EVALUATION OF THE GROWTH RESPONSE OF SOYBEAN (*Glycine max* L.) TO SOME COMMERCIAL FERTILIZERS IN THE GUINEA SAVANNAH AGRO - ECOLOGICAL ZONE OF GHANA

BY

DORCAS TINUKE EZEKIEL-ADEWOYIN

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By

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DECLARATION

I do hereby declare that this thesis was written by me and that it is the record of my own research work under supervision. It has neither in part nor in whole been presented for another degree elsewhere. Cited references have been duly acknowledged.

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ABSTRACT

Series of field experiments were undertaken in the Guinea Savanna agro - ecological zone of Ghana to assess the influence of some agricultural commercial products in optimizing soybean production. The field experiments were carried out using sixteen treatments at Akukayili, Cheshegu and Ghulahgu during the 2012 and 2013 cropping seasons. The treatments were replicated four times in a split – plot arranged in randomized complete block design. Soil chemical properties evaluated in the study areas before trial establishment indicated that the soil fertility status was below the optimum for soybean growth. The results showed that the use of starter N (25 kg N ha^{-1}) is essential to increase soybean yields in the study area. Grain yields of 3321 and 3056 kg ha^{-1} were recorded respectively for Boost Xtra (BX) + 25 kg N ha^{-1} and BX + 5O kg N ha^{-1} at Akukayili where 25 kg N ha^{-1} + BX emerged as the best treatment in enhancing both Agronomic Efficiency (AE) and Nutrient Use Efficiency (NUE) of nitrogen. Furthermore, the sole use of *Bradyrhizobium japonicum* inoculum did not significantly ($P > 0.05$) enhance grain yield. At Cheshegu, Fertisoil and *Bradyrhizobium japonicum* inoculum treatments increased nodule dry matter accumulation by 51% and 407% respectively relative to the control. Moreover, P_{90} + FS, P_{60} + FS and P_{30} + FS produced grain yields of 3588, 3583 and 3524 kg ha^{-1} respectively, which were significantly higher ($P < 0.05$) than the control. Phosphorus application increased N uptake in the order, 30 > 60 > 90 kg P ha^{-1}. The use of 30 kg P ha^{-1} enhanced the AE and NUE of phosphorus when combined with *Bradyrhizobium japonicum* inoculum (INO) and /or Fertisoil (FS). The emphasis on the benefit of combining more than one nutrient at a time was clearly observed across the study locations, where combined use of the various treatments (especially FS + $N_{25}P_{30}$) led to an increase in nodule dry weight, biomass and grain yield relative to their sole treatments. Also, the uptake of N and P were enhanced with the treatment combinations than the sole applications. At Ghulahgu, the application of Boost xtra

resulted in a value cost ratio (VCR) of 5 which was the highest relative to VCR's obtained at Akukayili and Cheshegu.

DEDICATION

This Thesis is dedicated to God Almighty,

My (late) father, my mother,

Three wonderful boys for being there for me throughout this academic pursuit.

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CHAPTER ONE

1.0 General Introduction

Soybean is well - known as an important source of protein in human diet and animal ration; containing substantial amounts of all the essential amino acids, oil, minerals and vitamins (Tefera, 2010). It is an economically important leguminous crop in Ghana widely cultivated in different agro - ecologies, yet its production still lags behind annual consumption (Plahar, 2006). Low fertility status of most of the cultivated tropical soils has been identified as a major factor causing low crop yield (Byerlee, 2007; Shiferaw *et al*., 2004). This is aggravated by inappropriate cropping practices such as continuous cropping with little or no external inputs as commonly observed among smallholder farmers particularly in northern Ghana (Shiferaw *et al.*, 2004). Inherently poor or nutrient depleted soils are characterized by low soil organic matter, available phosphorus and total nitrogen especially in the savannah and transition zones (FAO, 2005) of Ghana. Agricultural practices that augment or conserve these nutrient stocks are therefore required for sustainable soybean production.

Biological nitrogen fixation (BNF) offers an economically attractive and ecologically sound means of improving crop yield, reducing external N inputs and enhancing the quality of soil resources which consequently reduce the dependence on mineral fertilizers that could be costly and unavailable to smallholder farmers. Leguminous crops such as soybean hold promise in this regard. Solomon *et al*. (2012) reported that legumes including soybean can obtain between 50 and 80% of their nitrogen concentration requirements through BNF. Sanginga (2002), however, reported that the current promiscuous soybean genotypes cannot meet all their

demand for growth and seed development only by N_2 fixation. Okogun and Sanginga (2003) also reported that *Bradyrhizobium japonicum* may hardly exist in Ghanaian soils because soybean is an exotic crop. Moreover, populations of *Bradyrhizobia* are seldom available in soils where soybean crop has not been previously grown; therefore nodulation of soybean may require specific species of Bradyrhizobium for effective N_2 fixation (Abaidoo *et al*., 2007). However, the success of inoculation depends not only on high quality inoculants and good inoculation practices but also on the establishment of effective and efficient BNF through optimization of the factors that affect its performance such as legume genotype, climatic, edaphic and management factors (Giller, 2001; Giller and Wilson, 1991; Sanginga *et al*., 1995).

Some commercial agricultural products have proven to substantially enhance the yield of specific crops. However, there appears to be proliferation of new products on the markets that claim major impacts in increasing crop yield. In view of this, there is the need to rigorously assess these new products to ascertain whether they fulfill claims of the manufacturer to avoid the incidence of farmers acquiring low quality products which can reduce their profit and agricultural productivity. Evaluating the authenticity and performance of these products is also necessary for quality assurance of agricultural inputs (e.g. *Bradyrhyrhizobium japonicum* inoculum, Boost xtra, Fertisoil etc).

Enhanced crop yield is now linked to Integrated Soil Fertility Management (ISFM) (Sanginga *et al*., 2009, Vanlauwe *et al.*, 2010, FAO. 2011). Integrated soil fertility management has greater prospects in improving soil fertility status and achieving high crop yield because of the combination of mineral fertilizers, organic input and inoculants; which could synergistically improve the physical, chemical and microbiological properties of the soil. Little or no information is however available

on the complementary use of *Bradyrhizobium japonicum* inoculum with mineral and organic inputs particularly in the Northern part of Ghana. Thus the overall objective of this study was to evaluate the effectiveness of some commercial agricultural products in optimizing soybean yield.

The specific objectives of the study were to:

i. determine the influence of nitrogen and micro nutrients on soybean nodulation, growth and yield,

ii. evaluate the combined effect of phosphorus and Fertisoil on soybean nodulation, growth and yield,

iii. assess the effect of complementary use of mineral fertilizer, organic fertilizer (Fertisoil),

and inoculation (*Bradyrhizobium japonicum)* on soybean nodulation, growth and yield,

iv. estimate the profitability of mineral fertilizer (N, P and Boost xtra), organic fertilizer (Fertisoil) and *Bradyrhizobium* inoculum use for soybean nodulation, growth and yield.

