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ASSESSING THE NEED FOR INOCULATION OF SOYBEAN AND COWPEA AT TONO IN THE KASSENA NANKANA DISTRICT OF THE UPPER EAST REGION OF GHANA

BY

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BSc. Agriculture (Hons)

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IN

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CERTIFICATION

I hereby declare that this submission is my own work towards the MSc and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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DEDICATION

I dedicate this work to Prof R. C. Abaidoo, Madam Mary Awuah and Vida Donkor whose effort has brought me thus far.

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ABSTRACT

Soybean and cowpea can fix atmospheric nitrogen through symbiotic association with indigenous rhizobia but unfortunately, the amount of N_2 – fixed is usually not enough due to the presence of ineffective or low numbers of indigenous rhizobia. An experiment consisting of three treatments with four replications was set up in Randomized Complete Block Design to evaluate the effect of Legumefix on soybean and cowpea in a need to inoculate trial at Tono, in Kassena Nankana district in the Upper East region of Ghana. Rhizobia were isolated from the uninoculated fields and screened for effectiveness in the plant house at the soil microbiological section of soil research institute. Legumefix inoculated plants recorded shoot biomass that were lower than the urea – fertilized plants by 917 kg ha^{-1}. Legumefix inoculation increased biological nitrogen fixation in cowpea by 10.7% but the uninoculated plants recorded in excess of 10.3 kg N $ha^{-1}$of N_2 – fixed over the Legumefix inoculated soybean plants. Urea – fertilized plants produced significantly ($P > 0.05$) higher grain yield of soybean (2150 kg ha^{-1}) than Legumefix inoculated plants (1908 kg ha^{-1}) and that of uninoculated treatment (2029 kg ha^{-1}) was also higher than the inoculated plants but the differences were not significant. Legumefix inoculated treatment recorded the highest grain yield in cowpea (797 kg ha^{-1}) but the differences between this and the other treatments were not significant. The isolates obtained from the indigenous rhizobia population and tested on cowpea were classified into categories, effective (16%), moderately effective (42%); and ineffective (42%). Comparatively, 32% of the isolates from the indigenous population possessed symbiotic effectiveness superior to the reference strain, USDA 138. Isolates obtained from the indigenous rhizobia population and tested on soybean

were classified as highly effective (11%), effective (22%), moderately effective (11%) and ineffective (55%). Comparatively, 22% of the isolates from the indigenous rhizobia population possessed symbiotic effectiveness similar to the standard strain, USDA 138. This possibly explains why responses to Legumefix inoculation were not clearly significant.

CHAPTER ONE

1.0 INTRODUCTION

Nitrogen fixing bacteria are able to form symbiotic associations with legumes and fix nitrogen through Biological Nitrogen Fixation (BNF). The bacteria involved in this process is able to utilize molecular nitrogen with the help of the nitrogen fixing enzyme to convert atmospheric nitrogen into ammonia, a form that can easily be used by plants (FAO, 2006). The symbiotic association between the bacteria and the host legume is such that the host legume provides nutrition for the bacteria and the bacteria fix nitrogen (Keyser and Li, 1992; Unkovich and Baldock 2008). Although this association requires high amount of energy, its energy source is inexpensive and renewable and as such sustainable. BNF can improve soil fertility through the addition of nitrogen (Okogun *et al*., 2005). Herridge *et al*. (2008) reported that grain legumes contribute more than 20 million tons of fixed N each year indicating that the contributions of BNF cannot be undermined. Tahir *et al*. (2009) reported that the BNF capacity of legumes is a vital process for sustaining crop land management and is an effective and efficient source of N supply to plants under favourable atmospheric and environmental conditions.

Soybean and cowpea can fix atmospheric nitrogen through symbiosis with native rhizobia. Grain legumes including soybean and cowpea can fix over 200 kg N ha^{-1} (Giller, 2001). Soybean is specific with respect to the kind of rhizobia it forms symbiosis with and can only nodulate effectively with most *Bradyrhizobium japonicum* strains. *Bradyrhizobium japonicum* hardly exists in soils of Ghana since soybean is an introduced crop (Okogun and Sanginga, 2003). Promiscuous soybean varieties have been introduced to overcome specificity issues and to allow the plant to nodulate freely

with the native rhizobia (Okogun and Sanginga, 2003). Cowpea, on the other hand, is generally promiscuous but the amount of N_2 fixed by these promiscuous cultivars is not adequate to meet the plants demand for nitrogen thus affecting its yield. The numbers and the symbiotic effectiveness of indigenous rhizobia are important to successful inoculation as they can obviate significant response to rhizobia inoculation (Singleton and Tavares, 1986; Thies *et al*., 1991; Okogun and Sanginga, 2003).

Although, Fening and Danso (2002) assessed the symbiotic effectiveness of the native rhizobia in Ghanaian soils, the actual numbers and the symbiotic effectiveness of the native rhizobia in Tono, in the Kassena Nankana district in Ghana is not known. It is worth noting the numbers and effectiveness of the native rhizobia vary from one location to another (Fening and Danso, 2002). Nonetheless, it can be adjusted through inoculant application which introduces specific number of rhizobia into the rhizosphere for symbiosis (Keyser and Li, 1992). Rhizobia inoculation is not practised in Tono due to the unavailability of inoculants. Martin (1988) indicated that legumes grown without rhizobia inoculation may be retarded in growth and consequently affects the yield. It is therefore imperative to inoculate soybean and cowpea with superior exotic strains of bradyrhizobium and rhizobium, respectively.

The overall objective of this study therefore, was to evaluate the need for inoculation of cowpea and soybean respectively in Tono, in the Kassena Nankana district of the Upper East region of Ghana.

The specific objectives were to:

i. assess the rhizobia population in soils of the study area and test for their symbiotic effectiveness in the greenhouse.
ii. evaluate the effect of Legumefix on the growth and grain yield of soybean and cowpea.
iii. estimate the amount of nitrogen fixed in soybean and cowpea by the N – difference method.

The above specific objectives were based on the null hypothesis that

i. sufficient and effective indigenous rhizobia does not exist in the soils of the study area.
ii. Legumefix will not lead to improvement in growth and grain yield of soybean and cowpea.
iii. Legumefix will not increase the amount of nitrogen fixed in soybean and cowpea.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of soybean and cowpea

Legumes play an important role in natural ecosystems, agriculture, agro-forestry and industries. Soybean (*Glycine max* L. Merril) and cowpea (*Vigna unguiculata* (L.) Walp) are among the important legumes cultivated in Ghana. Both crops are used in the livestock industry to prepare feeds for livestock. Soybean is regarded as the most valuable grain in the world because of its source of oil and protein (Keyser and Li, 1992). Grain legumes are cheap sources of protein especially to the poor (Ennin *et al*., 2004). Soybean contains 40% protein, 30% carbohydrate and 20% oil (Tefera, 2010), and therefore has the potential for alleviating malnutrition problems. In sub Saharan Africa, cowpea is usually included in rotations and intercrops to fix atmospheric nitrogen. Cowpea has the ability to grow fast and more importantly the spreading type is able to control weeds and erosion (Harrison *et al*., 2007). Aside being processed into oil, cowpea and soybean can be used to prepare a variety of recipes.

2.2 Factors affecting rhizobia inoculation and biological nitrogen fixation

The introduction of superior strains of rhizobia into the soil does not guarantee a higher BNF hence higher yield (Lupwayi *et al*., 2000). However, in the absence of all other factors that affect nitrogen fixation, an introduced strain should be able to compete with the native rhizobia for nodulation. The efficiency and effectiveness of the introduced strain is limited by a number of factors; these factors have the tendency to influence the symbiotic relationship between the legume and the rhizobia. It reduces the ability of the rhizobia to form nodules with optimum N_2 - fixing capacity (Slattery and Pearce, 2002).

The success of inoculation, therefore, depends on a number of factors which are not excluded to the following: indigenous rhizobia, and N availability (Keyser and Li, 1992).

2.2.1 Indigenous / native rhizobia

The indigenous or the native rhizobia are the rhizobia inhabiting the soils of an area. Depending on the cropping history of the area and the type of crop being grown, symbiotically compatible rhizobia may or may not be present. The quality of the native rhizobia can affect plants response to inoculation (Giller and Cadisch, 1995; Peoples *et al*., 1995; Date, 2000).

A higher population of symbiotically effective indigenous rhizobia will have a competitive advantage over introduced strains because it is already adapted to the conditions of the area. According to Thies *et al*. (1991), "native rhizobia present a strong competition to the establishment of an introduced strain which sometimes leads to inoculation failure". Castro *et al*. (1999) reported that indigenous rhizobia are more competitive after studying nodulation of peanuts in the presence of indigenous rhizobia and introduced strains. To overcome this situation the introduced strains should be applied at a very high rate. Triplett and Sadowsky (1992) suggested that to overcome the competition presented by indigenous rhizobia and increase the competitive advantage of introduced strains, significant amounts of inoculants must be applied to legumes. An increase in the number of indigenous rhizobia decreases the possibility of enhancing yield with inoculant (Thies *et al*., 1991). Fening and Danso (2002) classified the native rhizobia in the soils across Ghana into effective, moderately effective and ineffective with respect to the organism's ability to nodulate cowpea. From their study,

6% were highly effective, 68% were moderately effective and the remaining 26% were ineffective. The study however, stressed that Bradyrhizobium populations and effectiveness vary considerably among locations in Ghana (Fening and Danso, 2002).

2.2.2 N availability

The amount of nitrogen fixed is usually high in soils with low mineral N but with sufficient water and enough of other nutrients capable of supporting plant growth (Unkovich *et al*., 2008). Nodule formation and functioning is suppressed as the level of soil mineral N in the rhizosphere increases (Keyser and Li, 1992). Ideally higher nodulation should increase the amount of nitrogen fixed but this could be limited by several environmental factors. For example, the legume - rhizobium symbiosis may not produce enough nitrogen during the early stages of growth to meet the N demand of the legume hence small application of chemical N is necessary to promote early growth (Keyser and Li, 1992). Nitrogen application at either vegetative or flowering stage can potentially increase pod and crop biomass by 44% and 16% respectively (Katulanda, 2011). There are several contradictory reports on the response of legumes to nitrogen application. There is a higher probability of obtaining positive response to inoculation when soil nitrate is low and the legume has a high potential for growth and in the same way high soil nitrate can potentially hinder N_2 fixation (Peoples *et al*., 1995). Response of legumes to nitrogen application depends on the time of application and the rates of application (Yinbo et al., 1997). Application of N fertilizer at the pod filing stage increases the proportion of plant N derived from the N fixation (Yinbo *et al*., 1997).

2.2.3 Nutritional and environmental constraints affecting BNF

Superior strains may be available but if there are nutritional and environmental constraints, the full potential of the strain may not be fully realised thus the amount of nitrogen fixed is strongly dependent on nutritional and environmental factors. Legume – rhizobium symbiosis leading to nitrogen fixation is limited by nutrient deficiencies and this has adverse effect on yield (O'Hara *et al*., 1988). Therefore, for optimum growth and effective symbiosis between the host and rhizobium hence N fixation, there must be an adequate supply of all the essential nutrients that affect the growth of the host legume and the rhizobia (FAO, 1984; O'Hara *et al*., 1988)

For example, aluminium and manganese produce toxic effects which adversely affect plants, hinder the survival of rhizobia and limits nitrogen fixation (FAO, 1984; Giller, 2001). Aluminium concentrations tend to be high in soils with low pH and with the exception of cowpea most legumes will not nodulate under such conditions (FAO, 1984). However, application of calcium is known to regulate the toxicities of aluminium and manganese and enhances the survival of rhizobia and increases the ability of the rhizobia to infect legumes (FAO, 1984; Giller, 2001).

Boron is involved in meristematic activity in both the host legume and the nodule; a deficiency of which causes nodule dysfunction (FAO, 1984). Sulphur has no direct effect on nodulation (Giller, 2001) but a deficiency will result in lower protein yield as its forms part of many amino acids (FAO, 1984). Zinc, chloride and cobalt have no effect on nodulation but are required for the growth of the host legume (FAO, 1984).

