spaink, 1995). It also further regulates nodule number in response to environmental factors such as the presence of nitrate or other sources of fixed nitrogen in the soil (Vandyk, 2003). The nodules which are red or pink in colour are effective while the nodules white in colour are ineffective, or have not yet developed to a stage at which they can fix nitrogen. This partnership is known as symbiotic N fixation (Adjei-Nsiah et al., 2008). BNF by legumes is a key process in External Input Agriculture (LEIA) technologies as it potentially results in a net addition of N to the system. However, the quantity of N fixed by legumes is difficult to quantify and varies according to the species involved and the location (Webster and Wilson, 1998). Since 1973 to 1988 the average global consumption of N- fertilizer has increased from 8 to 17 kg N/ha for agricultural purpose (FAO, 1990) and this significant increase has occurred in both developing and developed countries (Peoples et al., 1995). The requirement for fertilizer N are predicted to increase in future, however, with current technologies for fertilizer application both economic and ecological cost of fertilizer usage will eventually become prohibitive. The importance of BNF as a primary source of N for agriculture has diminished in recent decades as the amount

of N fertilizer increased for the production of food and cash crops (FAO, 1990). In recent years, the international emphasis on environmentally sustainable development focuses on the use of renewable resources, which include attention on the potential role of BNF for supplying N for agriculture (Zahran, 1999).

KNUST

2.12 Factors affecting BNF

There are several environmental factors affecting BNF. The process of N fixation is strongly related to the physiological states of the host plant. Severe environmental conditions such as salinity, unfavourable soil pH, nutrient deficiency, mineral toxicity, extreme temperature conditions, low or extremely high levels of soil moisture, inadequate photosynthates, and disease conditions can affect the plant growth and development (O"Hara et al., 1988). As a result of these stresses, the persistent rhizobium strains may not be able to perform root infection and N fixation in their full capacity (Zahran, 1999). The rate of BNF is highly variable and depends on bacterial strain, legume cultivar, soil, and environmental conditions (Shantharam and Mattoo, 1997). Moisture stress can adversely affect nodule functions. Drought conditions can reduce nodule weight and nitrogenase activity. After exposing to moisture stress for 10 days, the nodule cell wall starts to degrade resulting in senescence of bacteroids (Ramos et al., 2003). Under saline conditions, the accumulation of Na+ reduces plant growth, nodule formation and symbiotic N fixation capacity (Soussi et al., 1998; Kouas et al., 2010). High salt level can affect the early interaction between the rhizobium and legumes in nodule formation (Singleton and Bohlool, 1984). The plant nitrogenase activity reduces dramatically as a result of formation of ineffective nodules at high temperature (40oC) (Hungria and Franco, 1993). Extreme soil pH can reduce rhizobial colonization in the legume rhizosphere. Nitrogen fixation can be inhibited by low soil pH (van Jaarsveld, 2002). The characteristics of highly acidic soils (pH < 4) are low level of phosphorous, calcium and

molybdenum along with aluminium and manganese toxicity, which affects both plant and the rhizobia. As a result of low soil pH conditions, nodulation and N fixation is severely affected than the plant growth (FAO, 1984). Highly alkaline (pH > 8) soils tend to be high in sodium (Na+), chloride (Cl-), bicarbonate (HCO3-) and borate (BO3-) which reduces N fixation (Bordereau and Prévost, 1994). Uddin et al. (2008) reported that nodule number and size were significantly inhibited by the application of N fertilizer (urea). Symbiotic N fixation varies according to the carbon allocation to the nodules, in relation to endogenous factors, current photosynthesis, crop growth rate and other competing sinks for carbon (Voisin et al., 2003).

2.13 Effect of applied nitrogen in cowpea production

The application rate of fertilizer N to legumes (5 to 50 kg/ha) is generally low compared to cereals and cash crop (FAO,1992), however, even relatively low level of soil nitrate are capable of depressing BNF, which is the ability of legumes to convert atmospheric N into usable form by the plant. Although, small amounts of fertilizer N have been reported to stimulate growth and N2 fixation in some instances (Zahran, 1999) the use of starter N can jeopardize N2 fixation input in other situation (Jensen,1987). Ofori and Stern (1987) reviewed the influence of applied N on various cropping system. They found out that intercrop cereals yield increased progressively with N application, while seed yield of the legume either decreased or responded less because of the inability of cowpea in mixture to derive its entire N from N2 fixation (Jensen, 1987). They concluded that N application did not improve the land Equivalent ratio (LER) and, thus, the efficiency of the legume cropping system.

2.14 The need for inoculation in legume production

Although rhizobia seem to be widely distributed in the soil, soils in different places contain different strains of rhizobia and some of which may not be effective for nitrogen fixation. Some

soils may have effective rhizobial strains, but contains higher number of ineffective strains (Herridge et al., 2002). In both cases, inoculation of the seed may be required. Inoculation of legume seed is a simple and practical means of ensuring effective nitrogen fixation. The decision to inoculate is usually based on results from experimental plots. Date (1972) explained the experiments and trials necessary for recommending the use of rhizobial inoculant. The experiments included uninoculated control plants as well as those inoculated with effective rhizobia and N fertilizer control treatments. The uninoculated treatment evaluates the presence or absence of indigenous strains and, if indigenous strains are present, provides some assessment of their symbiotic effectiveness compared with plants inoculated with selected strains of rhizobia. The inoculated treatment assesses the ability of a known effective strain of rhizobia to colonize the rhizosphere and to compete for nodule forming sites with any indigenous strain that may be present. The nitrogen treatment is included to ensure that the legume has the potential to grow well when provided with adequate nitrogen and that growth is not limited by other factors such as phosphorus deficiency or low soil moisture availability.