The above specific objectives were based on the hypotheses that:

i. the use of nitrogen, Boost xtra and inoculation will increase soybean nodulation, growth and yield.

ii. the use of phosphorus, Fertisoil and inoculation will enhance soybean nodulation, growth and yield.

iii. the complementary use of mineral fertilizer (N, P and Boost xtra), organic fertilizer (Fertisoil) and inoculation (*Bradyrhizobium japonicum)* will enhance soybean nodulation, growth and yield.

iv. the use of mineral fertilizer (N, P and Boost xtra), organic fertilizer (Fertisoil) and *Bradyrhizobium* inoculum is profitable for soybean nodulation, growth and yield.

CHAPTER TWO

2.0 General literature review

2.1 Fertility of tropical soils

Tropical soils vary from young volcanic or alluvial soils to some of the oldest (Oxisols, Ultisols and less leached Alfisols), most highly weathered and leached soils in the world (Giller, 2001). The highly weathered and leached soils which are predominant (covering half of the land area) resulted from various parent materials, high temperatures and rainfall. These soils are highly susceptible to degradation resulting in low fertility that poses serious constraints to poverty alleviation and sustainable food security in many parts of the tropics. In the face of increasing population growth and concomitant decline in the area of land available for expansion of agriculture, many developing countries are confronted with diverse challenges of increasing agricultural production (FAO, 2002).

The previous act of leaving a land fallow for 10 - 12 years can no longer be accommodated as a result of this population pressure. Sadly, there is scarcely any productivity - enhancing investment accompanying increase in land use intensity. The extent and severity of land degradation in developing countries is not sufficiently known. Oldeman (1994) reports that an assessment carried out by the Global Assessment of soil Degradation (GLASOD), indicates that in Africa about 65% of agricultural lands are exposed to some degree (slight to extreme) of degradation.

Soil erosion by water and wind, depletion of soil nutrients, salinity, waterlogging, acidification and deforestation are the major agents of land degradation. The impact of such soil degradation is difficult to reverse, depending on the severity of the effect

on the soil (Salako, 2001). The high level of nutrient depletion and soil degradation in many smallholder systems, coupled with the high fertilizer prices that limit farmers' capacity to replenish soil fertility necessitate alternative nutrient management systems for the rehabilitation and reversal of soil degradation.

In the case of Ghana, soils develop from highly weathered parent materials (FAO, 2005). Alluvial and eroded shallow soils are common to all agro - ecological zones, most of which are inherently infertile, or infertile as a result of human activities (MOFA 1998). The northern half of Ghana is dominated by Luvisols which are described as having a mixed mineralogy, high nutrient content and good drainage (Bridges, 1997). The percent organic matter and nitrogen are particularly low in the Savannah and transition zones (FAO, 2005). It is generally recognized that most of Ghana's soils have low fertility with the following range of nutrients pH (4.5 – 6.7), organic matter (0.6 – 2.0), total nitrogen (0.02 – 0.05), available P (2.5 – 10.0 mg kg^{-1} soil) and available Ca (mg kg^{-1} soil) (AQUASTAT; FAO, 2005), which are responsible for low food production. In order to sustain soil and crop productivity, it is necessary to explore alternative soil fertility replenishing strategies different from what small-scale farmers are used to, which will be effective and affordable to support improved livelihoods.

2.2 Constraints to mineral fertilizer use in Ghana

In Ghana, the introduction of the Structural Adjustment Programme and the removal of most agricultural support resulted in a fall of fertilizer use since 1984. Decline in the use of mineral fertilizer can be attributed to policy changes by the Government. The CSIR-NARP (1998) identified privatization of importation and distribution of fertilizers and the removal of subsidies as one of the causes of low fertilizer use. However, there was an increase in fertilizer use in the second half of the 1990s

following an improvement in the national economy, but this fell again as a result of renewed financial problems and depreciation of the cedi. Nevertheless in 2002, it recovered to the level of the early 1980s. The average fertilizer nutrient use reported for Ghana is estimated at about 5 kg per hectare of cultivated land which is half that of sub-Saharan Africa and a quarter of Africa as a whole (FAOSTAT, 2008).

Considerably, more plant nutrients are being removed and lost than are being applied in Northern Ghana, with a consequent progressive impoverishment of soils, showing nutrient deficits. This implies that the difference between the quantities of plant nutrients applied and the quantities removed or lost is unbalanced, therefore, it is important to ensure that organic matter is added and incorporated into these soils to improve their structure and enhance their capacity to store adequate moisture and nutrients even after crops are harvested (Tabo *et al*., 2007). The negative nutrient balances represent a loss of potential yield and progressive soil impoverishment. Heisey and Mwangi (1996) and Larson and Frisvold (1996) reported that most smallholder farmers 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. Despite the high rate of fertilizer consumption in Northern Ghana as compared to the other regions in Ghana (Bonsu *et al.*, 1996), farmers would prefer to apply fertilizer to cereal crops rather than leguminous crops. However, recent research findings have shown that leguminous crops such as soybean requires fertilizer, especially N and P application and/or manure to facilitate and enhance nodulation and Biological Nitrogen Fixation (BNF) particularly on poor or nutrient depleted soils for initial root and vegetative growth before the nodule function is fully established.

2.3 Integrated soil fertility management

The acronym (ISFM) as defined by Fairhurst (2012) is a set of fertility management practices that necessarily include the use of fertilizers, organic inputs and improved germplasm combined with the knowledge on how to adapt these practices to local conditions, aiming at optimizing agronomic use efficiency of the applied nutrients and improving crop productivity. In order to achieve this all inputs need to be managed following sound agronomic and economic principles.

The approaches undertaken in sub - Sahara Africa to manage soil fertility problems in the past three decades have undergone substantial change due to improved knowledge, extensive field research and the overall social, economic and political environment. Agronomic trials have indicated that there are often large increases in crop yields when nutrients are added to the farm system. The use of mineral fertilizer has been recognized since 1960s and 1970s but have relatively little potential to enhance the soil organic matter (SOM) status. According to Vanlauwe *et al*. (2010), N fertilizer contaminates (ground) water resources when not used efficiently. Also, in the 1980s, emphasis was laid on the use of organic resources, partly due to the limitations encountered by smallholder farmers with the use of inorganic fertilizer. In addition, its sole application as suggested by Palm *et al*. (1997) might result in low and/or imbalanced nutrient content, unfavourable quality, or high labor demand for transporting bulky materials. Consequently, the combination of mineral fertilizer and organic resources is being advocated to enhance the benefit that would result in crop yield and efficient fertilizer use (Fairhurst, 2012). Indeed, there exist a large volume of literature reporting the efficiency and effectiveness of integrating farm yard manure and other inorganic nutrient sources in maintaining soil fertility, improving crop yields and, sustaining productivity especially on maize cultivation.

For example in Zimbabwe, Grant (1981) and Mugwira (1985) reported that the use of manure alone generally resulted in low crop yield, indicating a need for supplement with inorganic fertilizers on soils low in fertility while Heluf (2002) also reported increase in maize grain yield with the use of FYM over the control. Little is reported on the combined use of mineral fertilizer and organic fertilizer in the northern region of Ghana on soybean production; therefore necessiting the need for this study.