Phosphorus is one of the major essential nutrients required for plant growth. Under low or high pH conditions, large amount of phosphorus is fixed in the soil and these become

unavailable to plants (Chen, 2006). One major problem with the soils of the study area is phosphorus fixation. Survival of rhizobia in soils with low concentration of phosphorus is difficult and deficiency in plants prevents nodulation (Giller, 2001). Phosphorus forms part of the ATP which is required to provide energy for the rhizobia for fixation of nitrogen; about 25 – 28 molecules of ATP which is equivalent to 10 kg of carbohydrates is required by the rhizobia for each kilogram of nitrogen fixed (Keyser and Li, 1992). Low P may cause a reduction in yield by affecting N_2 fixation in nodules and consequently causing N deficiency in shoot (Singleton *et al*., 1985). Phosphorus - fertilization improves nodulation and plant growth in soils with P deficiency (Giller, 2001). Application of P - solubilizing bacteria (FAO, 2006) reduces soil pH and brings about dissolution of bound forms of phosphate thus making P available to the plants (Chen, 2006). Phosphorus solubilizing bacteria (PSB) is an ideal option for increasing concentrations of available P to plants as the application of chemical P is adsorbed by the soil particles causing a reduction in concentration of available P to the plants and reducing nitrogen fixation. Phosphorus solubilizing bacteria (PSB) can be applied to seeds just as inoculants before planting (Chen, 2006).

Soil moisture influences nitrogenase activity and nodulation (Danso *et al*., 1992). Biological nitrogen fixation is highly sensitive to moisture stress (Ledgard and Steele, 1992). The number of rhizobia in the soil decreases with drought causing a reduction in rates of N_2 fixation (Giller, 2001).

2.2.4 Biological agents

Pest and diseases can cause defoliation of leaves thus photosynthesis is impaired. The supply of nutrients (carbohydrates) which serves as energy for the rhizobia is reduced

hence reduction in the effectiveness of the symbiosis between the rhizobium and host legume and this can have adverse effects on nitrogen fixation (Giller, 2001). The host plants ability to tolerate these stress conditions is vital for the symbiotic relationship (Keyser and Li, 1992). Competition of plants with weeds for growth resources affects photosynthesis hence reduction in the nutritional supply to the rhizobia which affects the amount of N_2 fixed.

2.3 When 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 (Giller and Cadisch 1995; Herridge *et al*., 2002). The need to inoculate trials is normally set up with three treatments consisting of: inoculated treatment, non-inoculated treatments receiving no fertilizer and non-inoculated plants furnished with fertilizer nitrogen (FAO, 1984; Date, 2000). The inoculated treatment explains the maximum number of rhizobia that could be added to improve yield and also gives an opportunity to evaluate the effectiveness and the competitive ability of known superior strains; the uninoculated treatments measures the effect of soil N and gives an idea about the presence or otherwise of the native rhizobia as well as the symbiotic effectiveness of the native rhizobia and lastly the N fertilized treatments measures the maximum potential yield of the plants when N is not limiting (Thies *et al*., 1991; Date, 2000). 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. Inoculation may increase the

number of rhizobia in the soil for infection and promote symbiotic effectiveness between the host legume and the rhizobia, hence increased nodulation. 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).

2.4 Characteristics required for an effective symbiosis

In order to overcome the competition presented by the indigenous rhizobia, the selected rhizobia must be extremely effective and efficient with the legume it forms symbiotic association with (Keyser and Li, 1992). Brockwell *et al.* (1987) identified the following as the best attributes required by rhizobia for N_2 – fixation; competitive ability, ability to survive in seed pellets, persistence in soil, ability to multiply in broth and survive in inoculant carriers, ability to fix N in different environments, ability to adapt to adverse environmental conditions.

2.5 Approaches to enhancing N_2 – fixation

Approaches geared towards increasing biological nitrogen fixation depends on the interaction effects of the legume genotype, the rhizobium strain, the environment and the management of the aforementioned factors (Giller and Cadisch, 1995; Peoples *et al*., 1995; Keyser and Li, 1992). Breeding for improved cultivars of legumes may enhance the genetic potential of the plants in fixing nitrogen; this can cause 10% increase in N_2 – fixed relative to existing cultivars (Giller and Cadisch, 1995). Good growth of the legume is required for the symbiosis as it supplies nutrients to the rhizobia (Keyser and Li, 1992). Any mechanism aimed at breeding for superior strains or selection of the rhizobia should factor in the desirable qualities described in section 2.4 above.

Practices that regulate the population of rhizobia, decreases the inhibitory effects of soil nitrate and legume biomass can change the inputs of fixed N substantially (Peoples *et al*., 1995). Intercropping legumes with cereals in soils rich in nitrogen is one of the effective ways of increasing nitrogen fixation as its assumed that the cereal will establish effective rooting system than the legume thus utilizing most of the nitrogen in the soil before the legume becomes well established (Giller and Cadisch, 1995). Lupwayi *et al*. (2000) had suggested that carrier for rhizobia and methods of inoculant application should be reviewed under extensive conditions so that site or country specific recommendations can be made rather than generalised recommendations.

The most difficult factor to alter is the environment and therefore efforts must be geared towards maximizing systems that best fit a particular condition as well as using legumes and strains that are widely adapted to different climatic conditions (Giller and Cadisch, 1995). Edaphic, nutritional and climatic issues are among the environmental factors (Giller and Cadisch, 1995; Peoples *et al*., 1995).

Education about the benefits, availability, handling and the use of rhizobia inoculant can make a significant impact on improving BNF in developing countries (Keyser and Li, 1992). Without proper management and requisite skills by the farmer or the researcher, the above interventions will not yield the needed results.

2.6 N_2 – fixation in the maintenance of soil fertility

Losses of nutrients from the soil occur through leaching, crop removal through harvesting and soil erosion (Stoorvogel, 1993). In as much as these losses are inevitable,

it can be curtailed through the application of organic, inorganic and biofertilizers (Giller and Cadisch, 1995).

Legumes in farming systems can minimize the losses of nutrients through erosion as some of these legumes form canopy, which reduces the impact of rain drops (Giller and Cadisch, 1995). The litter also enriches the soil particularly with nitrogen thus legumes contribute to the maintenance of soil fertility through N_2 – fixation (Giller and Cadisch, 1995). Okogun *et al*. (2005) reported that soybean – rhizobium symbiosis can lead to nitrogen fixation of 253 kg N ha^{-1}. Giller *et al*. (1997) also indicated as high as 227 kg N ha^{-1} can be fixed through soybean – rhizobium symbiosis. Cowpea has been reported to fix 34 kg N ha^{-1} (Yusuf *et al*., 2006). Peoples *et al.* (1995) established that the plant remains after harvesting, if not transferred from the field can contribute to increasing the fertility of the soil.

2.7 Quantification of biological nitrogen fixation

The quantification of BNF is the estimation or measurement or the assessment of the amount of nitrogen derived from the atmosphere as a result of the symbiotic association between rhizobia and a host legume. There are many methods for quantifying BNF (Danso, 1995) but among the methods the following are commonly used; the total nitrogen difference (TND) method, ureide method (xylem-solute), acetylene reduction assay (ARA) technique, and the use of ^{15}N labelled compounds (Danso, 1995; Unkovich *et al*., 2008). Although some of the methods may be more accurate than others, none is perfect as each method has its own disadvantages. The quantification of BNF is necessary for the following reasons:

- to determine if the selected legume has the ability to fix biological N (Unkovich *et al*., 2008).
- ascertain the effects of management practices on the amount of biological N fixed and the amount it can fix s(Unkovich *et al*., 2008).
- to determine the effectiveness of the symbiotic association between an introduced strain and a host legume as well as the indigenous strain and a host legume.
- to determine the contribution of biological N to farming systems (Unkovich *et al*., 2008)

2.7.1 Total nitrogen difference method

This method compares the total N of N_2 fixing plant and that of a non N_2 fixing plant (usually known as a reference crop) - N_2 fixing plant is assumed to have two sources of nitrogen; soil mineral N and atmospheric N whereas the non N_2 fixing has only one source of nitrogen which is the soil mineral N and since both crops are assumed to use the same amount of soil mineral N, the difference between the two is estimated as the amount of nitrogen derived from the atmosphere (Danso *et al*., 1992; Unkovich *et al*., 2008).

Although the total nitrogen difference (TND) method is simple, of low cost (Danso, 1995; Unkovich *et al*., 2008) and does not require specialised equipment (Unkovich *et al*., 2008), it might either cause overestimation or underestimation of the amount of nitrogen derived from the atmosphere unless the N_2 fixing plant and the non N_2 fixing has the same rooting system to utilize the soil mineral N. TND method is more reliable under low soil N conditions (Danso, 1995; Unkovich *et al*., 2008).

2.7.2 Ureide (xylem-solute) method

According to Unkovich *et al*. (2008) this method is "versatile and useful and thus can be applied in glasshouse and field experiments, or used in farmers' fields, to assess N_2 fixation by ureide-exporting tropical and subtropical legumes". The xylem-solute technique is mostly used to measure BNF for those species that produce significant quantities of ureide as product of BNF (Danso, 1995). This technique is based on the principle that the N-solute composition in xylem sap and stem segments changes from one dominated by the ureides allantoin and allantoic acid in N_2 - fixing plants, to one dominated by nitrate and amino acids in plants utilising soil N so that the difference between incoming fixed N and the soil N can be distinguished based on the substantial differences in the principal forms of N transported in the xylem between symbiotic and non-symbiotic plants (Unkovich *et al*., 2008).

This technique is also simple and relatively inexpensive (Danso, 1995). With this technique, many samples can be collected and analysed in a day (Unkovich *et al*., 2008). This technique is however, restricted to ureide exporting legumes (Danso, 1995; Unkovich *et al*., 2008) and needs to be calibrated against a known method (Danso, 1995).

2.7.3 Acetylene reduction assay technique

The acetylene reduction assay is the most widely used method to study N_2 fixation in asymbiotic systems (Unkovich and Baldock, 2008). It is based on the principle that nitrogenase, which reduces N_2 to NH_3, is also capable of reducing acetylene (C_2H_2) to ethylene (C_2H_4). This technique measures the rate of acetylene conversion to ethylene by the nitrogenase enzyme; the amount of ethylene produced is used to estimate the

amount of nitrogen derived from the atmosphere by multiplying it by a conversion ratio (Danso, 1995).

ARA is highly sensitive and as such can be used to detect nitrogenase activity (Unkovich *et al*., 2008). It is also simple and relatively inexpensive (Danso, 1995). This technique is limited by the fact that measurements reflect nitrogenase activity for only the duration of the assay – there are variations in the diurnal and seasonal activities of the enzyme and as such many measurements are required for the correct estimations of the N_2 fixed (Unkovich et al., 2008). The validity of the ARA technique is questionable because of the use of conversion ratio (Danso, 1995). There is also auto – inhibition of acetylene conversion to ethylene (Danso, 1995). Acetylene is hazardous to man as it can explode (Unkovich et al., 2008).

2.7.4 ^{15}N Methods

This method comprises of the ^{15}N natural abundance and ^{15}N enrichment methods. This method provides an accurate estimate of BNF but it is expensive and requires specialised equipment and skills (Danso, 1995). It is generally based on the principle that the concentration of ^{15}N in the atmosphere is different from that of plant - available soil N and therefore the difference in the analyses of ^{15}N of the N_2 – fixing plant and the non - fixing plant is considered as the amount of N fixed.

2.8 Quality control of inoculants

Inoculant quality control is defined as a series of checks or activities put up to maintain the worth of the inoculant during and after production to ensure that the inoculant contains sufficient and viable number of rhizobia to colonise the rhizosphere for abundant nodulation (Beck *et al*., 1993). The inoculant carrier influences its quality but

the most important considerations are the rhizobia number and age; the inoculant should contain sufficient number of rhizobia with few dying with time (Herridge *et al*., 2002). There are several types of carriers but the peat is more desirable because of the protection it offers to the rhizobia coupled with its ability to nurture the organism (Herridge *et al*., 2002). Peats, be it sterile or non-sterile are commonly used but the former is preferred because it contains 100 – fold more rhizobia and also produces superior inoculant products than the latter but the cost of sterilization is high (Herridge *et al*., 2002 ; Lupwayi *et al*., 2000). Day (1991) reported cases of negative response and positive response by plants due to the application of non-sterile carrier inoculant and sterile carrier inoculant, respectively. This is due to the fact that non – sterile carrier inoculants contain low numbers of viable rhizobia or large number of contaminants (Lupwayi *et al*. 2000).