2.15 Inoculant strains and carriers

The bacterial inoculant strain and its carrier formulation influence the field performance and survivability of the bacteria (Albareda et al., 2009). Inoculants are available in different forms including powder (usually in peat carrier), granule and liquid. Rhizobial cells contained in the inoculant are living organisms and continue to grow and multiply (Xavier et al., 2004). The formulation of the inoculant must be such that the rhizobia survive in sufficient quantities to ensure the minimum quantity of living cells required for successful nodulation at planting (Xavier et al., 2004). In a study comparing liquid and peat based inoculants, both were shown to adequately nodulate soybeans in the field (Tittabutr et al., 2007). Most current inoculant products are supported in a liquid carrier due to the simplicity of production and application

(Xavier et al., 2004). Bacterial survival in liquid carriers has been greatly improved with new formulations. Without the protection of peat or related carriers, liquid carriers have been less consistent in maintaining high bacterial cell counts in the inoculant in the past. Rhizobial cells in liquid inoculants tend to experience starvation stress or nutrient depletion at a greater degree in comparison to those in peat (Tittabutr et al., 2007). However, quality liquid formulations currently available will maintain adequate population densities for soybean inoculation for at least three months of storage (Albareda et al., 2008). Liquid additives in these inoculant product formulations improve performance and can be customized to the individual bacterial strain (Tittabutr et al., 2007). Additives also are able to protect Bradyrhizobium japonicum on the seed when exposed to high temperatures (Tittabutr et al., 2007). Even with recent improvements to liquid formulations, peat carriers have proven to better protect rhizobia (Tittabutr et al., 2007). Companies have developed inoculants with superior bacterial strains for vigorous nodulation. However, if planting into a field with indigenous rhizobial populations, the introduced bacteria must be competitive against the resident bacterial in the soil (Berg et al., 1988). The number of root nodules per plant is directly correlated with the years since the last soybean crop. The more the number of years since the last soybean crop, the fewer the numbers of root nodules per plant. Despite new inoculants on the market that boast of improved bacterial strains, many times the naturalized rhizobia in the soil out compete the newer strains for infection of soybean roots. For improved competiveness with resident bacterial populations, in-furrow inoculants have proven to be superior (Lopez-Garcia et al., 2009). The competiveness of naturalized rhizobia may be the reason for lack of separation in the performance of inoculant products (Furseth et al. 2012). According to the authors,

soybean yield or oil and protein contents did not differ between inoculant products and the non-inoculated controls in soil with persisting B. japonicum populations. Therefore, if the strains

in the inoculant cannot compete with the naturalized populations, there is no benefit of inoculating the seed with superior rhizobia strains.

The minimum bacterial density for achieving adequate nodulation is 103 rhizobia per seed (Hiltbold et al., 1980). Nodulation in soil free of B. japonicum is directly related to the number of bacteria applied per seed. Lopez-Garcia et al. (2009) found that certain formulations or carriers out-perform others. In-furrow inoculants yielded slightly more than seed-applied inoculant on ground new to soybean in one study (Berg et al., 1988). On the other hand, Schulz and Thelen (2008) reported that liquid inoculants provided a significant yield advantage over other products in areas new to soybean production. Finally, inoculant product brand did not affect yields in a study that compared several products (Schulz and Thelen, 2008). With the ongoing inconsistencies in research results, inoculant product development will continue to be an area of research activity.

2.16 Importance of inoculant quality

Following the identification of the most effective rhizobial strain and the best inoculant carrier, it is imperative to maximize the numbers of rhizobia that colonize the seedling rhizosphere. This can be achieved by having large numbers of viable rhizobia in the inoculant itself (i.e. high quality inoculant), using higher-than-normal rates of inoculant or by minimizing the death of rhizobia between the times the seed or soil is inoculated and nodulation occurs (Kuykendall et al., 1982). All three strategies have merit, although using high-quality inoculants is more advantgeous (Brockwell et al. 1995). Roughley et al. (1993), in a field study of narrow – leafed lupin, reported that increasing the numbers of rhizobia applied to the seed from 1.9 x 104 to 1.9 x 10 increased nodule number from 8 to 26/plant; nodule dry weight from 65 to 393

mg/plant; % plants nodulated from 89 to 98%; shoot dry matter from 7.8 to 9 t/ha and most importantly, grain yield from 1.9 to 2.1 t/ha.

In similar studies of soybean, Brockwell et al. (1988) highlighted the strong, linear relationships between rhizosphere populations of rhizobia and nodulation, plant growth (shoot DM) and grain yield. Highest yields were only achieved when rhizosphere populations were >1× 105/plant. Hume and Blair (1992) reported that soybean yields in land that had not grown soybean before were increased by an average of 24% when rhizobial numbers on the seed were increased from 105 to 10.

In the narrow-leafed lupin study of Roughley et al. (1993), the survival of the inoculum through the various stages of inoculation, sowing and immediate post-sowing in the soil was quantified. Results indicated that 95% of the rhizobia died between inoculations and sowing and, of those surviving, 83% died after 23 h in the soil. Thus, only 1% of the original rhizobial cells had survived the first 24 h. Their results reinforce the need for the highest quality inoculants coupled with inoculation and sowing procedures that aid rhizobia survival. Hiltbold et al. (1980), on the other hand, examined commercial inoculants for quality and efficacy. In that study, rhizobial numbers in the commercial inoculants varied widely, from less than 103 /g to about 109/g. Nodulation of the soybean was directly related to numbers, with no nodulation produced by products supplying less than 103 rhizobia/seed, and abundant nodulation by products providing 105–106 /seed. Effects of inoculant quality on grain yield were similar. Yield increased linearly with increasing rhizobial numbers on the seed, in turn related to inoculant quality (Hiltbold et al., 1980). Another important consideration, with respect to inoculant efficacy is the age of the inoculant. Non- sterile inoculants will contain large numbers of contaminants and they depress numbers of rhizobia with time (Date and Roughley, 1977). Even in sterile carriers, numbers of viable rhizobia will decrease over time, although not at the same rate as in non- sterile carriers. Boonkerd (1991) reported differences between rhizobial

strains in storage characteristics and strong effects of storage temperature and peat treatment. The report showed that storage temperature was critical with survival of the rhizobia substantially greater at 10°C than at 30°C. The pre-treatment of the peat was also critical with numbers after 12 months storage in the irradiated peats about 3–5 times those in the autoclaved peats and 10–15 times the numbers in the non-sterile peats. Such effects are important if peat inoculants are not used immediately but stored for later use. The storage effects are of less consequence if the inoculants are used within a short time of manufacture (Boonkerd, 1991).