2.4 Need for nitrogen in soybean

A lot of contrasting reports have been published with regards to the response of legumes to nitrogen. Keyser *et al*. (1992) reported that as the level of mineral N in the rhizosphere increases, nodule formation and functioning is suppressed, apparently resulting in low amount of nitrogen fixed. With all things being equal, higher nodulation should increase the amount of nitrogen fixed. However, this is dependent on several environmental factors. Panchali (2011) reported that per adventure the legume–rhizobium symbioses due to such factors is not able to produce sufficient nitrogen during the early stages of growth to meet the plant N demand, then small application of mineral N becomes necessary.

Sosulski *et al*. (1989) suggested that the high demand of N by annual legumes may require a high level of soil N to achieve maximum yield. Katulanda (2011) also confirmed a potential increase of pod and crop biomass by 44 % and 16 % respectively, in response to nitrogen application at either vegetative or flowering stage. Kucey *et al*. (1989), Gan *et al*. (2003) and Osborne *et al*. (2006) were all in support of the use of nitrogen fertilizer to soybean at one stage of its growth or the other to boost its production. On the other hand, other researchers have not expressed

support of N use for soybean production. For example, Peoples *et al*. (1995) reported that high response to inoculation in a low nitrate soil by a legume with high potential for growth cannot be underestimated in a soil with low nitrate content, which implies that high soil nitrate can hinder N_2 fixation.

Schmitt *et al*. (2001) have also reported that soybean fertilized with mineral N did not result in high grain yield and oil content. Barker *et al. (*2005), Panchali (2011) and Gan *et al*. (2003) also reported that the use of N for soybean at certain growth stages might not be advisable. The use of N in soybean cannot be ruled out completely; so many factors (time of application, fertilizer type, rate of application and environment etc.) have to be put into consideration before conclusions can be drawn on these controversies.

2.5 Role of organic inputs in enhancing soil/crop productivity

Organic inputs are an important source of nutrients; nutrients such as nitrogen, phosphorus, magnesium and calcium are all released through mineralization (Fairhurst, 2012). Organic resources help the crop to respond better to the mineral fertilizer applied. It also helps in improving the soils capacity to store moisture, helps in regulating soil chemical and physical properties that affect nutrient storage and availability as well as root growth. Also organic inputs help in adding nutrients that are not contained in mineral fertilizers. They create a better rooting environment, improves the availability of phosphorus for plant uptake, ameliorates problems such as soil acidity and helps in soil organic matter replenishment (Fairhurst, 2012). However, the amount of nutrients contained in organic resources is insufficient to sustain the required levels of seasonal crop productivity and realize the full economic potential of a farmer's land and labour resources. The quality of organic

resources is often poor and the quality of manure or other organic materials is simply insufficient to meet the nutrient demand of crops. According to Fairhurst (2012) organic materials generally contain small amounts of nutrients compared with mineral fertilizer and are therefore more costly to store, transport and apply. For example, in livestock systems in West Africa, the present use of manure is very minimal (0.5 - 2.0 t ha^{-1}) therefore the potential transfer of nutrients in animal manure to crop fields is only about 2.5 kg N and 0.6 kg P ha^{-1} of cropland and not enough to meet the requirement of crops. These are some of the reasons that soil fertility sustainability and productivity improvement is not practicable, therefore, advocating for the use of external inputs either from organic or mineral fertilizer and especially their combination to satisfy plant nutrient needs.

2.6 The need for inoculation

The presence of compatible rhizobia in the soil and their effectiveness are the determining factors for need for inoculation. Promiscuous legumes usually have rhizobia strains with which they can form effective nodules. As a result, they rarely respond to inoculation (e.g. cowpea or groundnut). In grain legumes a response to inoculation is most common in soybean. However, many varieties are highly specific and do not always nodulate with indigenous rhizobia in Africa (Giller, 2001).

The Joint FAO/IAEA Programme of co-ordinated research showed that inoculation with a suitable strain of rhizobium at sowing was the single most useful agronomic practice in ensuring maximum legume yield (Gudni *et al*., 2003). Since the desired type of N_2-fixing micro-symbiotic may not exist in the required amounts in a given soil, inoculation with an appropriate strain suited for a specific crop and soil conditions is often required (Gudni *et al*., 2003). The use of inoculation is therefore

necessary when legumes are introduced into new regions. However, Giller (2001) reported that if the introduced legume crop can nodulate effectively with rhizobia that are present in the soil in sufficient numbers, then inoculation may not be necessary. The inoculation technology comes in different forms; powder or granular forms are common. The powder form is applied directly to the seeds before planting. The common problems with inoculations are their poor competitiveness with local strains; sensitivity to climatic and other stresses limiting their viability and number; and problems of packaging, transport and storage until end-use on the farm (Smith 1987; Bantilan and Johansen, 1995). Without refrigeration, the live microbial culture loses its potency fast making the use of the inoculants a difficult option under smallholder farming conditions in the tropics, especially in the rural communities (Smith 1987; Bantilan and Johansen 1995; Singleton *et al*., 1997; Montanez 2000). This notwithstanding, inoculums have often been used to increase the number of desirable strains of rhizobia in the rhizosphere (Lupwayi *et al*., 2000). Fening *et al*. (2002) confirmed that only 6 % of the indigenous rhizobia across Ghanaian soils are highly effective with 68 % and 26 % being moderate and ineffective, which therefore necessitate the need for the use of inoculation in Ghanaian soils. Therefore inoculation at times can be used as a form of insurance against crop failures. Deaker *et al*. (2004) and Herridge *et al*. (2002) reported that less problem is associated with over inoculation rather than not inoculating at all.

2.7 Soybean production requirement

2.7.1 Cultivation period

The time for cultivation of soybean is dependent on the type of agro - ecological zone. Typically in the northern region of Ghana, it has been suggested that the best time for soybean cultivation is mid - June to early July (SARI, 2005). Soybean yield

depends on several factors e.g. seed germination and seedling vigor which will be influenced by the conditions necessary for germination such as air, water and warmth. Crop establishment is another problem and is often cited as a production problem for soybean in both arid and semi-arid areas of Central and West Africa (ICRISAT, 1984).

2.7.2 Soil requirements

Soybean is well adapted to a variety of soils and soil conditions. However, it does well on, fertile workable, loose, well - drained loam soil, which will encourage air movement to roots and nitrogen for effective nitrogen fixation. It was reported by Njeze (1993) that soybean can also do well in fertile sandy soils with pH within the range of 5.5 and 7.0, and that the plant can tolerate acidity better than other legumes but does not grow well in waterlogged, saline and alkaline conditions.

Optimum soil pH range of 5.5 - 7.0 enhances nutrient availability, such as nitrogen and phosphorus, break down of residues and symbiotic nitrogen fixation by microbes (Ferguson et *al*., 2003). Rienke and Joke (2005) reported high yield in loamy textured soil and added that if the seeds are able to germinate they do better in clayey soils.