Inoculants with viable rhizobia are similar to inoculants with contaminants or dead rhizobia on appearance hence one cannot distinguish between quality and non-quality inoculants by mere observation; in view of this some producers are reluctant to institute quality control checks (Thompson, 1991). This therefore causes farmers to lose interest in inoculant because of limited response to inoculation (Lupwayi *et al*. 2000). For this reason there should be pragmatic measures to check the quality of inoculant to ensure that high quality inoculants are sold to farmers. Production of quality inoculants without any effective laid down procedures to check and maintain quality is as equally worse as producing low quality inoculant. Standards for regulating and maintaining inoculant quality may vary among countries (Herridge *et al*., 2002). Nonetheless, it serves the same purpose of ensuring the production and maintenance of high quality inoculants for

farmers. Countries like Canada and France have working standards which have contributed to the production of high quality inoculants to farmers (Herridge *et al*., 2002; Lupwayi *et al*., 2000). For instance, between 1974 and 1998 the number of viable rhizobia in inoculants sampled from the Canadian market increased from 15% to 95% as a result of effective quality control systems (Lupwayi *et al*., 2000).

(Lupwayi *et al*., 2000) highlighted the characteristics of quality inoculants as follows: inoculants should contain large cell numbers of superior rhizobia strain, less contaminant with no effect on its efficacy, be easy to apply, adequate shelf life, proper packaging, formulation that is effective and have clear labelling with instructions for use.

The quality of inoculants can be evaluated using any of the following methods (Lupwayi *et al.*, 2000): microscopic examination , plate counts of viable cells, most probable number (MPN) or plant infection, immunological techniques, cell agglutination reaction for rhizobia identity, immuno-spot blot and colony-lift immunoblot test for rhizobia identity, indirect fluorescent antibody identification of rhizobia in broth and syringe filter enzyme immunoassay.

2.9 Summary of literature review

Legume rhziobia symbiosis leading to the conversion of atmospheric nitrogen to ammonia for plants is limited by a number of factors. Among these factors are the population and effectiveness of native rhizobia; this has been identified to obviate significant response to rhizobia inoculation as in most cases they are able to out compete foreign strains for nodule occupancy. Higher soil mineral nitrogen has been reported to inhibit nodulation and N_2 – fixation. In addition, nutritional and environmental factors like phosphorus, pH, moisture, temperature and light also affects the symbiotic association between legumes and rhizobia. The native rhizobia are often low in numbers or ineffective and are therefore not able to fix enough nitrogen to meet the nitrogen demand of plants. A classical approach to increase biological nitrogen fixation is to study the interaction effect of legume genotypes, rhizobia strains and the environment under different management systems. Promiscuous legume varieties were introduced to nodulate freely with the native rhizobia because of the scarcity of rhizobia inoculants. The native rhizobia are often low in numbers or ineffective and are therefore not able to fix enough nitrogen with the promiscous legumes to meet the nitrogen demand of plants. It is therefore necessary to introduce foreign strains for symbiosis.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site

The laboratory work was carried out at the Microbiology section at Soil Research Institute, Kwadaso and the Soil Science laboratory at Kwame Nkrumah University of Science and Technology, Kumasi.

The field work was carried out at Tono (ICOUR) which stretches from latitude 10° 49' N to longitude 1° 05' Win the Kassena Nankana district of the Upper East region of Ghana. The area belongs to the Sudan savannah agro ecological zone with a unimodal rainfall; the rains last for 5 – 6 months starting from April or early May and reach its peak in August or early September whereas the dry periods last for 6 – 7 months starting from mid-November. The annual rainfall, temperature, relative humidity, wind speed, sunshine hours and solar radiation of the area are 885 mm, 28.6 °C, 54%, 81 km day^{-1}, 7.9 h and 20.4 M J $m^{-2}day^{-1}$, respectively. The field work was conducted between June and November, 2012.

3.2 Laboratory analyses

3.2.1 Soil sampling and sample preparation

Unless otherwise stated, all soil sampling and laboratory analysis reported in this section were carried out on both soybean and cowpea fields. Seven core samples were taken from each plot at a depth of 20 cm using an augur. The soil samples were then bulked and thoroughly mixed to obtain composite samples from which subsamples were taken for chemical analysis and enumeration of rhizobia. The samples were sieved with

a 2 mm mesh sieve to remove broken sticks and other debris before the physico-chemical analyses were carried out.

3.2.2 Determination of soil physical properties

3.2.2.1 Particle size analysis

Fifty – one grams of air dried soil was weighed into a 1L screw lid shaking bottle. Hundred millilitres distilled water was added and swirled thoroughly. Twenty millilitres of 30% H_2O_2 was added, followed by 50 ml of 5% sodium hexametaphosphate and drops of amyl alcohol and swirled gently. It was then shaken on a mechanical shaker for 2 h and the content transferred into a 1L sedimentation cylinder. The first hydrometer reading was recorded after 40 seconds and the first temperature reading was also taken with the help of a thermometer. The 1L sedimentation cylinder with its content was allowed to stand undisturbed for 3 h and the second hydrometer and temperature readings recorded respectively.

Calculation

$$\% \text{ Sand} = 100 - \left[H1 + 0.2\left(T1 - 20\right) - 2\right] \times 2$$

$$\% \text{ Clay} = \left[H2 + 0.2\left(T2 - 20\right) - 2\right] \times 2$$

$$\% \text{ Silt} = 100 - (\% \text{ Sand} + \% \text{ Clay})$$

Where

H1 = 1st hydrometer reading at 40 seconds

T1 = 1st temperature reading at 40 seconds

T2 = Temperature reading at 3 hours

H2 = 2nd hydrometer reading at 3 hours

-2 = Salt correction to be added to hydrometer reading

0.2 (T - 20) = Temperature correction to be added to hydrometer reading.

3.2.2.2 Bulk density

The bulk density was determined using the core sampling method (Blake and Hartge, 1986). With the aid of a mallet, a core sampler (5 cm diameter thin – sheet metal tube of known weight and volume) was driven 5 cm into the soil. It was then removed and the soil at both ends trimmed and flushed with a straight edged – knife. It was then transported to the laboratory where it was oven dried at 105 °C to a constant weight. The core samplers were removed and allowed to cool before it was weighed and recorded. The volume of the sampler was determined and the dry bulk density calculated as follows:

$$\text{Dry Bulk Density, } p_b\ (gcm^{-3}) = \frac{W_1 - W_2}{V}$$

Where:

ρ_b = Dry bulk density

W_1= Weight of core cylinder + oven dried soil

W_2= Weight of empty core cylinder

V= Volume of core cylinder

3.2.3 Determination of soil chemical properties

3.2.3.1 Soil pH

This was determined using the Eutech 510 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, 25 ml distilled water was added from a measuring cylinder, stirred thoroughly for 20 minutes. The soil

– 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.

3.2.3.2 Soil organic carbon

The modified Walkley and Black procedure as described by Nelson and Somers (1982) was used to determine organic carbon. The procedure 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. 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, 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 allowed to cool. One milliliter of diphenylamine indicator was added and titrated with 1.0 *M* ferrous sulphate solution.

Calculation:

$$\% \text{ Organic C} = \frac{M \times 0.39 \times mcf\ (V_1 - V_2)}{g}$$

where:

M = molarity of the ferrous sulphate solution

V_1 = ml ferrous sulphate solution required for blank titration

V_2 = ml ferrous sulphate solution required for sample titration o

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)

1.3 = a compensation factor for the incomplete combustion of organic matter

3.2.3.3 Total nitrogen

The Kjeldahl method involving digestion and distillation method 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 was 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 traces in the reagents as well as the water used.

Calculation:

14g of N contained in one equivalent weight of NH_3

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

where:

A = volume of standard HCl used in the sample titration

B = volume of standard HCl used in the blank titration

N = Normality of standard HCl

Mass of soil sample used, considering the dilution and the aliquot taken for distillation

$$= \frac{10 \text{ g} \text{ -} 10 \text{ ml}}{100 \text{ ml}}$$

$$= 1\text{g}$$

Thus, the percentage of nitrogen in the soil sample is,

$$\% \text{ Total N} = \frac{14 \times (\text{A} - \text{B}) \times \text{N} \times 100}{1000 \times 1}$$

Note:

When N = 0.1 and B = 0

% Total N = A x 0.14

3.2.3.4 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* NH_4F and 0.025 *M* HCl) was added. The bottle was placed in a reciprocal shaker and shaken for 10 minutes and filtered through 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 21D spectrophotometer at 660 nm wavelengths. The available phosphorus was extrapolated from a standard curve.

A standard series of 0, 1.2, 2.4, 3.6, 4.8, and 6.0 mg P/ l was 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.

Calculation:

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

Where:

a = mg P/l in the sample extract

b = mg P/l in the blank

g = sample weight in grams

mcf = moisture correction factor

35 = volume of extraction solution

15 = final volume of the sample solution

3.2.3.5 Extraction of exchangeable cations

Calcium, magnesium, potassium and sodium in the soil were determined in 1.0 *M* ammonium acetate (NH_4OAc) extract (Black, 1996). A 10 g sample was transferred into a leaching tube and leached with a 250 ml of buffered 1.0 *M* ammonium acetate (NH_4OAc) solution at pH 7. Hydrogen plus aluminum were determined in 1.0 *M* KCl extract as described by Page *et al.* (1982).

3.2.3.5 Determination of exchangeable calcium and magnesium

A 25 ml portion of the extract was transferred into a conical flask and the volume made to 50 ml with distilled water. Potassium ferrocyanide (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. The titre value was recorded. The titre value of calcium was subtracted from this value to get the titre value for magnesium.

Calculation:

$$\text{Ca + Mg (cmol (+) / kg} = \frac{0.01 \times (V1 - V2) \times 1000}{0.1 \times W}$$

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

3.2.3.6 Determination of exchangeable potassium and sodium

Potassium and sodium in the percolate were determined using flame photometry as described by Helmke and Sparks (1996). A standard series of potassium and sodium were prepared by diluting 1000 mg/l for both potassium and sodium solutions to 100 mg/l. This was done by taking 25 mg portion of each into one 250 ml volumetric flask and made to volume with water. Portions of 0, 5, 10, 15 and 20 ml of the 100 mg/l standard solutions were put into 200 ml volumetric flasks respectively. Hundred millilitres of 1.0 *M* NH_4OAc solution was added to each flask and made to volume with distilled water. The standard series obtained was 0, 2.5, 5.0, 7.5, 10.0 mg/l for potassium and sodium. Potassium and sodium were measured directly in the percolate by the flame photometry at wavelengths of 766.5 and 589.0 respectively.

Calculations:

$$\text{Exchangeable K (cmol / kg soil)} = \frac{(A - B) \times 250 \times mcf)}{(10 \times 39.1 \times g)}$$

$$\text{Exchangeable Na (cmol/kg soil)} = \frac{(A - B) \times 250 \times mcf)}{(10 \times 23 \times g)}$$

where:

A = mg/l K or Na in the diluted sample

B = mg/l K or Na in the blank sample

g = air – dried sample weight of soil in grams

mcf = moisture correction factor

3.2.3.7 Determination of Cu, Fe and Mn by Diethylenetriamine pentaacetic acid (DTPA) extraction.

Ten grams air dried soil was weighed into plastic bottles for each of the elements above. Hundred millilitres DPTA extractant was added. It was shaken for 2 hours and filtered with Whatman No. 42 filter paper. The values were read on Atomic Absorption Spectrophotometer with the appropriate standards.

3.3 Plant tissue analysis

The shoots as well as the seeds of the plants were milled in a miller, after which nitrogen and phosphorus contents were determined.

Total nitrogen was determined according to the procedure described in section 3.2.3.3.

Total phosphorus was determined by using the spectrophotometric vanadium phosphomolybdate method. One gram of plant sample was weighed into the digestion tube. One millilitres of digestion mixture ($HCIO_4HNO_3$) was added. It was digested and made up to 500 ml in a volumetric flask. Ten millilitres of the digest was measured into a 50 ml volumetric flask. Ten millilitres of vanadomolybdate was then added. Distilled water was added to make the required volume. It was shaken vigorously and kept for 30 minutes. It was read on 430 nm spectrophotometer after a yellow colour had developed. The percentage transmittance was recorded. The absorbance and the P content were determined from a standard curve.