2.17 Factors limiting the quality of inoculants

The aim of legume inoculation is to provide high numbers of viable effective rhizobia in the rhizosphere to allow rapid colonization, nodulation and nitrogen fixation by the selected inoculants strain in order to maximize legume yield potential (Deaker, 2006). Survival is affected by the initial condition of the cells in the inoculant, particularly the moisture status, age, purity, the initial number, the strain and the type of inoculant. Changes in the physiological and morphological characteristics of cells during the maturation of the inoculant have been shown to affect survival (Lucy et al., 2004). Inoculants are usually suspended in polymeric adhesive, often containing other additives such as dyes or pigments, plant nutrients and seed protection agents, before being applied to seed.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site

The field work was carried out at the Plantation section, Faculty of Agriculture which lies on 6˚ 43′N, 1˚ 36′W with rainfall pattern being bimodal. The area has high temperatures during the day and cool temperatures at night. The soil is sandy loam. All laboratory procedures were carried out at the Soil Microbiology and Soil Science Laboratories of the Kwame Nkrumah University of Science and Technology, Kumasi.

3.2 Field work

3.2.1 Source of planting materials

The cowpea seeds used for the study were obtained from the Crops Research Institute, Fumesua, Ghana. The variety of the test crop used was „Tona". It is one of the non-creeping hybrid varieties developed by Crops Research Institute. The seed is usually pinkish in colour, ovoid in shape, smooth in texture and matures at approximately 71-80 days.

3.2.2 Biofertilizer products used

- Eco-Rhiz-Soya (a rhizobial inoculant containing the Bradyrhizobium japonicum strain WB74 for fixing atmospheric nitrogen in the root nodules of cowpea. This strain was imported from Plant Health Products (Pty) Ltd from South Africa).
- BR 3262 (is a plant growth promoting inoculant). It was developed in Brazil and was obtained from SARI (Savannah Agriculture Research Institute), Ghana.

3.2.3 Land preparation, inoculant application and planting

The field was ploughed, harrowed and manually levelled with a hoe after which the field layout was done. Plot sizes measuring 5 m x 1.8 m were demarcated. The Eco-Rhiz-Soya was applied by dissolving 12.5 g of it in 50 ml of water after which 5 ml of the resulting solution was added to 1 kg of seed in a sealed container. This was then vigorously shaken to ensure that all the seeds were adequately coated with the solution. The BR 3262 strain was applied by adding 5 g of it onto 1 kg of seed in a container with a lid and about 50 ml sugar solution was added and covered. This was then vigorously shaken to ensure that all the seeds were adequately coated with the solution. The inoculated seeds were then allowed to air dry under shade for about 30 minutes before planting.

Seeds were planted at three per hill at a planting distance of 60 cm x 20 cm. Hands were thoroughly washed with soap after planting plots with the same inoculant treatment to prevent cross contamination. The cowpea seedlings were thinned to two seedlings per hill two weeks after planting. During planting, it was ensured that the control plots were planted before the inoculated plots to prevent contamination.

3.2.4 Treatments and experimental design

The experiment was laid out in a 4 x 2 factorial, with the treatments arranged in a Randomised Complete Block Design. The first factor was fertilizer N application rates, which were 0, 15, 30 and 45 kg N/ha. The second factor was inoculant (two types; Eco-Rhiz Soya and BR 3262).

The Eco-Rhiz-Soya and BR 3262 were applied to the seeds before planting whiles the Sulphate of ammonia was applied two weeks after planting. The fertilizers were

applied by the band placement method to ensure fertilizer use efficiency and also to reduce weed growth.

3.3 Soil sampling and sample preparation

Samples were taken from each plot using an auger at a depth of 0 - 30 cm. The soil samples from the twelve plots were then bulked and thoroughly mixed to obtain homogenous samples from which sub-samples were taken for physico-chemical analyses and enumeration of rhizobia. The samples were sieved with a 2 mm mesh sieve to remove broken sticks and other debris before the analyses were carried out.

3.4 Laboratory analysis

3.4.1 Determination of soil chemical properties

3.4.1.1 Soil pH

The initial pH of the soil was 5.10 and this was determined using a glass electrode (H19017 Microprocessor) pH meter in a 1:2.5 soil to distilled water ratio. A 10 g air-dried soil was weighed into a 100 ml beaker. To this, 50 ml distilled water was added and stirred vigorously for 20 minutes. The soil – water suspension was allowed to stand for 30 minutes. After calibrating the pH meter with buffer solution of pH 4.0 and 7.0, the pH was read by immersing the electrode into the upper part of the suspension.

3.4.1.2 Soil organic carbon

The modified Walkley and Black procedure as described by Nelson and Sommers (1982) was used to determine organic carbon (0.56 %). The procedure used involves a wet combustion of the organic matter with a mixture of potassium dichromate and sulphuric acid after which the excess dichromate was titrated against ferrous sulphate. One gram soil was weighed into a conical flask. A reference sample and a blank were included in separate conical flasks. Ten millilitres of 0.166 M (1.0 N) potassium dichromate solution was added to the soil and the blank flask. To this, 20 ml of concentrated sulphuric acid was carefully

added from a measuring cylinder; contents were then swirled and allowed to stand for 30 minutes on an asbestos mat. Distilled water (250 ml) and 10 ml concentrated orthophosphoric acid were added and the mixture allowed to cool. One milliliter of diphenylamine indicator was added and titrated with 1.0 M ferrous sulphate solution.

Calculation:

Organic
0.39 mcf (12)g

KNUST

where:

M = molarity of the ferrous sulphate solution

V1 = ml ferrous sulphate solution required for blank titration

V2 = ml ferrous sulphate solution required for sample titration

g = weight of air – dry sample in grams

mcf = moisture correction factor (100 + % moisture) / 100

0.39 = 3 x 0.001 x 100 % x 1.33 (3 = equivalent weight of C)

3.4.1.3 Organic matter

Organic matter content was determined by multiplying the percentage organic carbon by 1.724 (van Bemellen factor).

3.4.1.4 Soil total nitrogen

An initial total nitrogen of 0.104 % was obtained using the Kjeldahl method; involving digestion and distillation as described by Bremner and Mulvancy (1982) was used to determine the total nitrogen. Ten grams of soil sample was weighed into a Kjeldahl digestion flask and 10 ml distilled water added to it. After 30 minutes, 5 ml concentrated sulphuric acid and selenium mixture were added, mixed carefully and digested for 3 hours until a colourless solution was observed. The digest was diluted with 50 ml distilled water and allowed to cool. The digest was made to 100 ml with

distilled water and mixed well. A 10 ml aliquot of the digest was transferred to the reaction chamber and 20 ml of 40% NaOH solution was added followed by distillation. The distillate was collected over 4% boric acid. Using bromocresol green as an indicator, the distillate was titrated with 0.02 N HCl solution. A blank distillation and titration was also carried out to take care of N traces in the reagents as well as the water used.