2.8 Biological nitrogen fixation

Biological nitrogen fixation is a process used by microorganisms living in the soil to fix nitrogen in leguminous plants (Gregoire, 2003). It involves association of rhizobia and legumes. The rhizobium - legume symbiosis plays an important role in agriculture, because it offers the ability to convert atmospheric molecular nitrogen into forms useable by the plant (Jensen and Nielsen, 2003). 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 bright 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.

Soybeans are nodulated by the slow growing *Bradyrhizobium japonicum* (Jordan, 1982), *Bradyrhizobium elkanii* (Kuykendall *et al*., 1992), *Bradyrhizobium liaoningese* (Xu *et al*., 1995) as well as the fast growing *Sinorhizobium fredii* (Scholla and Elkan, 1984). Promiscuous soybean varieties are known to nodulate with a wide range of rhizobial strains and therefore, are likely to be widely adopted by farmers (Okereke *et al*., 2000; Fening and Danso, 2002; Okogun and Sanginga, 2003). The foregoing researchers have only dealt with the type of Bradyrhizobium that fix nitrogen with the soybean, but they have not shown which one is more effective in fixing nitrogen, under varying conditions of host and non - host factors.

2.9 Legume contribution in biological nitrogen fixation

Symbiotic nitrogen fixation by legumes plays an important role in sustaining crop productivity and maintaining fertility of marginal lands in smallholder farming

systems. The most important nitrogen - fixing symbiotic associations are the relationships between legumes and *Rhizobium* bacteria. Leguminous plants provide the major N input into the biosphere as a result of their ability to convert atmospheric N (N_2) to a form that can be assimilated by plants (Hardarson *et al*., 2003)

By providing N through fixation, legumes reduce mineral N inputs and the cost of production. Nitrogen fixation is variable in different grain legumes. Some legumes such as Faba bean (*Vicia faba*) and Lupin (*Lupinus spp*) are known for their effectiveness (i.e. up to 200 kg N ha^{-1} of their N in one season) under suitable field conditions, while soybean (*Glycine max*) can only fix on the average approximately about 100 kg N ha^{-1} (Hardarson *et al*., 2003). Sanginga (2003) reported the use of promiscuous soybeans for the development of sustainable cropping systems in the moist Savannahs of West Africa to alleviate the serious food production threat in N depleted soils. The actual amounts of N_2 fixed by soybeans and their residual N benefits to subsequent cereal crops varied between 38 and 126 kg N ha^{-1}, when only seeds of soybeans were removed from the plots while the net N accrual of soil nitrogen ranged between - 8 and + 47 kg ha^{-1} depending on soybean cultivar (Sanginga, 2003). Likewise Eaglesham *et al*. (1982) indicated that soybean derived less than 60% of their N from fixation which resulted in a negative contribution of – 36 kg N ha^{-1} to the N balance compared to a positive balance of 53 kg N by *Vigna unguiculata* (cowpea) in cropping systems in Nigeria.

2.10 Factors influencing rhizobia inoculation in legumes

The introduction of superior strains of rhizobia into the soil does not guarantee a higher BNF hence higher yield (Lupwayi *et al*., 200). 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 – fixation capacity (Slattery and Pearce, 2002). The success of inoculation, therefore, depends on a number of factors which are not excluded to indigenous rhizobia and N availability (Keyser and Li, 1992).

2.10.1 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 not be present. The quality of the native rhizobia can affect a plant's response to inoculation (Giller and Chadisch, 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*. (1991) 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 indigenious 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 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 varied considerably among locations in Ghana (Fening and Danso, 2002).

2.10.2 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 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 filling stage increases the proportion of plant N derived from the N fixation (Yinbo *et al*., 1997).

2.11 Nutrient management systems and their deficiencies in legume - rhizobia symbiosis

In Rhizobium - legume symbiosis, the essential mineral nutrients are those required for the normal establishment and functioning of the symbiosis. Based on this definition adapted from Arnon *et al*. (1939), the following chemical elements C, H, O, N, P, S, K, Ca, Mg, Fe, Mn, Cu, Zn, Mo, B, C1, Ni and Co are known to be essential for the legume - *Rhizobium* symbiosis. Each essential nutrient has specific physiological and biochemical role with minimal nutrient concentrations required within both legumes and rhizobia to sustain metabolic function at rates which do not limit growth (Graham *et al*., 1988).

Mineral nutrients influencing nitrogen fixation in leguminous plants can result in both positive and negative effects. For example, the presence of mineral nitrogen in the soil inhibits both nodule formation and nitrogenase activity (Sprent *et al*., 1988) though there are contradicting reports to this. Other researchers have reported the need for mineral nutrient to establish the plant before nodulation commences (Becker *et al*., 1991; Keyser *et al*., 1992; Hardarson, 1993; Carsky *et al*., 2001).

The enhancing effect of low levels of combined nitrogen on N_2 fixation in legumes is related to the lag phase between root infection and the onset of N_2 fixation. Phosphorus (P) is second only to nitrogen as an essential mineral fertilizer for crop production. At any given time, a substantial component of soil P is in the form of poorly soluble mineral phosphates. A high phosphorus supply is needed for nodulation. When legumes dependent on symbiotic nitrogen receive an inadequate

supply of phosphorus, they may suffer from nitrogen deficiency. Weisaney *et al.* (2013) reported that the deficiency of phosphorous supply and availability remains a severe limitation to nitrogen fixation and symbiotic interactions. Potassium and sulphur are not usually limiting nutrients for nodulated legumes, although a K^+ supplement for osmoadaptation has to be considered for growth in saline soils. Among mineral nutrients, boron (B) and calcium (Ca) are undoubtedly the nutrients with a major effect on legume symbiosis. Both nodulation and nitrogen fixation depend on B and Ca^{2+}, with calcium being more necessary for early symbiotic events and B for nodule maturation (Delgado, 1998) Copper (Cu) plays a role in proteins that are required for N_2 fixation in rhizobia. Copper deficiency decreased nitrogen fixation in subterranean clover. Iron is required for several key enzymes of the nitrogenase complex as well as for the electron carrier ferredoxin and for some hydrogenases. A particular high iron requirement exists in legumes for the heme component of haemoglobin (Tang *et al.*, 1992).

Molybdenum is a metal component of nitrogenase; all N_2-fixing systems have a specific high molybdenum requirement. As reported by Brodrick *et al.* (1991), molybdenum deficiency induced nitrogen deficiency in legumes. Relying on N_2 fixation is widespread, particularly in acid mineral soils of the humid and sub humid tropics. A specific role for nickel in nitrogen fixing bacteria according to Buerkert (1990) is now well established with the determination that a nickel - dependent hydrogenase is active in many rhizobial bacteria. Ahmed *et al*. (1960) also reported that cobalt is required for the synthesis of leghemoglobin and for the growth of legumes relying on symbiotically fixed nitrogen. It has been established that rhizobium and other N_2 - fixing microorganisms have an absolute cobalt requirement whether or not they are growing within nodules and regardless of whether they are

dependent on a nitrogen supply from N_2 fixation or from mineral nitrogen. Therefore, in sustainable BNF in agriculture systems, the use of mineral fertilizers is one of the most important principles though it has to be done minimally and specifically according to the requirement of each farm location.