3.4 Enumeration of rhizobia population

The estimation of the rhizobia populations for both soybean and cowpea fields were carried out using the most probable number method (MPN) (Vincent, 1970). Uniform clean seeds of good viability were surfaced sterilized with alcohol and hydrogen

peroxide as described by Somasegaran and Hoben (1994). The seeds were pre - germinated in Petri dishes that contained moist sterile cotton wool and incubated between the temperatures of 20 °C and 30 °C. Seeds were then transferred to plastic growth pouches containing Broughton and Dilworth N-free plant nutrient solution aseptically with the help of forceps. The growth pouches were arranged in a wooden rack and kept at the greenhouse awaiting inoculation.

Five – fold dilution of each of the sub samples for both soybean and cowpea were made; five different test tubes were filled with 20 ml distilled water. With the help of a pipette, 1 ml solution was transferred from a 500 ml solution that had been shaken vigorously into one of the five different test tubes. Series of dilution were made from 1^1 to 1^6. Each growth pouch was inoculated with 1 ml of the diluent replicated four times using different pipette tips to prevent contamination. The plants were watered with sufficient N – free nutrient solution when required. Nodulation was assessed after twenty eight days.

3.5 Preparation of Congo red yeast extract mannitol medium.

The above was prepared by weighing the exact amount of each chemical required (Appendix IV) with an analytical balance and dissolved sequentially in a 1 litre beaker that was filled with 200 ml of distilled water

The beaker was placed on a magnetic stirrer and a heater (the heater was to prevent the medium from solidifying because of the agar) and made up to 1 litre. After thorough mixing, the pH of the medium was adjusted to 6.8 with a drop – wise addition of either

0.5 *N* NaOH or 0.5 *N* HCl. The medium was divided into 500 ml each before adding the agar and 1 ml of Congo red to one of the solutions.

The beaker was covered with cotton and aluminium foil and autoclaved at a temperature of 121 °C and a pressure of 1.33 bars for 30 minutes. After autoclaving, the medium was left to cool under the Laminar flow. The Congo red yeast extract mannitol agar was dispensed into Petri dishes whiles the yeast extract mannitol broth was dispensed into beakers.

3.6 Isolation and culturing of rhizobia from the indigenous population

The nodules were sampled from growth pouches following inoculation of soybean and cowpea with soils from the uninoculated plots of the study area. The isolated nodules were surface sterilized with 70% alcohol for 3 minutes and rinsed with several changes of sterilized distilled water and then placed in 20% H_2O_2 for 3 minutes followed by repeated rinses with several changes of sterilized distilled water.

The nodules were crushed in a drop of sterile distilled water in a Petri dish with a sterilized forceps. The nodule content was streaked with a wire loop onto the Congo red yeast extract mannitol agar plates (Somasegaran and Hoben, 1994) and incubated at a temperature of 28 °C. The Petri dishes were sealed with parafilm and labeled accordingly.

The forceps and the loops were flamed intermittently to minimize contamination. The inner part of the laminar flow was also surface sterilized with 70% alcohol before the isolation of rhizobia.

3.7 Testing the effectiveness of the isolated rhizobia from the indigenous population

The experiment were conducted in pots containing acid – washed sand (Somasegaran and Hoben, 1994) that had been autoclaved at a temperature of 121 °C and a pressure of 1.33 bar for 1 h. There were two seeds per pot and the pots were arranged in a randomized complete block design. The cultured isolates were looped into the yeast extract mannitol broth and placed in an orbital incubator at a temperature of 28 °C. The orbital incubator was set at 125 revolutions per minute. The broth was kept in the orbital incubator until it became turbid. One millitre of the turbid yeast extract mannitol broth was used to inoculate each plant in a pot. There were four treatments; the test isolates from the indigenous rhizobia population, USDA 138 which served as the standard strain and uninoculated plants with or without nitrogen. Each treatment was replicated three times. Cowpea plants were supplied with 0.5 g l^{-1} KNO_3 whiles soybean plants were supplied with 0.25 g l^{-1} KNO_3. The inoculated plants as well as the uninoculated without nitrogen were kept supplied with N – free nutrient solution whereas the uninoculated with N were supplied with nitrogen at 0.5 / 0.25 g l^{-1} KNO_3. The following parameters were measured accordingly; nodulation and shoot dry weight. Plant dry weight values of each isolate was compared with those of N controls and the LSD at 5% level was used to establish significance differences between treatments (Beck *et al*., 1994). Classes of effectiveness were defined from comparison with the positive control as follows: symbiotic effectiveness was high when the isolate produced plant yield equal (80% - 100%) to or greater (above 100 %) than N – fertilized plants, moderately

effective (60% - 79%) when slightly less than N controls and ineffective (below 60%) when isolates produced yields similar to uninoculated controls (Beck *et al*., 1994)

The following parameters were estimated accordingly:

Index of effectiveness (*E*) according to Ferreira and Marques, (1992) expressed as:

$$E = \frac{(Xa - Xc)}{(Xb - Xc)}$$

where:

a = the shoot dry weight of inoculated test isolates

c = the shoot dry weight of uninoculated control

b = the shoot dry weight of nitrogen control

Relative effectiveness (R.E.) of isolates in fixing nitrogen expressed as:

$$R.E = \frac{\text{shoot dry of weight inoculated test strain}}{\text{shoot dry of weight inoculated standard strain}} \times 100$$

3.8 Field work

3.8.1 Source of planting materials

Both soybean and cowpea seeds as well as the chemical fertilizers and inoculants were obtained from Savannah Agricultural Research Institute.

The soybean variety (Jenguma) is a medium maturing variety (105-110 days maturity) and takes 45 days to attain 50 % flowering. Cowpea variety (IT90K-277-2) is semi erect

and takes 80-89 days to reach full maturity (medium maturing variety) (Dugje *et al.* 2009).

3.8.2 Land preparation

The field was ploughed, harrowed and ridged at a spacing of 75 cm apart. Each plot measured 3 m x 5 m for both soybean and cowpea.

3.8.3 Planting and inoculant application

Soybean and cowpea seeds were sown on ridges at a spacing of 75 cm x 5 cm; and 75 cm x 20 cm, respectively. Soybean seeds were sown at two seeds per hill and thinned to one seed per hill two weeks later. Cowpea seeds were sown at three seeds per hill and thinned to two seedlings per hill two weeks later. The reference crop (maize) was also sown on ridges at a spacing of 75 cm x 40 cm. It was sown at three seeds per hill and thinned to two seeds, two weeks later.

The Legumefix was used as follows; for every one kilogram of seeds, five grams of the inoculant was added to it and the slurry method of inoculation was employed. It was kept under shade for 30 - 45 minutes with intermittent mixing before planting.

3.8.4 Treatments and experimental design

The experiments consisted of three treatments for both soybean and cowpea designated as follows:

- T 1 – Legumefix (5 g per 1 kg of seeds)
- T 2 – Urea (100 kg N)
- T 3 – Uninoculated (0 Legumefix, 0 kg N)

All the treatments received basal application of 30 kg P and 30 kg K from triple superphosphate and muriate of potash respectively.

The urea was applied in splits; 50 kg N at two weeks after planting and 50 kg N at 50% flowering for both soybean and cowpea. The band method of fertilizer application was used to ensure fertilizer use efficiency and also to reduce weed growth.

The experiments were laid out in a Randomised Complete Block Design (RCBD) with four replications each. Each replication had reference plot (Maize) for BNF assessment.

3.9 Data collected

3.9.1 Nodule count

At 50 % flowering, ten consecutive plants were harvested from the two middle rows of each plot of soybean and cowpea. The plants were cut at about 5 cm above the ground. The roots of the plants were carefully dug out, put in polythene bags, together with detached nodules collected from the soil. The roots were then put in a 1 mm mesh sieve and washed under running tap water to remove adhered soil. The nodules were gently removed, washed and counted.

3.9.2 Shoot dry weight

At 50% flowering, ten consecutive plants were harvested from the two middle rows of each plot for soybean and cowpea. The plants were cut at about 5 cm above the ground. The shoots were oven dried for 72 h at 60 °C. The dry weights of the shoots were recorded and later milled for laboratory analysis.

3.9.3 Number of pods per plant and grain yield

Both soybean and cowpea were harvested at physiological maturity from an area measuring 1.5 m x 2.5 m and the number of pods and grain yield determined. Pods were removed from the plants after harvesting and counted. The pods were then air dried and threshed. The grains were oven dried at 60 °C for 72 h and the dry weights recorded. The dry weights were then used to estimate the grain yield per hectare (Okogun *et al*., 2005).

3.9.4 BNF assessment

The amount of biological nitrogen fixed was assessed using the Total Nitrogen Difference (TND) method. The total amount of nitrogen in both legumes (cowpea and soybean) and the maize (reference crop) were determined and the amount of N fixed calculated using the modified equations of Mary *et al*. (1995).

$$\text{Total N in plants} = \frac{\text{shoot dry weight} \times \%\,\text{N in shoots}}{100}$$

$$\text{Amount of N fixed} = \text{Total N in legume} - \text{Total N in reference crop}$$

$$\%\,\text{NDFA} = \frac{\text{Total N in legume} - \text{Total N in reference crop}}{\text{Total N in reference crop}} \times 100$$

where NDFA = nitrogen derived from the atmosphere.

3.10 Statistical analysis

The data generated were subjected to Analysis of Variance (ANOVA) using Genstat statistical software version 12. Significant differences were assessed at 5% ($p = 0.05$)

level of significance and the means separated using least significance difference (LSD) procedure. All count data were transformed logarithmically (Kihara *et al*., 2011) before being subjected to ANOVA.

CHAPTER FOUR

4. 0 RESULTS

4.1 Symbiotic effectiveness of the isolated rhizobia from the indigenous population on cowpea at the greenhouse.

4.1.1 Nodulation

Of all the isolates from the indigenous rhizobia population, approximately, 32% produced more than 50 nodules per pot. Isolate 9 produced significantly higher number of nodules than all the isolates except isolate 1 (Table 4.1). Twenty one percent of the isolates produced nodules that were significantly ($P < 0.05$) higher than the standard strain, USDA 138. Eleven percent of the isolates produced less than 10 nodules per pot (Table 4.1). The uninoculated control with or without nitrogen did not nodulate.

Nearly 37% of the isolates produced nodules with dry weights less than 100 mg per pot and which was significantly ($P < 0.05$) lower than the dry weight of the standard strain, USDA 138. The remaining 63% recorded nodule dry weights that were not significantly different from the standard strain, USDA 138.

Table 4.1. Nodules on cowpea inoculated with USDA 138 and isolates from indigenous rhizobia population

Isolates	Nodule number pot^{-1}	Nodule dry weight mg pot^{-1}
1	63.3	110.0
2	35.3	53.3
3	17.0	120.0
4	2.3	26.7
5	29.7	53.3
6	48.0	80.0
7	37.7	20.0
8	33.0	23.3
9	83.0	146.7
11	6.0	40.0
12	24.3	196.7
14	36.7	160.0
15	45.7	186.7
16	60.3	143.3
17	30.3	143.3
18	45.3	163.3
19	53.0	133.3
20	67.0	186.7
21	51.3	136.7
USDA 138	34.7	170.0
LSD (0.05)	20.58	64.83
CV %	34.3	37.7

Values represent means of two plants per pot.

4.1.3 Index of effectiveness of isolates

The isolates from the indigenous rhizobia population were classified as effective (16%), moderately effective (42%) and ineffective (42%) based on their performance relative to the uninoculated controls with (+N) or without nitrogen (-N) (Fig. 4.1). Isolate 9 produced the highest number of nodules but had 60.4% effectiveness and was classified as moderately effective. Isolates 19, 1 and 7 were effective whereas isolates 4, 3, 11, 12, 8, 15, 14 and 5 were ineffective (Fig. 4.1).