Calculation:

Weight of N in the soil
( )

1000

where:

A = volume of standard HCl used in the sample titration

B = volume of standard HCl used in the blank titration

N = Normality of standard HCl

3.4.1.5 Available phosphorus

The readily acid – soluble forms of phosphorus were extracted with Bray No. 1 solution as outlined by Olsen and Sommers (1982). Phosphorus in the sample was determined on a spectrophotometer (210 VGP Buck scientific) by the blue ammonium molybdate with ascorbic acid as a reducing agent. A 5 g soil was weighed into 100 ml extraction bottle and 35 ml of Bray 1 solution (0.03 M NH4F and 0.025 M HCl) was added. The bottle was placed on a reciprocal shaker and shaken for 10 minutes and filtered through a Whatman No. 42 filter paper. An aliquot of 5 ml of the filterate was pipetted into 25 ml flask and 10 ml colouring reagent (ammonium paramolybdate) was added followed by a pinch of ascorbic acid. After mixing well, the mixture was allowed to stand for 15 minutes to develop a blue colour. The colour was measured using a spectrophotometer at 660 nm wavelength. A standard series of 0, 1.2, 2.4, 3.6, 4.8, and 6.0 mg P/L were prepared by pipetting respectively 0, 10, 20, 30, 40 and 50 ml of 12.0 mg P/L in 100 ml volumetric flask and made to volume with distilled water. The initial available phosphorus was then extrapolated from the standard curve which gave 6.91 mg/kg.

Calculation:
P (mg kg)
( )g

where:

a = mg P/l in the sample extract

b = mg P/l in the blank

g = sample weight in grams

mcf = moisture correction factor

35 = volume of extraction solution

15 = final volume of the sample solution

KNUST

3.4.1.6 Extraction of exchangeable cations

Potassium in the soil was determined in 1.0 M ammonium acetate (NH4OAc) extract. A 10 g sample was transferred into a leaching tube and leached with a 250 ml of buffered 1.0 M ammonium acetate (NH4OAc) solution at pH 7. Hydrogen plus aluminium were determined in 1.0 M KCl extract as described by Page et al. (1982).

3.4.1.6.1 Determination of exchangeable potassium

Potassium in the percolate was determined using flame photometry as described by Helmke and Sparks (1996). Standard series of potassium was prepared by diluting 1000 mg/l potassium solution to 100 mg/l. Portions of 0, 5, 10, 15 and 20 ml of the 100 mg/l standard solutions were put into 200 ml volumetric flasks. One hundred millilitres of 1.0 M NH4OAc solution was added to the flask and made to 200 ml with distilled water. The standard series obtained were 0, 2.5, 5.0, 7.5, 10.0 mg/l for potassium. Potassium was measured directly in the percolate by flame photometry at wavelength of 766.5 nm.

Calculation:

xchangeable K (cmol( ) kg soil)
( ))

(10 x 39.1 x g)

where:

A = mg/l K in the diluted sample

B = mg/l K in the blank sample

g = air – dried sample weight of soil in grams

mcf = moisture correction factor

KNUST

3.4.1.6.2 Determination of exchangeable calcium and magnesium

Twenty five millilitre portion of the extract was removed into a conical flask and the volume made to 50 ml with distilled water. Potassium ferro-cyanide (1 ml) at 2%, hydroxylamine hydrochloride (1 ml), potassium cyanide (1 ml) at 2% (from a burrette), ethanolamine buffer (10 ml) and 0.2 ml Eriochrome Black T solution were added. The mixture was titrated with 0.01 M ethylene diamine tetraacetic acid (EDTA) to a pure turquoise blue colour. A 20 ml 0.01 M EDTA in the presence of 25 ml of 1.0 M ammonium acetate solution was added to provide a standard blue colour for titration and the titre value recorded. The titre value of calcium was subtracted from this value to get the titre value for magnesium.

Calculation:

$$a \quad g\ (cmol\ (\ )\ kg)$$
$$(\quad)$$

$$0.1 \times W$$

where:

V1 = ml of 0.01 M EDTA used in the sample titration

V2 = ml of 0.01 M EDTA used in the blank titration

W = weight in grams of air – dry soil extraction

0.01 = concentration of EDTA used

3.4.2 Plant total nitrogen

Plant total nitrogen was obtained using the Kjeldahl method; involving digestion and distillation as described by Bremner and Mulvancy (1982) was used to determine the plant total nitrogen. Four grams of ground dry plant sample was weighed and passed

through a mesh sieve into a Kjeldahl digestion flask and 10 ml distilled water added. One spatula of a catalyst was added followed by an addition of 5 ml of concentrated sulphuric acid. The mixture was heated strongly to digest the plant material to a permanent green colour. The digest was diluted with 50 ml distilled water and allowed to cool. The digest was made to 100 ml with distilled water and mixed well. A 10 ml aliquot of the digest was transferred to the reaction chamber and 20 ml of 40% NaOH solution was added followed by distillation. The distillate was collected over 4% boric acid. Using bromocresol green and methyl red as indicators, the distillate was titrated with 0.01 N HCl solution. A blank distillation and titration was also carried out to take care of N traces in the reagents as well as the water used.

Calculation:

Weight of N in the plant

( )

1000

where:

A = volume of standard HCl used in the sample titration

B = volume of standard HCl used in the blank titration

N = Normality of standard HCl

3.5 Number of days to emergence

The initial planting date was noted and the date of seedling emergence from each plot was also noted. The difference between the dates from the plots were calculated to determine the number of days for seedling emergence on each plot.

3.6 Plant height

Five randomly selected plants in the second row of each plot were tagged. Their heights were measured at 15, 25 and 35 days after planting (DAP). The plant height

was measured using a graduated meter rule from the soil level to the last terminal leaf of the plant. The mean height of the five plants was recorded to represent each treatment.

3.7 Plant leaf area

A manual and non-destructive method was used to determine the leaf area at the flowering stage. The three leaflets from the tagged plants in each plot were taken for the exercise. The length (L) was taken along the midrib of the leaf from the point of attachment to the petiole to the tip of the leaf. The breadth (B) was taken by measuring the maximum width of the leaf (Wilhelm and Nelson, 2000). The leaf area (LA) was estimated and their mean from the five tagged plants was used to represent the leaf area.