2.12 Optimization of N fixation

Biological N fixation presents economic, environmental, and agronomic benefits and could be used to a larger degree as an alternative to synthetic fertilizers (Silva and Uchida, 2000). However, nitrogen fixation in legumes requires the symbiotic interaction of plants with rhizobia bacteria. Increasing the quantity and efficiency of the N fixation process could increase crop productivity and reduce fertilizer costs. Optimizing this symbiosis may require improving the selection of the host and rhizobia participating in this interaction. Breeding for improved cultivars of legumes may enhance the genetic potential of the plants in fixing nitrogen which can result in 10% increase in N_2 - fixed relative to existing cultivars according to Giller and Cadish (1995).

Biological nitrogen fixation may be increased by repeated rhizobial inoculation (Vessey, 2004; Athar, 1998), use of more effective strains (Hynes *et al*., 1995), or co - inoculation with “helper organisms” such as mychorrhizae (Dileep - Kumar *et al*., 2001). The efficiency of N_2 fixation is not only dependent on the selection of the most robust strains of rhizobia but is also related to crop varieties and the interactions of specific strains with specific varieties. Good growth of the legume is also of importance (Keyser and Li, 1992). The environment also plays an important role, because it is the soil and climatic condition that will determine the plant growth and indirectly nodulation and root development. Giller and Cadish (1995) and

People *et al*. (1995) suggest that conditions that will render the soil non - productive should be guarded against. The legume and the inoculum strain should be able to survive and function optimally in the environment in question.

CHAPTER THREE

3.0 General Materials and Methods

3.1. Determination of soil chemical properties

3.1.1 Soil pH

Soil pH was determined using the glass electrode HT 9017 pH meter in a 1: 2.5 soil to distilled water (soil: water) ratio. A 20 g soil sample was weighed into a 100 ml plastic beaker. To this 50 ml distilled water was added from a measuring cylinder, stirred thoroughly and allowed to stand for 30 minutes. After calibrating the pH meter with buffer solutions at pH 4.0 and 7.0, the pH was read by immersing the electrode into the upper part of the suspension.

3.1.2 Soil organic carbon

The modified Walkley and Black procedure as described by Nelson and Sommers (1982) was used to determine organic carbon. The procedure involved 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 millilitre of diphenylamine indicator was added and titrated with 1.0 *M* ferrous sulphate solution.

Calculation:

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

M = molarity of ferrous sulphate solution

V_1 = ml ferrous sulphate solution required for blank titration

V_2 = ml ferrous sulphate solution required for sample titration

s = weight of air-dry sample in gram

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

0.39 = 3 x 0.001 x 100% x 1.3 (3 = equivalent weight of C)

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

3.1.3 Total nitrogen

The macro Kjeldahl method involving digestion and distillation as described by Soil Laboratory Staff (1984) was used in the determination of total nitrogen. A 0.5 g soil sample was weighed and put into a Kjeldahl digestion flask and 5 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. 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 25 ml aliquot of the digest was transferred to the reaction chamber and 10 ml of 40% NaOH solution was added followed by distillation. The distillate was collected in 2% 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 of nitrogen 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:

M = concentration of HCl used in titration.

a = ml HCl used in sample titration

b = ml HCl used in blank titration

s = weight of air-dried sample in grams

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

1.4 = 14 x 0.001 x 100% (14 = atomic weight of nitrogen)

v = total volume of digest

t = volume of aliquot taken for distillation

3.1.4 Available phosphorus

The readily acid – soluble forms of phosphorus were extracted with Bray No. 1 solution

(HCl: NH_4F mixture) (Bray and Kurtz, 1945; Olsen and Sommers, 1982). Phosphorus in the sample was determined on a spectrophotometer 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's no. 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 about 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 wavelength. 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 100ml volumetric flask and made to volume with distilled water.

Calculation:

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

where:

a = mg P/l in the sample extract

b = mg P/l in the blank

s = sample weight in gram

mcf = moisture correction factor

35 = volume of extracting solution

15 = final volume of sample solution.

3.1.5 Extraction of exchangeable cations

Calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}) and sodium (Na^{+}) in the soil were determined in 1.0 *M* ammonium acetate (NH_4OAc) extract (Black, 1986). 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 aluminium were determined in 1.0 *M* KCI extract as described by page *et al*. (1982).

3.1.6 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 burette), ethanolamine buffer (10 ml) and 0.2 ml Eriochrome Black T solutions 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 again was recorded. The titre value of calcium was subtracted from this value to get the titre value for magnesium.

Calculation:

$$\text{Ca + Mg (cmol(+)/kg} = \frac{0.01 \times (V_1 - V_2) \times 1000}{0.I \times W}$$

where:

W = weight in grams of air - dry soil extraction.

V_1 = ml of 0.01 *M* EDTA used in the sample titration.

V_2 = ml of 0.01 *M* EDTA used in the blank titration.

0.01 = concentration of EDTA used

3.1.7 Determination of calcium only

A 25 ml portion of the extract was transferred to a 250 ml conical flask and the volume made to 50 ml with distilled water. Hydroxylamine hydrochloride (1 ml), potassium cyanide (1 ml of 2% solution) and potassium ferro cyanide (1 ml of 2%) were added. After a few minutes, 4 ml of 8 *M* potassium hydroxide and a spatula of murexide indicator were added. The solution obtained was titrated with 0.01 *M* EDTA solution to a pure blue colour. Twenty milliliters of 0.01 *M* calcium chloride solution was titrated with 0.01 *M* EDTA in the presence of 25 ml 1.0 *M* ammonium acetate solution to provide a standard pure blue colour. The titre value of calcium was recorded.

3.1.8 Determination of exchangeable potassium and sodium

Potassium and sodium in the percolate were determined by flame photometry. A standard series of potassium and sodium were prepared by diluting both 1000 mg/l potassium and sodium solutions to 100 mg/l. This was done by taking a 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 solution were put into 200 ml volumetric flasks respectively. 100 milliliters 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 flame photometry at wavelengths of 766.5 and 589.0 nm 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 diluted blank sample.

s = air-dried sample weight of soil in grams.

mcf = moisture correcting factor

3.1.9 Determination of exchangeable acidity

Exchangeable acidity (defined as the sum of Al and H) was determined by titration method after extraction with 1.0 *M* potassium chloride (Page *et al*., 1982). A 50 g soil sample was put in 200 ml plastic bottle and 100 ml of 1.0 *M* KCl solution added.

The bottle was capped and shaken for 1 hour on a mechanical-electric shaker and then filtered. A 50ml portion of the filtrate was taken with a pipette into a 250ml conical flask and 2 – 3 drops of phenolphthalein indicator solution added. The solution was titrated with 0.1 *M* NaOH until the colour just turned permanently pink. A blank was included in the titration.