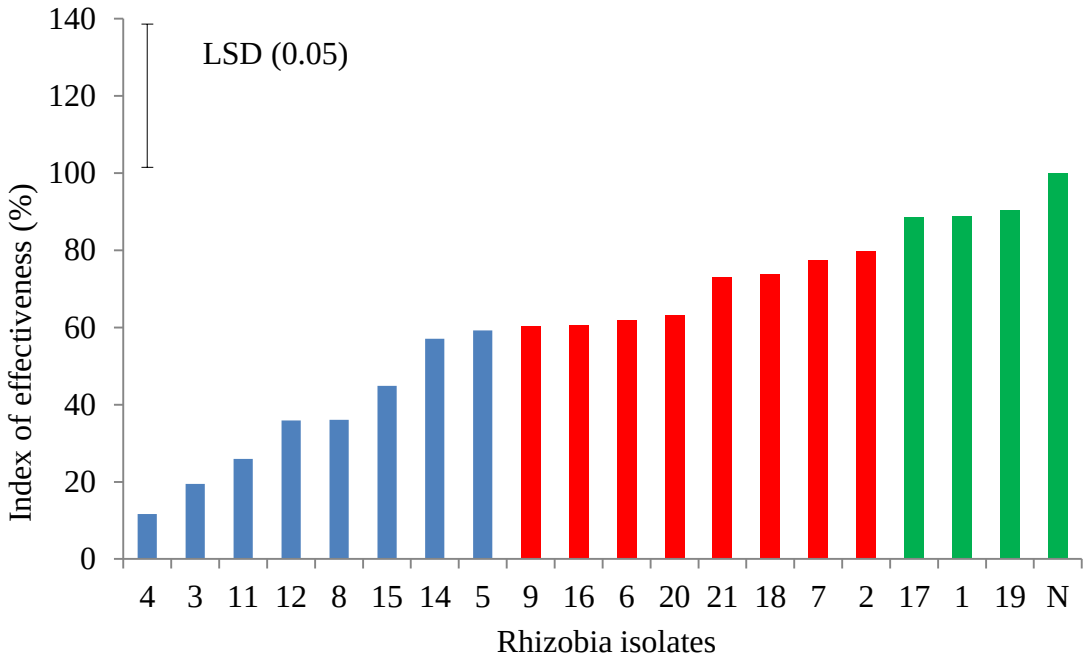

Figure 4.1. Index of effectiveness of the isolates from the indigenous rhizobia population.
1 – 19 = indigenous rhizobia. N= KNO_3 Blue bars = ineffectiveness, Red bars = moderately effective and Green bars = effective

4.1.4 Relative effectiveness of isolates from the indigenous rhizobia population

Fifteen (79%) out of the nineteen isolates from the indigenous rhizobia population that were compared to the standard strain, USDA 138 possessed symbiotic effectiveness superior to the standard strain but only five (33%) of them had symbiotic effectiveness significantly ($P < 0.05$) higher than the standard strain, USDA 138 (Fig. 4.3). Four (21%) of isolates from the indigenous rhizobia population possessed symbiotic effectiveness inferior to the standard strain, USDA 138 but the difference between them were not statistically significant (Fig. 4.3).

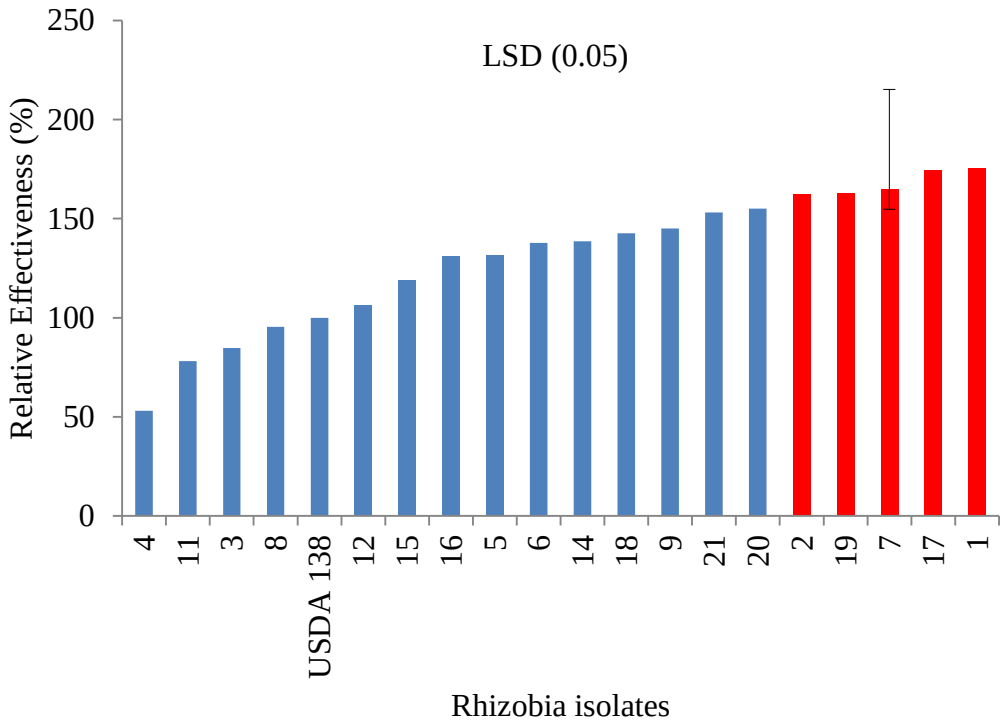

Figure 4.2. Relative effectiveness of the isolates from the indigenous rhizobia population to an adopted standard strain, USDA 138

4.2 Symbiotic effectiveness of the isolated rhizobia from the indigenous population on soybean at the greenhouse

4.2.1 Nodulation

Approximately 78% of the isolates from the indigenous rhizobia population produced nodule numbers that were significantly ($P < 0.05$) lower than the nodule number produced by the standard strain, USDA 138 (Table 4.2). Nonetheless, the difference between the means of nodule numbers produced by approximately 22% of the isolates from the indigenous rhizobia population and that of the standard strain, USDA 138 were not statistically significant ($P > 0.05$).

Approximately, 11% of the isolates from the indigenous rhizobia population produced significantly ($P < 0.05$) higher nodule dry weight (283 mg pot^{-1}**)** than the nodule dry weight (157 mg pot^{-1}**)** produced by the standard strain, USDA 138. The differences between the means of nodule dry weights produced by approximately 89% of the isolates from the indigenous rhizobia and that of the standard strain, USDA 138, were not statistically significant (Table 4.2).

Table 4.2. Nodules on soybean inoculated with USDA 138 and isolates from indigenous rhizobia population

Isolates	**Nodule number pot^{-1}**	**Nodule dry weight mg pot^{-1}**
18	11.3	93
21	20.3	167
22	49.0	283
23	23.3	90
24	22.3	123
26	13.3	90
27	15.3	153
29	16.7	163
30	39.3	153
USDA 138	62.0	157
LSD (0.05)	20.38	99.9
CV (%)	43.5	39.5

Values represent means of two plants per pot

4.2.2 Index of effectiveness

Isolates from the indigenous rhizobia population were classified as highly effective (11%), effective (22%), moderately effective (11%) and ineffective (55%) (Fig. 4.3).

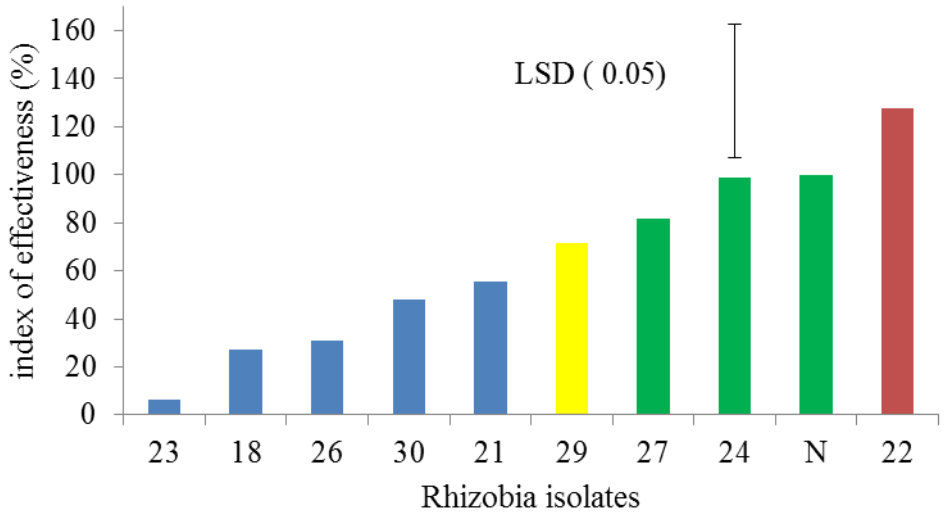

Figure 4.3. Index of effectiveness of the isolates from the indigenous rhizobia population.
18 – 30 = indigenous rhizobia. N = KNO_3. Blue bars = ineffectiveness, Yellow bar = moderately effective and Green bars = effective and Red bar = highly effective

4.2.3 Relative effectiveness of the isolates from the indigenous rhizobia population

None of the isolates from the indigenous rhizobia showed symbiotic effectiveness superior to the standard, USDA 138. However, approximately 22% (22 and 24) of the isolates from the indigenous rhizobia population possessed symbiotic effectiveness that was not statistically different from the standard strain (Fig. 4.4). Approximately 78% of the isolates from the indigenous rhizobia population showed symbiotic effectiveness that was significantly inferior to the standard strain, USDA 138 (Fig. 4.4).

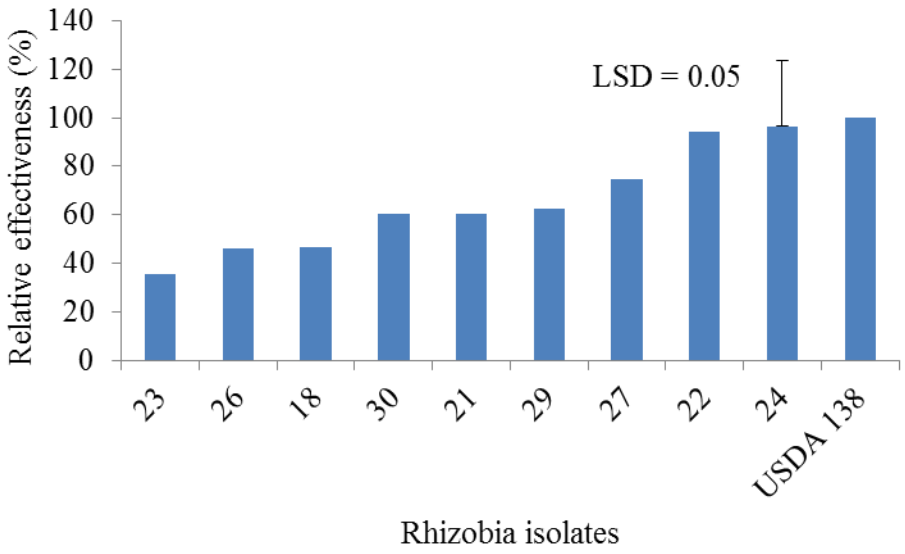

Fig 4.4. Relative effectiveness of the isolates from the indigenous rhizobia population to an adopted standard strain, USDA 138

4.3. Field work

4.3.1 Physico – chemical properties and the most probable number count

The physico – chemical properties of the study site is as shown below in Table 4.3

The estimated numbers of the indigenous rhizobia at the study area was 5.71 x 10^1 cells g^{-1} soil and 7.5 x 10^1 cells g^{-1} soil for soybean and cowpea, respectively (Table 4.3).

Table 4.3. Physico - chemical analyses and MPN count at the experimental site

Soil parameters	Soybean	Remarks	Cowpea	Remarks
pH(1:2.5) (H_2O)	5.04	*strongly acidic	4.87	*strongly acidic
Total N (%)	0.013	*very low	0.012	*very low
Available P (mg kg^{-1})	10.64	#low	12.12	#low
Exchangeable K (cmol (+) kg^{-1})	1.39	*high	0.95	*high
Organic C (%)	0.062	#very low	0.05	#very low
Exchangeable Ca (cmol (+) kg^{-1})	7.73	*moderate	11.6	*high
Exchangeable Na (cmol (+) kg^{-1})	0.73	*high	0.63	*moderate
Exchangeable Mg (cmol(+) kg^{-1})	0.28	*low	5.2	*high
Mn (mg kg^{-1})	7.33		8.33	
Cu (mg kg^{-1})	6.00		6.67	
Fe (mg kg^{-1})	29.33		26.33	
Sand (%)	88.12		86.12	
Silt (%)	5.08		7.08	
Clay (%)	6.8		6.8	
Textural class	Loamy sand		Loamy sand	
MPN (Rhizobia cell g^{-1}soil)	5.71×10^1		7.5×10^1	

*Pam and Brian (2007).
#Hills laboratories, technical notes

4.3.2 Shoot biomass of soybean and cowpea

The shoot biomass of soybean and cowpea were not significantly ($P > 0.05$) increased by the application of Legumefix and urea (Figs. 4.5 and 4.6). However, the application

of urea to soybean increased shoot biomass over Legumefix and uninoculated treatments by 917 kg ha^{-1} and 763 kg ha^{-1}, respectively (Fig. 4.5). The uninoculated treatment produced 154 kg ha^{-1} shoot biomass in excess of that of Legumefix (Fig. 4.5). The urea fertilized plots recorded the highest shoot biomass in cowpea; producing 296 kg ha^{-1} and 294 kg ha^{-1} more than the inoculated and uninoculated plants, respectively (Fig. 4.6).