3.8 Nodule count

During sampling at 20 and 40 DAP, the roots of five plants were carefully dug using a shovel. The soil was carefully removed and all nodules picked into a white envelope

and sealed. The nodules attached to root hairs were gently washed under a tap, over a fine sieved to remove all remaining nodules and soil particles. The nodules were then finally counted and their mean recorded.

3.9 Effective nodules and nodule dry weight

The nodules were taken and cut open with a blade to determine the effective ones. Those with reddish and pinkish colour were considered effective while those with green, grey or dark colour were considered ineffective (Gwata et al., 2003). Nodule dry weights were then determined using an electronic scale after oven dried at 65 °C for 48 hours.

3.10 BNF determination

The total nitrogen difference method (TND) as described by People et al. (1989) was used to determine the amount of N fixed. Nitrogen fixed was calculated using the formula modified by Mary et al. (1995).

N fixed (kg/ha) =Total N in legumes – Total N in reference crop

Total N in plants= ( ) /100

3.11 Number of pods per plant, seeds per pod and 100 seed weight

Five plants were randomly taken from the outer rows and put in large brown envelopes and taken to the laboratory. The pods were separated from the five plants, counted and the mean recorded for each plot. All the pods were threshed and their seeds removed, counted and recorded. The number of seeds per pod is calculated as follows:

The seeds from each treatment were then put in the brown envelopes and oven dried for 72 hours at 65 °C. One hundred (100) seeds were counted from each envelope and weighed on an electronic scale and the weights were recorded.

3.12 Grain yield

Grain yield was determined from a net plot of 5 m x 0.45 m (2.25 m^2) measured within two middle rows of each plot (9 m^2). Plants within the net plot (the central rows) were harvested, threshed and dried in an oven for 96 hours at 65 °C and weighed. The grain yields were extrapolated from the dry weight of the grain as suggested by Okogun et al. (2005).

3.13 Crop residue dry weight

The residue from five plants of each plot were weighed after oven drying for 72 hours at 65 °C.

3.14 Quality assessment of inoculants used in the study

3.14.1 Viable cell count

The spread plated technique employed by Zuberer (1994) was used to estimate the number of viable cells in the inoculants. Physiological saline solution (0.85% NaCl) was used as diluents for all ten-fold dilution series. A 10-fold serial dilution from 10^{-1} to 10^{-10}

was prepared with each inoculant for which 100 ul of aliquots of each dilution were spread onto Yeast Extract Mannitol Agar and Potato Dextrose Agar for the Eco-Rhiz-Soya and Eco-T respectively starting from the highest dilution. Each dilution plate culture was replicated three times. The plates were then kept for 8 days at 28 ˚ in an incubator after which the dilution level having colonies between 30 - 300 were used for the estimation. The number of colony forming units (Cfu) was determined using the formula below:

fu

Aliquot plated

3.14.2 pH

This was determined using glass electrode (H19017 Microprocessor) pH meter in a 1:2.5 inoculant to distilled water ratio. Ten grams of the inoculant was weighed into a 100 ml beaker. To this, 25 ml distilled water was added from a measuring cylinder and stirred thoroughly for 20 minutes. The inoculant – water suspension was allowed to stand for 15 minutes. After calibrating the pH meter with buffer solution at pH 4.0

and 7.0, the pH was read by immersing the electrode into the upper part of the suspension.

3.14.3 Moisture content

The moisture content of the inoculants were determined according to the procedure described in America Association of Cereal Chemists (AACC, 2000). Five grams of the sample was weighed into a moisture dish which had been previously dried in an oven and weighed. The uncovered dish was then dried in the oven for 3 hours at a

temperature of 105±5
°
C. The dish was covered and was transferred to desiccators and

weighed quickly as soon as the dish was cooled. The heating and weighing procedure was repeated until successive weights did not differ by more than one milligram. The moisture content was determined using the relation below;

Calculation

oisture ( )

Weight of sample

where;

M1 = weight of empty dish

M2 = weight of empty dish + weight of sample before drying

M3 = weight of empty dish + weight of sample after drying

3.15 Statistical analysis

The data collected were subjected to Analysis of Variance (ANOVA) using Genstat statistical software version 12. Significant differences among treatments were determined using Least Significance Difference (LSD) procedure at 5 % probability. All count data were transformed logarithmically (Kihara et al., 2011) before being

subjected to ANOVA.

KNUST

CHAPTER FOUR

RESULTS

4.0 Growth parameters

4.1 Plant height

Results of plant height are presented in Table 4.1. The Nitrogen rates (15 kg N/ha, 30 kg N/ha and 45 kg N/ha) and inoculation significantly affected plant height on all sampling times. At 15 DAP, plant height was highest in the 45 kg/ha N rate, and this was significantly higher than all other treatment effects. The control treatment was significantly lower than the 30 kg/ha N rate. Eco-Rhiz Soya and BR 3262 supported plant height which were at par and either effect was significantly higher than the control treatment effect.

At 25 and 35 DAP, plants that received 45 kg N were the tallest, and this was significantly higher than all other treatment means. The 30 kg N rate also produced taller plants than the control and 15 kg N rate on 15 and 35 DAP. The control treatment effect was similar to that of 15 kg N/ha at 25 DAP, but lower at 35 DAP. At 25 DAP, both inoculants produced similar effects, and either effect was significantly higher than the control. At 35 DAP, plant height of the Eco-Rhiz soya was greater than the other treatments, while the BR 3262 produced significantly higher plant height than the control treatment.

Table 4.1 Effect of N fertilizer application and inoculation on plant height

Plant height (cm)			
Treatments	15 DAP	25 DAP	35 DAP
Nitrogen level (kg/ha)			
0	10.48	13.47	17.22
15	10.60	13.35	19.00
30	11.92	14.90	22.07
45	13.00	16.89	25.98
LSD (5 %)	0.57	0.42	0.53
Inoculants			
Control	10.85	14.03	19.85
Eco-Rhiz Soya	11.64	14.97	22.10
BR 3262	12.01	14.96	21.25
LSD (5 %)	0.49	0.36	0.46
CV (%)	6.00	3.50	6.20

DAP = Days After Planting

4.2 Number of branches

The results of number of branches at 15, 25 and 35 DAP are presented in Table 4.2. The number of branches at 15 DAP differed significantly ($P < 0.05$) among the nitrogen fertilizer levels. The 30 kg N /ha rate produced the greatest number of branches, and this was significantly higher than those of the 15 kg N /ha and control treatments only. The number of branches were not significantly ($P > 0.05$) different

among the inoculants treatments.