Calculation:

$$\text{Exchangeable acidity (cmol/kg soil)} = \frac{(a - b) \times M \times 2 \times 100 \text{ mcf}}{s}$$

where:

a = ml NaOH used to titrate with sample

b = ml NaOH used to titrate with blank.

M = molarity of NaOH solution

s = air-dried soil sample weight in gram

2 = aliquot factor (100/50)

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

3.1.10 Effective Cation Exchange Capacity (ECEC)

This was calculated by the summation of the exchangeable bases (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}) and exchangeable acidity ($Al^{+} + H^{+}$).

3.1.11 Determination of copper, iron and manganese

Copper, iron and manganese in the soil were determined using the diethylenetriamine pentaacetic extraction method. Ten (10) grammes air dried soil was weighed into separate plastic bottles for Cu, Fe and Mn after which hundred milliliters DPTA extract was added to each. It was shaken for 2 hours and filtered with Whatman No. 42 filter paper. Their values were all read on an Atomic Absorption Spectrophotometer using the appropriate standards.

3.2 Plant tissue analysis of soybean

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 for the determination of total nitrogen in soil. Total phosphorus was determined using the spectrophotometric vanadium phosphomolybdate method. One gram of plant sample was weighed into the digestion tube. One millilitre of digestion mixture ($HCIO_4$ and HNO_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 and 10 ml of vanadomolybdate added. Distilled water was then added to make the required volume. The mixture was then shaken vigorously and kept for 30 minutes. This was then read on a 430 nm spectrophotometer after a yellow colour had developed to record the percentage absorbance. The absorbance and the P content were determined from a standard curve.

3.3 Enumeration of soil rhizobia population

The estimation of the rhizobia populations in the study fields were carried out using the most probable number method (MPN) (Vincent, 1970). Uniform 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 containing 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 (Broughton and Dilworth, 1970) plant nutrient solution aseptically with the help of forceps. The growth pouches were arranged in a wooden rack and kept in the greenhouse awaiting inoculation.

Five – fold dilutions of each of the samples were made as follows: Five different test tubes were filled with 20 ml distilled water. With a pipette, 5 ml solution was transferred from the 10^{-1} dilution (which was prepared by vigorously shaking 100 g of the sample in 400 ml of the sterile distilled water) into one of the five different test tubes. Series of dilutions were then made from 10^{-1} to finally achieve 10^{-6}. Each growth pouch was inoculated with 1 ml of the dilutions replicated four times for each dilution series, using different pipette tips and started from the highest dilution to prevent contamination. The plants were watered with sufficient N – free nutrient solution when required. Nodulation was assessed after twenty eight days after which the total number of pouches that nodulated for each replicated dilution unit was used to determine the number of rhizobia per gram of soil using charts generated by MPNES software (Woomer *et al*., 1990)

3.4 Determination of soil physical properties

3.4.1 Particle size distribution

This was determined by the Bouyoucos hydrometer method (Bouyoucos, 1936). A 40 g soil was weighed into 250 ml beaker and oven dried at 105 ^{0}C overnight. The sample was removed from the oven and placed in a desiccator to cool, after which the oven dry weight was taken. A 100 ml of dispersing agent sodium hexa-metaphosphate was added to the soil. It was then placed on a hot plate and heated until the first sign of boiling was observed. The content of the beaker was weighed into a shaking cap and fitted to a shaking machine and shaken for 5 minutes. The sample was sieved through a 50 μm sieve mesh into a 1.0 L cylinder. The sand portion was dried and further separated using graded sieves of varying sizes into coarse, medium, and fine sand. These were weighed and their weights taken. The 1.0

L cylinder containing the dispersed sample were placed on a vibration - less bench and then filled to the mark. It was covered with a watch glass and allowed to stand overnight. The hydrometer method was used to determine the silt and the clay contents. The cylinder with its content was agitated to allow the particles to be in suspension. It was then placed on the bench and hydrometer readings taken at 40 seconds and 6 hours interval. At each hydrometer reading, the temperature was also taken. The percent sand, silt and clay were calculated as follows:

% Clay = corrected hydrometer reading at 6 hours x 100/weight of sample

% Silt = corrected hydrometer reading at 40 seconds x 100/weight of sample - % clay.

% Sand = 100 % - % silt - % clay

The various portions were expressed in percentage and using the textural triangle, the texture was determined.

3.4.2 Determination of bulk density

About 1 – 2 cm surface soil was removed from the sampling spot and the spot levelled. A 5 cm diameter thin-sheet metal tube of known weight (W_1) and volume V was driven 5 cm into the soil surface. The soil around the tube was excavated and excess soil trimmed from the tube ends. The soil was put in an oven at 105^{o}C for 2 days and its weight (W_2) recorded.

Calculation:

$$\text{Bulk density (g cm}^{-3}) = \frac{W_2 - W_1}{V}$$

3.4.3 Moisture content

The moisture content of the soil was 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 ^{0}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:

$$\text{Moisture (\%)} = \frac{\text{Weight loss}}{\text{Weight of sample}} \text{ x } 100$$

$$= \frac{M_2 - M_3}{M_2 - M_1} \, x \, 100$$

where;

M_1 = weight of empty dish

M_2 = weight of empty dish + weight of sample before drying

M_3 = weight of empty dish + weight of sample after drying

3.5 Cultural practices

Weeding was done manually (hoeing) as and when necessary. Insecticide was also sprayed as and when required using knapsack.

3.6 Data collection

3.6.1 Nodulation

Ten soybean plants at 50% flowering growth stage from each plot were carefully uprooted from each experimental plot by digging around the plant using a spade and washed with clean tap water to remove all attached soil from the roots and the nodules. The nodules were then detached from the roots and counted and oven – dried at 70 ^{o}C for 48 hours. The dry weights of the nodules were then recorded.

3.6.2 Determination of shoot dry weight

The shoots of the ten plants used for the nodule sampling were separated from the roots. They were then dried in the oven at 70 °C for 72 hours. The dry weights of the shoots were recorded and later milled for laboratory analysis.

3.6.3 Determination of grain yield

Harvesting of the soybean was done according to the plot size at harvest maturity, air-dried, threshed and winnowed. The grains were then dried in an oven at 60 °C for 72 hours and the dry weight recorded. The grain yield was then estimated from the dry weight of the grains as suggested by Okogun *et al*. (2005).

3.6.4 Determination of harvest index

Harvest index is the ratio of crop economic yield (grain yield) to the biological yield at harvest (biomass yields). Harvest index (HI) of soybean was calculated using Bange *et al*. (1998) equation as follows:

$$\text{HI} = \frac{\text{Economic yield}}{\text{Biological yield}}$$

where :

Economic yield = grain yield

Biological yield = biomass yield

3.6.5 Agronomic efficiency

The agronomic efficiency of nitrogen in soybean biomass harvested at flowering was calculated as described by Dobbermann (2005)**:**

$$AE = \frac{(YN - YO)}{F}$$

where:

AE - agronomic efficiency

F - amount of (fertilizer) nutrient applied (kg N ha^{-1})

YN - crop yield with nutrient (kg N ha^{-1}) application

Y0 - crop yield (kg ha^{-1}) from the control plot

3.6.6 Nutrient use efficiency

This is the total biomass or grain yield produced per unit of fertilizer applied.