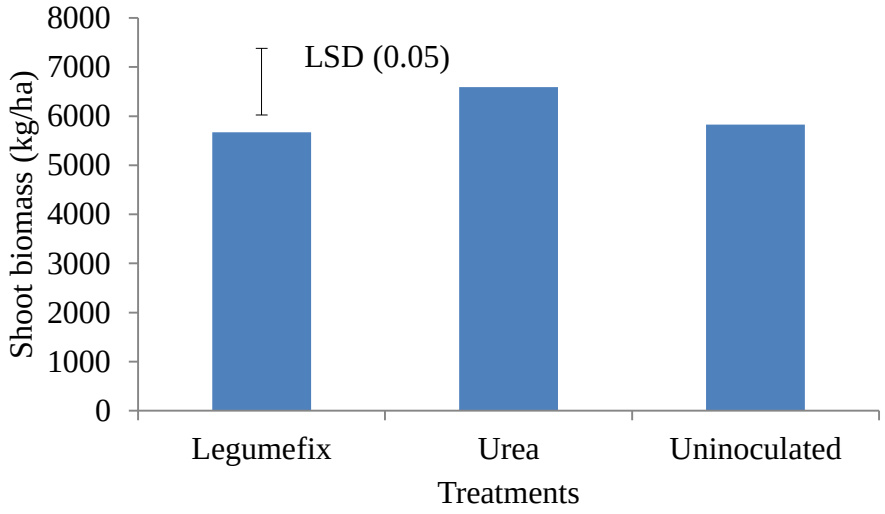

Figure 4.5. Effect of Legumefix inoculation on shoot biomass of soybean

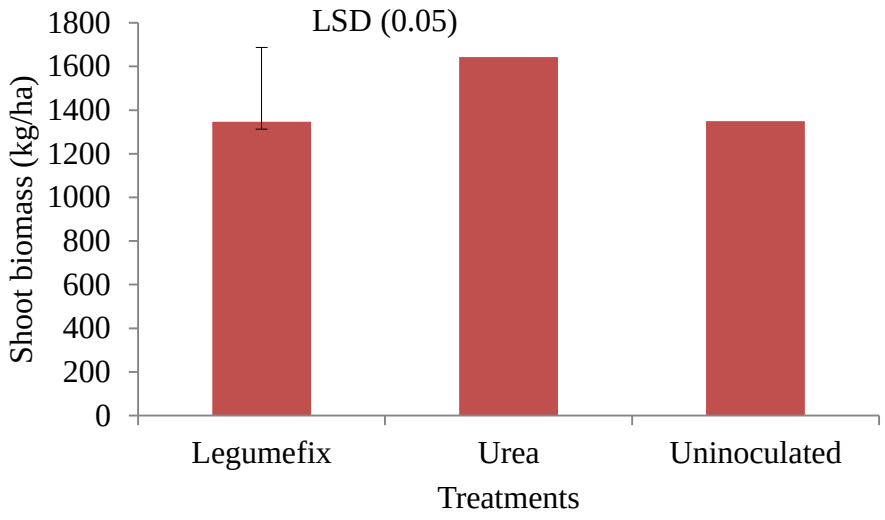

Figure 4.6. Effect of Legumefix inoculation on shoot biomass of cowpea

There was a strong positive correlation (R^2 = 0.8633) between shoot biomass and the amount of nitrogen fixed in soybean (Fig. 4.7).

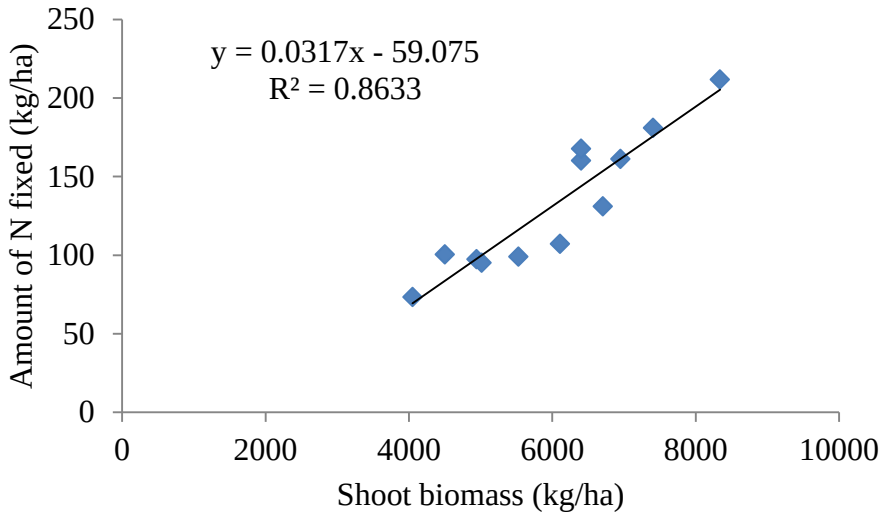

Figure 4.7. Relationship between shoot biomass and nitrogen fixed in soybean

There was a positive correlation (R^2 = 0.6209) between shoot biomass and the amount of nitrogen fixed in cowpea (Fig. 4.8).

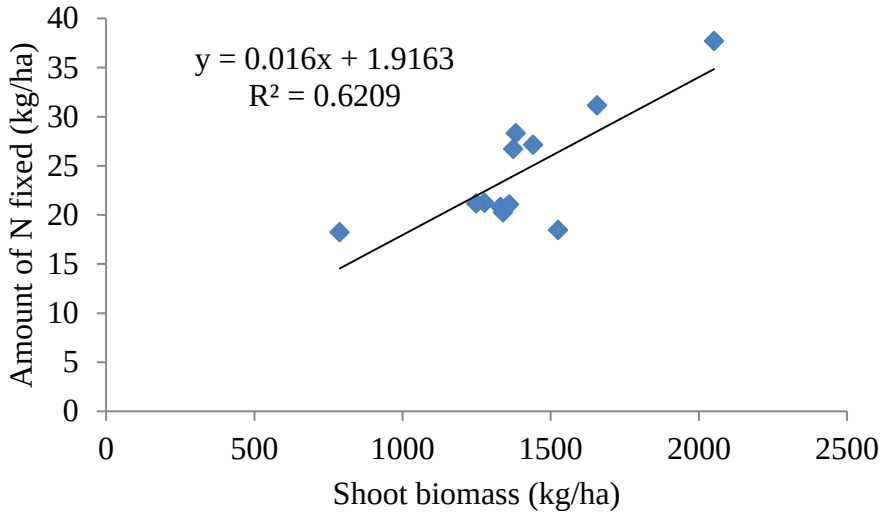

Figure 4.8. Relationship between shoot biomass and nitrogen fixed in cowpea

4.3.3 Nodulation of soybean and cowpea

Soybean and cowpea plants amended with Legumefix produced the highest number of nodules (1.98) and (2.03) per plant for soybean and cowpea respectively but was not significantly (P > 0.05) different from the urea fertilized plots and uninoculated controls (Table 4.4). The urea fertilized plots produced the lowest number of nodules in soybean while the uninoculated plants recorded the lowest number of nodules in cowpea (Table 4.4). Application of Legumefix to soybean and cowpea increased nodule number by 15.12% and 5.12% respectively over the urea fertilized plots; and by 8.79% and 5.18%, respectively over the uninoculated plants but these differences were not statistically significant (P > 0.05) (Table 4.4).

Table 4.4. Effect of Legumefix on nodule count of soybean and cowpea

Treatments	Number of nodules	
	Soybean	Cowpea
Legumefix	1.98	2.03
Urea (100 kg N)	1.72	1.95
Uninoculated	1.82	1.93
LSD (0.05)	0.39	0.25
CV %	12.4	7.4

Values represent means of ten plants. Number of nodules was log transformed

4.3.4 Nitrogen fixation of soybean and cowpea

Although the amount of nitrogen supplied through biological nitrogen fixation for soybean and cowpea was not significantly ($P > 0.05$) affected by the application of urea and Legumefix, urea increased biological nitrogen fixation by 22.1 kg N ha^{-1} in soybean (Fig. 4.9) and Legumefix inoculation increased biological nitrogen fixation in cowpea by 10.7 % (Fig. 4.10). The uninoculated plants recorded in excess of 10. 3 kg N ha^{-1} of N_2 – fixed over the legumefix plants in soybean (Fig. 4.10).

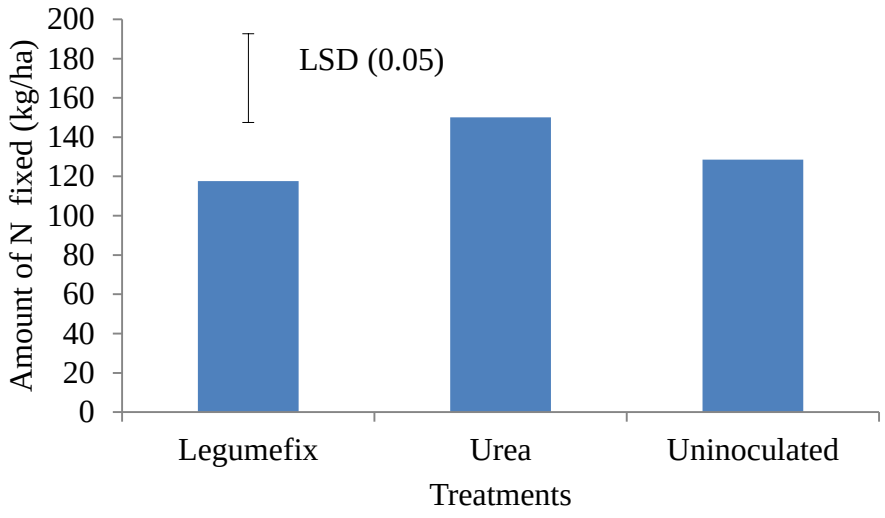

Figure 4.9. Effect of Legumefix inoculation on nitrogen fixation of soybean

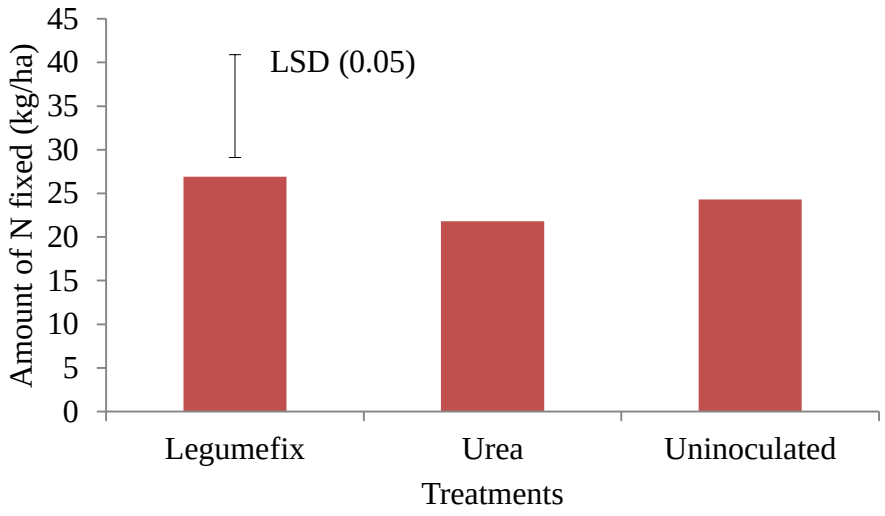

Figure 4.10. Effect of Legumefix inoculation on nitrogen fixation of cowpea

4.3.5 Number of pods of soybean and cowpea

Number of pods produced by all the treatments were not significantly ($P > 0.05$) different for both soybean and cowpea (Table 4.5). Urea and uninoculated control increased pod number by approximately 7% and 4%in soybean, respectively over Legumefix (Table 4.5). However, Legumefix increased pod number by 18% and 25% in cowpea over urea fertilized plots and uninoculated control, respectively (Table 4.5)

Table 4.5. Effect of Legumefix inoculation on pod number of soybean and cowpea

Treatments	**Pod number**	
	Soybean	Cowpea
Legumefix	1535	109
Urea	1642	92
Uninoculated	1592	87
LSD (0.05)	442.4	55.63
CV %	16.1	34.1

Values represent means of ten plants.