At 25 and 35 DAP, the number of branches were significantly different (P <0.05) among the nitrogen fertilizer levels. The rate of 45 kg N /ha produced the greatest number of branches, and this was significantly higher than the other treatments at both sampling periods. Number of branches from the 30 kg N /ha rate was also higher than

KNUST

those of 15 kg N/ha and the control treatments at 25 DAP, but only that of the control treatment at 35 DAP. The control treatment and 15 kg N/ha treatment effects were similar on sampling periods. Inoculation did not significantly (P>0.05) affect branch production at 35 DAP. However, at 25 DAP, both inoculations supported similar branch production, but either effect was greater than that of the control treatment.

Table 4.2 Effect of N fertilizer application and inoculation on number of branches

Number of branches			
Treatments	15 DAP	25 DAP	35 DAP
Nitrogen level (kg/ha)			
0	2.43	3.93	4.81
15	2.51	4.16	5.49
30	2.73	5.00	6.23
45	2.65	5.59	7.13
LSD (5 %)	0.14	0.40	0.80
Inoculants			
Control	2.51	4.12	5.35

Eco-Rhiz Soya 2.58 4.96 6.25

BR 3262 2.65 4.93 6.15

LSD (5 %) NS 0.35 NS

CV (%) 6.50 10.40 16.40

NS= Not significant at 5% probability

KNUST

4.3 Number of leaves

The number of leaves at 15, 25 and 35 DAP are indicated in Table 4.3. The number of leaves at 15 DAP was significant (P <0.05) among the nitrogen fertilizer levels. The number of leaves in all N applied treatments was significantly higher than the control treatment effect. During sampling at 25 and 35 DAP, treatment effect of the 45 kg N/ha rate was significantly higher than all treatment effects at 35 DAP, control and 15

kg N/ha treatments at 25 DAP. The control treatment effect was the lowest on all sampling occasions.

Inoculant application did not significantly affect (P>0.05) leaf production at 15 and 35 DAP (Table 4.3). At 25 DAP sampling, however, effects of both inoculant types were statistically similar, and either effect was significantly higher than the control.

4.4 Number of nodules and nodule dry weight

Nodule number and nodule dry weight during sampling at 20 and 40 DAP are presented in Table 4.4. Nodule numbers were significantly (P<0.05) different among the nitrogen fertilizer levels at 20 DAP, with the 30 kg N/ha treatment effect being significantly higher (P< 0.05) than all other treatment effects. The 45 kg N/ha treatment effect was also greater than the control and 15 kg N/ha treatment effects, whilst that of the 15 kg N/ha treatment effect was also greater than the control

treatment effect.

At 40 DAP, nodule number was greatest in the 15 kg N/ha treatment, and this was significantly higher than those of the 45 kg N/ha and the control. Additionally, the 45 kg N/ha treatment effect was significantly lower than those of 30 kg N/ha and the control treatments.

Nodule number was not significantly (P>0.05) affected by inoculation. Nodule dry weight was not significantly (P>0.05) affected by N application (Table 4.4).

Inoculation did not affect nodule dry weight at 40 DAP. At 20 DAP, the Eco Rhiz soya was significantly higher than the control.

Table 4.3 Effect of N fertilizer application and inoculation types on number of leaves

Table 4.4 Effect of N fertilizer application and inoculation on number of nodules per plant and nodule dry weight

NS= Not significant at 5% probability

4.5 Nodule effectiveness, leaf area and leaf area index

Table 4.5 shows the percentage nodule effectiveness percent at 20 and 40 DAP. The percentage nodule effectiveness at 20 and 40 DAP were significantly (P <0.05)

Number of leaves

Treatments 15 DAP 25 DAP 35 DAP

Nitrogen level (kg/ha)

0 2.42 3.83 4.85

15 2.57 4.08 5.52

30 2.65 4.94 6.07

45 2.57 5.27 6.98

LSD (5 %) 0.11 0.34 0.75

KNUST

Inoculant

Control 2.52 4.04 5.31

Eco-Rhiz Soya 2.54 4.71 6.19

BR 3262 2.59 4.83 6.06

LSD (5 %) NS 0.29 NS

CV (%) 5.5 9.1 15.4

Number of nodules per plant Nodule dry weight (g)

Treatments 20 DAP 40 DAP 20 DAP 40 DAP

Nitrogen level (kg/ha)

0 1.78 3.87 30.00 280.00

15 2.30 4.16 45.00 468.00

30 2.98 3.93 66.70 161.00

45 2.66 2.87 56.70 143.00

LSD (5 %) 0.31 0.28 NS NS

Inoculants

Control 2.30 3.69 33.80 293.00

Eco-Rhiz Soya 2.42 3.69 68.80 288.00

BR 3262 2.57 3.74 46.30 208.00

LSD (5 %) NS NS 31.11 NS

CV (%) 15.50 9.20 87.20 75.80

different among the N rates. The 30 kg N /ha treatment produced the highest effective nodules percent on both sampling periods, and this was significantly higher than all other treatment effects at 20 DAP, and only 15 kg N/ha rate at 40 DAP. The control was significantly lower than all others at 20 DAP, but at 40 DAP, it was the second best treatment.

The effective nodules percent were also significantly ($P<0.05$) different among the inoculant treatment effects only at 20 DAP, where Eco Rhiz soya inoculation produced significantly higher effective nodules percent than all other treatments. The control treatment effect was similar to that of the BR 3262 inoculant. Both leaf area and leaf area index were not significantly ($P>0.05$) affected by all treatments (Table 4.5).