Nutrient use efficiency of soybean for nitrogen was calculated as:

$$NUE = \frac{Total\ grain\ or\ biomass\ yield}{Fertilizer\ nutrient\ rate\ applied}$$

3.6.7 Nitrogen and phosphorus uptake

Nutrient uptake was calculated by multiplying the nitrogen and phosphorus content of soybean shoot biomass and grain with their respective yield.

Calculation:

$$\text{N OR P uptake (kg ha}^{-1}) = \frac{\%N \text{ or } P \times Yield}{100}$$

where:

Yield = shoot biomass or grain yield (kg ha^{-1})

%N or P = N or P concentration in shoot biomass or grain

3.7 Statistical analysis

Collected data were subjected to mixed model (REML) for variance analysis using Genstat statistical software version 9. Treatment means were separated using Standard Error of Difference (SED) and significant differences were assessed at 5% (P = 0.05) level of significance using Chi^2 probability. Nodule count was transformed logarithmically (Kihara *et al*., 2011) before being subjected to analysis. Regression analysis between some measured variables was also undertaken.

CHAPTER FOUR

Effect of mineral fertilizer and *Bradyrhizobium japonicum* inoculant on soybean nodulation, growth and yield.

4.0 Introduction

Plants, like other living things, need food (both macro and micro nutrients) for their growth and development. The availability and uptake of nutrients are therefore very important especially in depleted soils of sub - Saharan Africa of which Ghana is no exception. The need to improve and increase grain yield of soybean cannot be overemphasized because soybean possess a lot of benefits to human, animal and soil fertility replenishment programmes. Soybean (*Glycine max (*L) Merrill) is a major oilseed crop with considerable nutritional, industrial, medicinal and of high economic importance (Graham and Vance, 2003) after groundnut and mustard. Soybean is an introduced crop in Ghana, yet it is becoming popular among smallholder farmers especially in the northern regions. Soybean can regulate the process which usually under low soil nitrogen conditions triggers nodule formation through a highly energy demanding process. Microbes derive this energy from carbohydrates which is photosynthetically made by the plant (Matt, 2009). It has been suggested that when a nutrient such as nitrogen is deficient in soil, it is more energy efficient for the plant to take up from available soil nitrogen sources, organic matter, manure or fertilizer application (Matt, 2009).

Despite the known ability of legumes to fix atmospheric nitrogen in symbiotic association with rhizobia, it has been demonstrated that supplementary fertilization can lead to improved performance of these crops (Mallarino, 2005). Apart from soil application, foliar spray of nutrients has also shown to be a practical means of replenishing the reservoir of nutrients in the leaves of legumes during pod

development, since the efficiency of nutrient uptake by roots as well as symbiotic fixation activities are known to decline at this stage (Ashour and Thalooth, 1983). Alternatively, the production of nitrogen by legumes in symbiosis with rhizobium reduces the cost of production, but for a legume (soybean) to be efficient in growth and yield improvement, production sustainability measures aimed at maintaining soil health and sustaining crop production become paramount.

It is therefore important that an enabling environment is created by supplying the required nutrients at the right time, in the required quantity coupled with the application of rhizobium inoculant strain, especially when the soil has low indigenous rhizobia population and fertility due to many years of soil degradation (Hansen *et al*., 1995). Although the required nutrients for soil fertility replenishment are available in the market for farmers, the efficacy of these products has often not been broadly investigated. Farmers lack substantial knowledge on the benefits of agro inputs such as nitrogen, Boost xtra and inoculats available in the markets for soybean production. It is thus important that these commercial products are evaluated before being recommended for farmers use in areas where the knowledge of their use is minimal.

4.1 Objective

To evaluate the effectiveness of Boost xtra, mineral nitrogen and *Bradyrhizobium japonicum* inoculation on nodulation, growth and yield of soybean

4.2 Literature review

4.2.1 Soybean response to inoculation

Inoculation is a technology used for the manipulation of rhizobia populations for improved crop productivity and soil fertility (Keyser and Li, 1992). Peoples *et al*.

(1995) reported that inoculation can lead to the establishment of large populations of rhizobia in the rhizosphere and improve nodulation and N_2 - fixation. Soybean response to inoculation is dependent on so many factors including the inherent field variability and differences in environmental and edaphic conditions (van Kessel and Hartley, 2000). Thies *et al.* (1991) reported that the response of legumes to inoculation depends to a large extent on the number of rhizobia already established in the soil, the availability of soil nitrogen and the management practices put in place. In general, and as confirmed by Araujo *et al*. (1994), the effective and efficient use of inoculation occur in soils which are depleted or contain low indigenious rhizobia population and when there is an established but inefficient rhizobia population. Dorivar *et al*. (2009) reported a positive response of rhizobia inoculation to nodulation, shoot biomass and grain yield while Otieno *et al*. (2009) reported increased nodule number, nodule dry weight but not shoot biomass, root dry weight and grain yield. These variations in response to inoculation could be due to many factors including soil pH, temperature, moisture content and soil nutrient status.

4.2.2 The response of soybean to mineral nitrogen and foliar fertilizer

Rathke *et al*. (2005) reported that mineral N fertilization is a crucial factor in oil - seed legume production. The N requirement of soybean can be met by both mineral N assimilation and symbiotic N_2 fixation (Hartwig, 1974). However, farmers have difficulty in satisfying the N demands of soybean because of its high N demand. Studies carried out on the effect of fertilizer - N on soybean growth and N_2 fixation showed that N fertilization increased growth but reduced N_2 fixation by causing reduction in nodule number and nodule weight (Chen *et al*., 1992; Starling *et al*., 1998). This, however, is dependent on quite a number of factors including the

amount of N applied, soil type, climate and farming system and the farmer's ability to afford fertilizer and using it at the required rate and time. Few studies have evaluated the determinants of successful starter N at planting and top dressing with N and foliar application of macro and micro nutrients at the late vegetative stage of soybean production in the savanna agro ecological zone of Ghana.