4.3.6 Grain yield

The application urea at 100 kg ha^{-1} produced significantly ($P < 0.05$) higher grain yield (2150 kg ha^{-1}) of soybean than inoculation with legumefix (1,908 kg ha^{-1}) and the grain yield of the uninoculated treatment (2,029 kg ha^{-1}) was also higher than that of the Legumefix but the differences were not statistically significant (Fig. 4.11).

Unlike soybean, Legumefix inoculation produced the highest grain yield in cowpea (797 kg ha^{-1}) but was not statistically significant ($P > 0.05$) from the grain yield (629 kg ha^{-1}) obtained from the urea fertilized plots (Fig. 4.12).

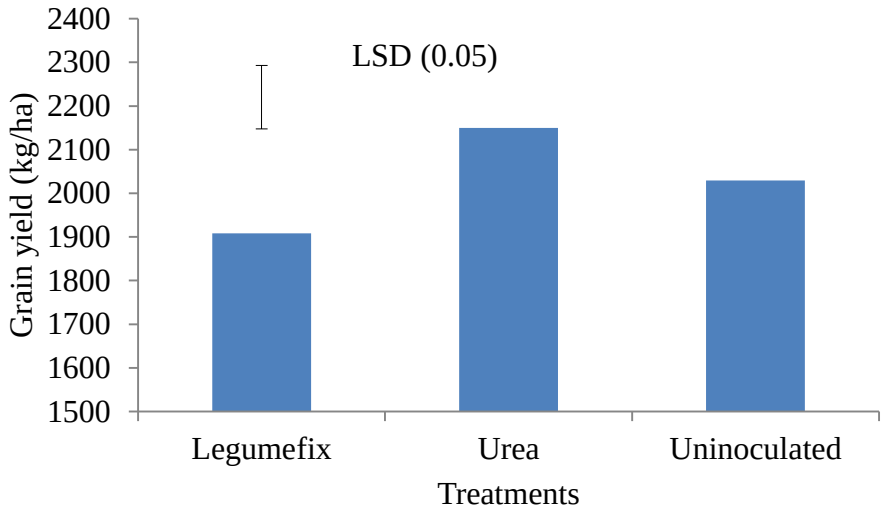

Figure 4.11. Effect of Legumefix inoculation on grain yield of soybean

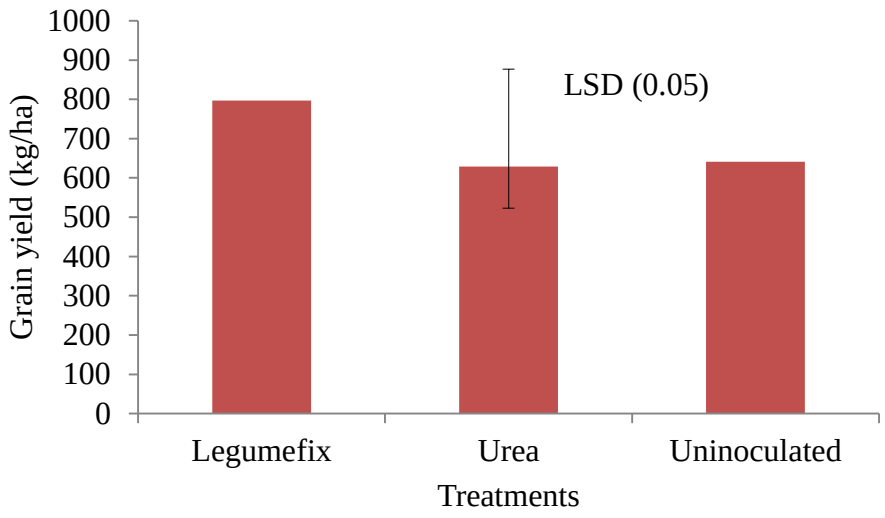

Figure 4.12. Effect of Legumefix inoculation on grain yield of cowpea

4.3.7 Grain phosphorus

Application of Legumefix did not significantly (P > 0.05) increase grain phosphorus uptake in soybean and cowpea seeds over the urea fertilized plots and the uninoculated control (Table 4.6). Application of Legumefix to soybean increased grain phosphorus uptake over urea fertilized plots and uninoculated control by 18.93% and 3.4% respectively (Table 4.6). Similarly, the seeds from inoculated cowpea plants recorded in excess of 11.38% and 1.51% phosphorus over the urea fertilized plots and uninoculated plants respectively (Table 4.6).

Table 4.6. Effect of Legumefix inoculation on grain phosphorus of soybean and cowpea

Treatments	**Grain P (kg ha $^{-1}$)**	
	Soybean	Cowpea
Legumefix	6.91	6.07
Urea	5.81	5.45
Uninoculated	6.68	5.98
LSD (0.05)	1.43	3.46
CV %	12.8	34.3

Values represent means of ten plants

4.3.8 Grain nitrogen

The amount of nitrogen in the cowpea grain (seed) was not significantly ($P > 0.05$) affected by the treatments that were imposed (Fig. 4.14). The inoculated cowpea plants increased the grain nitrogen over the urea and uninoculated plants by 20.6% and 20.1%, respectively

The amount of nitrogen in the soybean grain was significantly ($P < 0.05$) influenced by the various treatments that were imposed (Fig. 4.13). Application of urea produced significantly ($P < 0.05$) higher grain nitrogen (137.5 kg ha^{-1}) than that of Legumefix treatment which recorded grain nitrogen of 124.8 kg ha^{-1}. The uninoculated treatment produced higher grain nitrogen (131.6 kg ha^{-1}) than Legumefix (124.8 kg ha^{-1}) but the differences between the treatments were not statistically significant (Fig. 4.13).

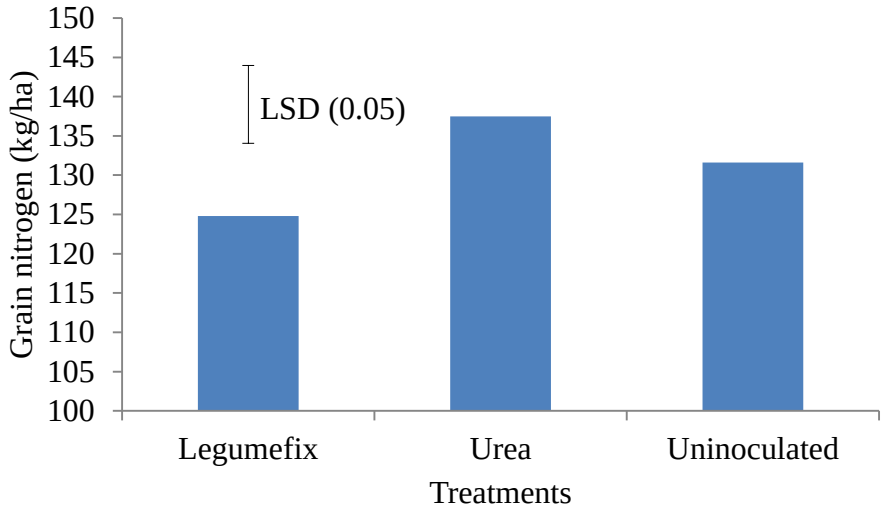

Figure 4.13. Effect of Legumefix inoculation on grain nitrogen content of soybean

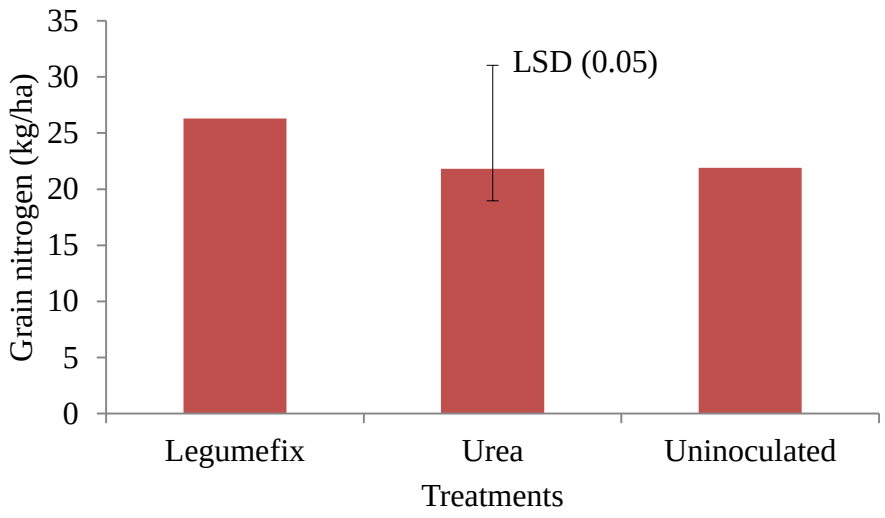

Figure 4.14. Effect of Legumefix inoculation on grain nitrogen content of cowpea

CHAPTER FIVE

5.0 Discussion

5.1 Symbiotic effectiveness of isolated rhizobia from the indigenous population

The results of this work that 16% of the isolates from the indigenous rhizobia population tested on cowpea were effective is contrary to the studies of Fening and Danso (2002) which reported that 6% of the indigenous rhizobia in Ghanaian soils are highly effective on cowpea. Meanwhile 11% of the isolates from the indigenous rhizobia population tested on soybean was classified as highly effective. The growth of uninoculated soybean plants with nitrogen appears to be low because the required amount was halved. This was based on previous experience where full dose application scorched the plants.

The inability of the uninoculated controls with or without nitrogen to nodulate in the greenhouse evaluation study suggests that nodulation can only occur in the presence of compatible rhizobia. This also signifies that nodulated plants did not possibly results from cross contamination.

Although all the isolates nodulated, the variation in nodulation and shoot biomass indicate that some of the isolates from the indigenous rhizobia population are infective and effective whereas others are infective but not effective. The symbiotic effectiveness of the indigenous rhizobia is an important tool in determining the response of plants to inoculation (Singleton and Tavares, 1986; Thies *et al*., 1991). The native rhizobia are more persistent (Fening and Danso, 2002), well adapted to local conditions and this gives them added advantage of competing successfully at the expense of introduced strains for nodule occupancy. One of the challenges of inoculant application in the

tropical regions is the presence of competitive naturally occurring rhizobia (Sarkodie – Addo *et al*., 2006). This explains why the introduced Legumefix inoculum strains did not significantly increase grain yield in soybean and cowpea at Tono.

5.2 Shoot biomass of soybean and cowpea

Application of urea did not cause a significant ($P > 0.05$) increase in shoot biomass of cowpea and soybean (Figs. 4.5 and 4.6). This observation is in contrast to the work of Abayomi *et al*. (2008) who reported a significant increase in dry matter due to mineral N application. It is however worth noting that Abayomi *et al*. (2008) used a higher concentration of mineral N (150 and 300 kg ha^{-1}). It was observed that soybean and cowpea plants that were furnished with urea grew larger with more leaves than the plants that depended on the atmospheric nitrogen (Figs. 4.5 and 4.6). This may have resulted in the urea - fertilized plants producing more shoot biomass of 917 and 296 kg ha^{-1} than Legumefix inoculated soybean and cowpea plants, respectively. The amount of nitrogen fixed is a function of shoot dry weight (Keyser and Li, 1992). This was reflected by the strong positive correlations between the amounts of nitrogen fixed and shoots biomass of soybean and cowpea (Figs. 4.7 and 4.8).