Table 4.5 Effect of N fertilizer application and inoculation on percent effective nodules, leaf area and leaf area index

	Effective nodules percent			
Treatments	20 DAP	40 DAP	Leaf area (cm^2)	Leaf area index
Nitrogen level (kg/ha)				
0	85.95	92.51	62.30	7.11
15	91.13	83.41	72.30	8.03
30	91.61	94.91	71.30	7.92
45	86.48	88.26	73.30	8.14
LSD (5 %)	0.26	0.35	NS	NS

Inoculants

Control 90.00 96.75 67.60 7.65

Eco-Rhiz Soya 98.34 90.24 70.20 7.80

BR 3262 76.65 88.24 71.60 7.96

LSD (5 %) 0.22 NS NS NS

CV (%) 14.20 12.60 14.3 15.8

NS= Not significant at 5 % probability

4.6 Days to emergence and number of days to 50 % flowering

Days to emergence was not significantly different (P>0.05) among the N fertilizer levels, as well as inoculation (Table 4.6). Number of days to 50 % flowering significantly differed (P<0.05) among the nitrogen fertilizer levels. Number of days to 50 % flowering of 45 kg N ha^{-1} treatment was significantly lower than all other treatment means. The 15 kg N/ha rate gave the highest number of days to 50 % flowering.

Inoculation did not significantly affect days to 50 % flowering.

Table 4.6 Effect of N fertilizer application and inoculation on days to emergence and number of days to 50 % flowering

Treatments Days of emergence No. of days to 50 % flowering

Nitrogen level (kg/ha)

0 4.92 8.92

15 4.92 9.83

30 4.83 8.79

45 4.75 7.92

LSD (5 %) NS 0.63

KNUST

Inoculants

Control 4.88 9.12

Eco-Rhiz Soya 4.93 8.78

BR 3262 4.75 8.69

LSD (5 %) NS NS

CV (%) 7.6 8.60

NS= Not significant at 5 % probability

45

4.7 Yield and yield components

4.7.1 Number of pods per plant and number of seeds per pod

The number of pods per plant and number of seeds per pod are presented in Table 4.7. For number of pods per plant, the control treatment produced significantly the lowest number of pods than all the N applied treatments. The number of pod per plant of 45 kg N /ha

treatment was significantly higher than all N-applied treatments (Table 4.7). Among the inoculants, the BR 3262 produced the greatest number of pods, which was significantly higher than those of the control and Eco Rhiz soya treatments. Eco Rhiz soya treatment produced significantly lower number of pods than the control treatment. Number of seeds per pod of 30 kg N /ha

treatment was significantly higher

than all other N applied treatments. The difference between the control and 15 kg N/ha treatments was not significant at 5 % level of probability. The Eco-Rhiz soya treatment effect was significantly higher than that of the BR 3262 inoculant only.

Table 4.7 Effect of N fertilizer application and inoculation on number of pods per plant and number of seeds per pod

Treatments	Number of pods per plant	Number of seeds per pod
Nitrogen level (kg/ha)		
0	5.54	8.19
15	6.46	7.92
30	8.05	9.18
45	8.88	8.71
LSD (5 %)	0.24	0.42
Inoculants		
Control	7.22	8.54
Eco-Rhiz Soya	7.01	8.83
BR 3262	7.47	8.13
LSD (5 %)	0.20	0.36
CV (%)	4.00	6.00

4.7.2 Hundred seed weight and grain yield

The 100 seed weight and grain yield as influenced by the different treatments are

indicated in Table 4.8. Hundred seed weight was significantly (P<0.05) different among the nitrogen fertilizer levels. The 100 seed weight from the 15 kg N/ha treatment was significantly higher than all other treatment effects. Additionally, the control treatment effect was significantly lower than that of the 30 kg N/ha application. Inoculation significantly affected mean seed weight, while BR 3262 was greater than that of the control and the Eco Rhiz soya inoculant.

Grain yield differed significantly (P<0.05) among the nitrogen fertilizer levels. The 45 kg N ha^{-1} treatment recorded the greatest yield of 1171.09 kg/ha which was significantly higher than all other treatment effects. The control treatment effect was also significantly lower than those of the 15 and 30 kg N/ha treatments.

Grain yield following inoculation was greatest in the BR 3262 inoculant, which was significantly higher than those from the control and Eco Rhiz soya inoculant. The control treatment effect was significantly lower than that of the Eco Rhiz soya inoculant.

4.7.3 Residue and seed N content

Both N application and inoculation did not significantly (P>0.05) affect residue and seed N contents (Table 4.9).

Table 4.8 Effect of N fertilizer application and inoculation on 100 seed weight and grain yield

Table 4.9 Effect of N fertilizer application and inoculation on total nitrogen in

residue and seed

NS= Not significant at 5 % probability

KNUST

100 seed weight (g)	Grain yield (kg/ha)
Treatments	
Nitrogen level (kg/ha)	
0	8.93 917.16
15	10.04 1036.32
30	9.35 1109.57
45	9.02 1171.09
LSD (5 %)	0.34 59.56
Inoculants	
Control	9.18 982.13
Eco-Rhiz Soya	9.00 1054.44
BR 3262	9.83 1139.04
LSD (5 %)	0.29 51.58
CV (%)	4.30 6.80

Total nitrogen in residue (%)	Total nitrogen in seed (%)
Treatments	
Nitrogen level (kg/ha)	
0	1.92 9.50
15	2.11 9.46

30 2.05 8.64

45 2.18 9.39

LSD (5 %) NS NS

KNUST

Inoculants

Control 2.13 9.56

Eco-Rhiz Soya 2.00 8.98

BR 3262 2.06 9.21

LSD (5 %) NS NS

CV (%) 15.80 7.30

4.7.4 Nitrogen fixed and N derived from the atmosphere

Table 4.10 shows N fixed and N derived from the atmosphere. Both N fixed and N derived from the atmosphere were significantly ($P<0.05$) different among the nitrogen fertilizer levels. The N fixed and N derived from the atmosphere were greatest in the 45 kg N/ha treatment, and were significantly higher than all other treatment effects. Additionally, the control treatment effect was significantly lower than all the N-applied treatments. Inoculation significantly affected N fixed and N derived from the atmosphere. The BR 3262 fixed significantly greater N than the Eco Rhiz soya inoculant and the control treatment.

Nitrogen derived from the atmosphere was also significantly ($P<0.05$) higher in BR 3262 inoculant than in the control and Eco Rhiz soya inoculant.