5.3 Nodulation and biological nitrogen fixation of soybean and cowpea

Rhizobia inoculation could not elicit significant increase in nodulation (Table 4.4). This agrees with the studies of Okogun *et al*. (2005) and Chemining'wa *et al*. (2007) who reported no significant increase in nodulation following rhizobia inoculation. However, Katulande (2011) and Albareda *et al*. (2009) reported a significant increase in nodule number due to rhizobia inoculation. Singleton and Tavares (1986) observed that rhizobia inoculation did not increase nodule number when the native rhizobia

population was 1 x 10^2 rhizobial cell g^{-1} soil. A similar trend was observed in this current study except that the indigenous rhizobia population of the study area (5.71 x 10^1 cells g^{-1} soil and 7.5 x 10^1 cells g^{-1}) was less than 1 x 10^2 rhizobial cell per gram of soil (Table 4.3). However, Okogun and Sanginga (2003) indicated that response to rhizobia inoculation is likely to occur in soils with indigenous rhizobia population of less than 10 cell g^{-1} soil. Similarly, Thies *et al.* (1991) reported that positive response to rhizobia inoculation and the ability of an introduced strain to compete with and overcome the indigenous rhizobia is inversely related to the number of the indigenous rhizobia. It is therefore likely that the indigenous rhizobia population were more competitive than the introduced inoculum strains.

Studies on competitiveness of the indigenous rhizobia population are very limited due to methodological challenges which could not have been resolved in this study. Uddin *et al*. (2008) reported that the application of N fertilizer (urea) significantly inhibited nodule number. Although there was no significant (P = 0.37) increase in nodule number, urea – fertilized plants produced nodule numbers close to the numbers produced by the inoculated and uninoculated plants (Table 4.4). Table 4.3 showed that the initial amounts of nitrogen (0.013% and 0.012%) in the soil were low and could not have suppressed nodulation. Differences in nodule numbers and the amount of nitrogen fixed in soybean and cowpea indicates that even though all the plants may have succeeded in forming nodules, not all the nodules contained effective bacteria that contributed effectively to nitrogen fixation. Moisture stress could have influenced the activities of nitrogenase, the enzyme that catalyses the reaction thus affecting nodulation (Danso *et al*., 1992). Giller (2001) reported that moisture deficit decreases

the number of rhizobia in the soil which in turn causes reduction in N_2 fixation. The amount of nitrogen fixed by soybean (117.7 – 128 kg N ha^{-1}) (Fig. 4.9) was lower than what has been reported by Giller *et al*. (1997) (159 – 227 kg N ha^{-1}), however it falls within the range reported by Okogun *et al*. (2005) (19.2 – 253 kg N ha^{-1}). The N difference method of estimating nitrogen fixation is reported to be less accurate and effective than the ureide method used by Okogun *et al.* (2005) (Danso, 1995; Unkovich *et al*., 2008). This may partly account for the observed differences in the amount of nitrogen fixed in this study and what was reported by Okogun *et al*. (2005). The amount of nitrogen fixed by the cowpea (24 – 26.9 kg N ha^{-1}) (Fig. 4.10) conforms to the range of 16 - 34 kg N ha^{-1} reported by Yusuf *et al*. (2006) who used the N difference method in their measurement. The percentage of nitrogen derived from the atmosphere by the soybean and cowpea inoculated with Legumefix were 89.62% and 60.2%, respectively whiles those of the uninoculated control treatment were 89.85% and 62.6% respectively (Figs. 4.9 and 4.10). This may suggest that nodulation of inoculated plants might have been initiated by native rhizobia. Higher nodulation does not always translate into higher nitrogen fixation and this has been reported by many researchers including Singleton and Tavares (1986) and Sarkodie – Addo *et al*. (2006). Singleton and Tavares (1986) and Sarkodie –Addo *et al*. (2006) in unrelated situations, observed an increase in nodule number without a corresponding increase in nitrogen fixation. Singleton and Tavares (1986) reported that inoculated plants must have 2.5 times more nodules than unioculated plants before a corresponding increase in nitrogen fixation could be achieved.

5.4 Pod and grain yield of soybean and cowpea

The study did not record any significant difference in pod number among the treatments for soybean and cowpea (Table 4.5). This result is in contrast to that of Katulande (2011) who reported that pod number increase significantly with rhizobia inoculation. However, similar result of no significant increase in pod number was observed by Yinbo *et al.* (1997) when 100 kg N ha^{-1} was applied. The low grain yield of cowpea (629 – 797 kg ha^{-1}) could largely be the result of poor pod filling and damage to a large proportion of seed during the seed maturation period.

Significant increase in grain yield due to rhizobia inoculation has been reported by several authors (eg., Thies *et al*., 1991; Seneviratne *et al*., 2000 ; Albareda *et al*., 2009; Katulande, 2011). This study showed that the introduced strains were not effective to significantly increase grain yield as compared to urea - fertilized and uninoculated controls in soybean and cowpea (Figs. 4.11 and 4.12).

These findings are in contrast to the work of Thies *et al*. (1991), Seneviratne *et al*.(2000), Albareda *et al*. (2009) and Katulande, (2011). Similar results of the inability of introduced strains to elicit significant response have also been reported (Thies *et al*., 1991; Chemining'wa *et al*., 2007). Differences in results of this study and other researchers could be attributed to varietal differences, environmental conditions and types of rhizobia inoculant used. Different varieties have different yield potentials and respond differently to rhizobia inoculant application and the potency of different inoculants may not be the same. It is worth noting that response to rhizobia inoculation is not predictable but highly variable and site specific (Date, 2000). Application of rhizobia inoculants does not always produce the desired results (Chemining'wa *et al*.,

2007). Failure of the two legumes (soybean and cowpea) to respond to rhizobia inoculation could also be due to the inability of an introduced strain to survive on the seeds as well as to successfully compete with native rhizobia to colonize the rhizosphere.

Uninoculated soybean plants were in whole better than inoculated soybean plants (Fig. 4.11). This was probably due to more number of plants within the harvested area in the uninoculated plots than the inoculated plots. It appeared that inoculation pretreatment had adverse effect on soybean germination as in most times the plant population of inoculated treatment tended to be lower than uninoculated treatments.

Another reason for this finding perhaps might be due to the fact the number of viable rhizobia in the inoculum might have been reduced as of the time of application (Chemining'wa *et al*. 2007). Brokwell *et al*. (1987) reported that there could be a hearty decrease in the population of the introduced strains in an inoculum due to mortalities following its introduction into the soil thus causing a reduction in the optimum numbers required for symbiosis. This coupled with the competition from the indigenous rhizobia and the promiscuous nature of the cowpea and soybean genotype used might have contributed to no significant increase in grain yield of both Legumefix inoculated soybean and cowpea.

Also, the low pH of the experimental site (5.04 and 4.87) may have partly contributed to the observation of no significant increase in grain yield of soybean and cowpea due to Legumefix inoculation; as detrimental effects of pH on inoculum survival, root infection and nodulation has been established by Blamey *et al*. (1983). Below pH of 5.5, nitrogen fixation is impaired (Blamey *et al*., 1983) and some nutrients such as

phosphorus become less available (Chen, 2006). The cowpea variety, IT90K-277-2 could not tolerate the low pH of the study area and this partly accounted for the generally low yield recorded by the treatments imposed on cowpea (Dugje *et al*., 2009). Thies *et al*. (1991) reported a significantly higher yield due to nitrogen fertilizer application than inoculation. On the contrary, Abayomi *et al*. (2008) reported a decreased in yield and final yield loss of 24% due to the application of N, P and K. Reasons for variation in the results obtained by different researchers are not too clear; management practices and growth conditions are among the factors that have been cited (Yinbo *et al*., 1997). Once the application of nitrogen did not improve the yield of cowpea, is an indication that other factors aside nitrogen are limiting and as such rhizobium inoculation alone will not increase grain yield unless the limiting factors are identified and addressed accordingly.

The potential yield for both Jenguma and IT90K-277-2 are 2.5 t ha^{-1} and 1.5 t ha^{-1}, respectively (Dugje *et al*., 2009). The application of urea produced grain yield (2.1 t ha^{-1}) which was almost equivalent to the potential yield of Jenguma (soybean) but not the IT90K-277-2 (cowpea). Legumefix was not able to meet the potential yield of both soybean and cowpea but recorded yields greater than the current yield of soybean which is 1.6 t ha^{-1} and lower than that of cowpea which is 1.4 t ha^{-1}.

5.5 Grain N and P uptake

The findings of this study that Legumefix inoculation had no significant effect on grain nitrogen are in contrast to the results of Okogun *et al*. (2005) that significant increase in grain nitrogen in inoculated plants over uninoculated plants. However, grain nitrogen uptake due to Legumefix inoculation (124.8 kg ha^{-1}) was within the range reported by

Okogun *et al.* (2005) (2.4 - 284.7 kg ha^{-1}). The significant increase in grain nitrogen by the urea – fertilized plants over the Legumefix inoculated plants could be as a result of the combined effect of fixed nitrogen and applied nitrogen. The results of no significant difference in grain phosphorus uptake (Table 4.6) are also in contrast to the results of Okogun *et al*. (2005) who reported a significance increase in seed P due to rhizobium inoculation.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The soils of the study area had low numbers of native rhizobia (5.71×10^1 and 7.5×10^1) as the populations did not exceed 1×10^2. Isolates 19, 1 and 17 from the indigenous rhizobia population tested on cowpea were classified as effective. Comparatively, isolates 2, 19, 7, 17 and 1 from the indigenous rhizobia population tested on cowpea showed symbiotic effectiveness superior to the standard strain, USDA 138. Isolate 22 from the indigenous rhizobia population tested on soybean was classified as highly effective whereas isolates 27 and 24 were classified as effective. The native rhizobia were effective to obviate response to Legumefix inoculation. This confirms the null hypothesis that sufficient rhizobia do not exist in the soils of the study area but it invalidates the null hypothesis that the indigenous rhizobia of the study area are not effective.

Inoculation with Legumefix inoculant at a rate of 5 g kg^{-1} of seed had no significant effect on shoot biomass, pod number and grain yield of soybean and cowpea. This outcome affirms the null hypothesis that Legumefix will not lead to improvement in growth and yield of soybean and cowpea at Tono.

Legumefix inoculation had no significant effect on the amount of nitrogen supplied through BNF in soybean and cowpea. This result reasserts the null hypothesis that Legumefix will not increase the amount of nitrogen supplied through BNF in soybean and cowpea at Tono.

6.2. RECOMMENDATIONS

The following recommendations are worth considering:

1. conduct further studies on the characterization as well as field testing of the native rhizobia at the study area.

2. conduct studies on the appropriate rate of the Legumefix that needs to be applied in order to elicit significant response.

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APPENDICES

Appendix 1: Broughton and Dilworth N-free Plant Nutrient Solution

Stock Solutions	Element	Form	g/l
1	Ca	$CaCl_2 \cdot 2H_2O$	294.1
2	P	KH_2PO_4	136.1
3	Fe	Fe-citrate	6.7
	Mg	$MgSo_4 \cdot 7H_2O$	123.3
	K	K_2SO_4	87.0
	Mn	$MnSO_4 \cdot H_2O$	0.338
4	B	H_3BO_3	0.247
	Zn	$ZnSO_4 \cdot 7H_2O$	0.288
	Cu	$CuSO_4 \cdot 5H_2O$	0.100
	Co	$CoSO_4 \cdot 7H_2O$	0.056
	Mo	$Na_2MoO_2 \cdot 2H_2O$	0.048

Source: N2 Africa Technical Training Manual

Appendix 2: Most probable number count for cowpea

	REPLICATIONS				
	1	2	3	4	TOTAL
5^{-1}	+	+	+	+	4
5^{-2}	+	+	+	+	4
5^{-3}	+	-	-	-	1
5^{-4}	+	-	-	-	1
5^{-5}	-	-	-	-	0
5^{-6}	-	-	-	-	0
TOTAL					10

P = 0.05. Confidence interval = 25.9 – 215.0

Appendix 3: Most probable number count for soybean

	REPLICATIONS				
	1	2	3	4	TOTAL
5^{-1}	+	+	+	+	4
5^{-2}	+	+	+	+	4
5^{-3}	+	-	-	-	1
5^{-4}	-	-	-	-	0
5^{-5}	-	-	-	-	0
5^{-6}	-	-	-	-	0
TOTAL					9

P = 0.05. Confidence interval = 19.8 – 164.7

Appendix 4: Constituent of yeast extract mannitol medium

YEAST EXTRACT MANNITOL AGAR		YEAST EXTRACT MANNITOL BROTH	
CHEMICAL	g/L	CHEMICAL	g/L
K_2HPO_4	0.5	K_2HPO_4	0.5
NaCl	0.2	NaCl	0.2
$MgSO_4.7H_2O$	0.1	$MgSO_4.7H_2O$	0.1
Mannitol	10	Mannitol	10
Yeast extract	0.5	Yeast extract	0.5
Agar	15		