Table 4.10 Effect of N fertilizer application and inoculation on nitrogen fixed and percent nitrogen (N) derived from the atmosphere

	N fixed (kg/ha)	N derived from atmosphere (%)
Treatments		
Nitrogen level (kg/ha)		
0	48.87	50.56
15	69.99	54.69
30	68.05	53.76
45	92.66	59.06
LSD (5 %)	2.86	1.90
Inoculants		
Control	66.79	53.43
Eco-Rhiz Soya	68.32	53.63
BR 3262	74.27	56.35
LSD (5 %)	2.48	1.60
CV (%)	3.20	2.80

KNUST

CHAPTER FIVE

DISCUSSION

5.1 Starter N and inoculation on growth of cowpea

Cowpea growth parameters measured were plant height, number of branches, leaf area and leaf area index. Generally, it was observed that plant height increased as the nitrogen rate increased. The maximum plant height were obtained with application rate of 45 kg N/ ha and this can be attributed to the fact that nitrogen promoted vegetative growth (Sharma et al., 2002). Plant height results also showed an increase in all N treatment application across 15, 25 and 35 DAP. This has been observed by several workers in several agricultural crops. Plants are usually small at the beginning of growth and therefore are not able to use all the growth resources at the initial stages (Salisbury and Ross, 1985; Gardener et al., 1985). During the early stage, their energy requirements are also small. However, with increase in plant size, more resources are used, and therefore growth becomes rapid. Plant height increased greatly (about 100 %) between 25 and 35 DAP. This meant that the growth was greater between 25 and 35 DAP than 15 and 25 DAP even though the plants were getting to reproductive stage. Generally, plant height from greater N rate treatment was higher than the inoculant treatment effect which implies more N availability from such treatments. This confirms the findings of other workers who reported that legume plant height increases with increasing fertilizer N application rates (Starling et al., 2000; Varon et al., 1984). Plant height from the inoculant treatment was greater than that of the control treatment. This is in accordance with the reports from Oad et al. (2004) and Malik et al. (2006), which showed that inoculants effect on plant height was higher than the control. Thies et al. (1991) also reported that, Eco –Rhiz soya (Rhizobium japonicum) inoculation in legumes increased plant height during growth. Number of branches were significantly different among the N applied treatment at 25 and 35 DAP. The significant influence of N fertilization on number of

branches confirm the work of Darth et al. (1977) in London, Bekere et al. (2013) in Ethiopia and Siam et al. (2012) in Egypt where N fertilization of legumes significantly affected branch production. The effect from both inoculants (BR 3262 and Eco-Rhiz Soya) did not differ significantly at 15 and 35 DAP but differed significantly at 25 DAP. The study showed that the introduced strains were not effective to significantly increase the number of branches as compared to the fertilizer treatment at 15 and 35 DAPS, respectively (Table 4.2). These findings are in contrast to the work of Date (2000). Similar result of the inability of an introduced strain to elicit significant response have been attributed to several workers (Minchin et al. (1981); Martin et al. (2003)). Leaf area and index did not differ significantly among the N and both inoculant applications, even though leaf area and index from BR 3262 inoculant were significantly higher than from the control treatments. Additionally, among the N-applied treatments, the 45 kg N/ha treatment effect was the greatest at 50 % flowering period (Table 4.6). The importance of N in leaf production is well known in plants (Hopkins, 1993; Gardner et al., 1985). Results from Lambon (2016) and Ahmed et al. (2013) suggest increase in leaf area and index as well as number of branches following the availability of N in crop production. The reports of Varon et al. (1984) and Umeh et al. (2011) also showed increasing number of leaves and branches due to availability of N following inoculation.

5.2 Starter N application and inoculation on nodulation and N-fixation of cowpea

5.2.1 Nodulation

The nodule numbers were not significantly different among the treatments and this disagrees with observations of earlier researchers who reported significant increase in nodule numbers as a result of inoculation (hemining‟wa et al., 2012; Okogun et al.,2005; Sarkodie-Addo et al., 2006). It has also been established that the environment has a severe impact on crop and its nodulation (Salvucci et al., 2012). Thies et al. (1991) also reported positive response to rhizobia

inoculation and the ability of introduced strains to compete and overcome the indigenous rhizobia is inversely related to the number of the indigenous rhizobia. It was therefore assumed that the indigenous rhizobia populations were more competitive than the introduced inoculants strains. The application of N fertilizer to the cowpea resulted in the significant difference in nodule number per plant as 30 kg N/ha treatment at 20 DAP and 15 kg N /ha at 40 DAP recorded the greatest nodule numbers, respectively. The 45 kg N/ha treatment recorded the least number of nodules per plant at 40 DAP. The results showed that application of N fertilizer at higher rates inhibited nodulation and this agrees with the finding of Uddin et al. (2008). The results also confirm the work of Chen et al. (1992) who reported that N fertilizer reduces nodulation. Due to this, several workers had recommended N fertilization of cowpea at small amounts at early stage as a starter, especially in N deficient soils which improved growth and subsequently yield, but reduced nodulation (Osborne and Riedell, 2006; Pikul et al.,2001). The results suggest that, in order to ensure good cowpea growth in the study area, N fertilizer should be applied at the rate of 15 to 30 kg N/ha to soil with low N content to enhance plant growth, nodulation and N fixation.

An important observation was that treatments that produced greater number of nodules had greater nodule dry weight, meaning such nodules were larger in size, although researchers like Blair (1989), Sarkodie-Addo (1991) and Giller (2001) had made contrary observations. Additional observation in nodule weight showed that, nodule dry weight in the N- applied treatments and the inoculant treatments were greater than the control treatment effects. The N-applied treatments provided the plant the needed start-up energy which supported greater nodulation than the control treatment. The report of Asei et al. (2015) where inoculation significantly increased nodule dry weight over the control treatments were confirmed by this present study.

5.2.2 Nitrogen fixed and N derived from the atmosphere

There have been several positive effects of fertilizer N and inoculant use on legume field investigations (Adjei-Nsiah et al., 2008). The study undertaken in the savanna zone of Ghana showed positive effects of N fertilizer and inoculant applications on N-fixed by cowpea. The soil which is inherently very low in organic matter and, therefore, soil N limit the yield of both leguminous and cereal crops, which require several management strategies to enhance crop yield and at the same time maintain or improve soil fertility.

The present results showed that inoculation and N fertilizer application to cowpea improved N-fixed as well as Ndfa. The N-fixed following N application and inoculant treatments ranged from 48.87 - 92.66 N kg/ha and 66.79 - 74.27 kg N/ha, respectively. These results are similar to figures obtained by earlier workers such as Lambon.

KNUST