4.2.3 Need for fertilizer top dressing at flowering growth stage of soybean

Soybean flowering phase is followed by pod formation, leaf senescence, seed filling and morphological and physiological maturity**.** The peak of flowering is the peak of nodule activities (BNF) after which the nodules rupture and leaf falls. Studies have shown that the use of nutrient supplements at late vegetative, early or late reproductive stage prolongs plant vegetative stage and therefore complements biological nitrogen fixation, which tends to decline at this stage to sustain pod formation and seed filling (Ashour and Thalooth, 1993). The response of crop to foliar fertilizer application rapidly reflects in 3 - 4 days while nutrients applied to the soil takes five to six days (Fageria *et al*., 2009). The authors further reported that foliar fertilizer supplement soil fertilization because nutrients penetrate the cuticle of the leaf or the stomata and then into the cells more readily. This rate of ion passage through the cuticle and the epidermal tissues of the leaves depend on many factors such as the concentration, physical and chemical properties of the sprayed ion and micronutrients which are needed in limited quantities (Fageria *et al*., 2009). Garcia and Hanway (1976) reported yield increases of 27 to 31% when liquid N – P – K - S fertilizer was applied at late reproductive stages (R5 to R6). Wesley *et al*. (1998) and Mallarino *et al*. (2001) also reported an increase in yield due to the use of foliar fertilizer. On the contrary, Boote *et al*. (1978) and Parker and Boswell (1980) reported neither increase nor decrease in yield of soybean to the use of foliar

fertilizer. Clement *et al*. (2013) reported that the application of foliar micronutrients on the double inoculation of fungi - Rhizobium increased grain yield. Similarly, Ross *et al*. (2006) and Bellaloui *et al*. (2010) indicated the importance of some micronutrients such as boron on soybean nitrogen fixation and seed yield.

4.2.4 Fertilizer use efficiency

Fertilizer use efficiency of a crop is associated with many factors according to Aulakh and Benbi (2008), such as management practices that enhance fertilizer use efficiency, which includes best source of fertilizer, adequate rate and diagnostic techniques, proper method and right time of application, balanced fertilization, nutrient interrelationships, integrated nutrient management, time of seeding of crops and utilization of residual nutrients. Similarly, Bationo and Waswa (2012) attributed fertilizer use efficiency to mode of application, suggesting hill placement to be the most efficient method. Studies have also proofed that high fertilizer use efficiency is usually attributed to low fertilizer rates of application (Karim and Ramasamy, 2000). However, maximum profitability of fertilizer use is not only based on the use of low rate of fertilizer but some other factors such as, the soil fertility status, the soil pH, the environmental factor, the time of application, the growth stage of the plant and the demand for a particular nutrient at a particular point in time and the availability of other cheap nutrient resources that could make up for the limitations of inorganic fertilizer. Therefore there is need to evaluate the different resources available in the market to ascertain their efficiency in soybean growth more so that soybean has been declared to require all the essential nutrients for achieving its full potential.

4.3 Materials and Methods

4.3.1 Experimental site

This experiment was carried out at the experimental field of Savanna Agricultural Research Institute, located about 16 km west of Tamale, and lies on latitude 09^{o} 23'22.4'' N and longitude 01^{o} 00' 12.1" W, at an elevation of 195 m above mean sea level of the interior Guinea Savanna agro - ecological zone of Ghana. The rainfall is mono - modal (April / May – October), and a dry season with severe harmmattan wind occurring between December and January. The total annual rainfall ranges from about 800 to 1,500 mm (SARI, 2009) and the annual temperature ranges from a minimum of 13 ^{o}C to a maximum of 40 ^{o}C, with a mean of 28 ^{o}C. The experimental field had been previously cultivated to hot pepper for three consecutive years. The soil of the study area is Tingoli series classified as Ferric Luvisol (FAO/UNESCO, 1988).

4.3.2 Soil sampling and preparation

Composite soil samples for laboratory analysis were taken from (0 - 20 cm depth) the experimental site prior to land preparation. The samples were taken randomly across the field using a soil augur. Samples were then air dried, thoroughly mixed and passed through a 2 mm mesh sieve and packaged for laboratory analyses.

4.3.3 Determination of soil chemical and physical properties

Soils collected from the experimental field were analyzed for pH in a 1:2.5 suspension of soil to water ratio using the glass electrode method, organic carbon content by the modified Walkley Black procedure (Nelson and Sommers. 1982), total N Kjeldahl by distillation procedure (Bremner and Mulvaney, 1982), available phosphorus by Bray 1 (Bray and and Kurtz, 1954) and potassium using flame

photometry as described by Helmke and Sparks (1996). Micro nutrients were determined using the protocol described in sections 3.1.11. Soil physical properties such as particle size analysis and bulk density were also determined using the procedures described in sections 3.4.

4.3.4 Land preparation and layout

The land was ploughed, harrowed and ridges were constructed mechanically. Plots measuring 7 m by 7 m were demarcated for planting. An alley of 2 m between plots and 3 m between blocks were also constructed.

4.3.5 Inoculation

Soybean seeds (var. Jenguma) were inoculated prior to planting with a peat - based inoculum of *Bradyrhizobium japonicum* at the rate of 5 g per one kilogram of seeds using the slurry method as described by Woomer *et al*. (1994).

4.3.6 Planting

Soybean seeds were planted at two seeds per hill on ridges made at 0.05 m within rows and 0.75 m between rows and covered with soil and thinned to one seed per hill two weeks later. Planting was done in June 2012 starting with the un-inoculated plots followed by the inoculated plots to avoid contamination.

4.3.7 Treatments

The treatments used for the study were: T_1=Boost xtra (BX), T_2=BX + *Bradyrhizobium japonicum,* T_3=25 kg N ha^{-1}, T_4=25 kg N ha^{-1} + *B. japonicum* (INO), T_5=50 kg N ha^{-1}, T_6=50 kg N ha^{-1} + INO, T_7=75 kg N ha^{-1}, T_8=75 kg N ha^{-1} + INO, T_9=25 kg N ha^{-1} + BX + INO, T_{10}=25 kg N ha^{-1} + BX, T_{11}=50 kg N ha^{-1} +

BX + INO, T_{12}=50 kg N ha^{-1} + BX, T_{13}=75 kg N ha^{-1} + BX + INO, T_{14}=75 kg N ha^{-1} + BX, T_{15}= INO and T_{16}=Control

4.3.8 Fertilizer application

Nitrogen was applied as ammonium sulphate. The 50 and 75 kg N ha^{-1} treatments were applied in two splits; 25 kg N ha^{-1} was applied seven days after planting and top dressed at 50% flowering with 25 and 50 kg N ha^{-1} for 50 and 75 kg N ha^{-1} treatments, respectively. Triple super phosphate and muriate of potash were applied basally (30 kg ha^{-1}) before planting. Boost Xtra (foliar fertilizer) was applied at two weeks intervals (4 L ha^{-1}) from 50% flowering growth stage to advanced podding stage giving a total rate of 600 mls ha^{-1} corresponding to 20% NPK, 1.5% MgO, 0.15%, 0.075% Mn, Fe and Zn, 0.0012% Co and Mo.

4.3.9 Experimental design

Each treatment plot of 7 × 7 m^2 was made of nine rows with 1 m between plots. The plots were laid in split - plot design with treatment combinations (BX and N) as main plot factors while *Bradyrhizobia japonicum* inoculant was the sub - plot factor. The treatments were replicated four times.

4.3.10 Statistical analytical procedure

Data obtained from the trial was analyzed with GenStat 9^{th} edition (2007), using general linear model (GLM) (mixed model). The various levels of significance (5%) and the standard errors (WALD Statistic) were determined. The use of mixed model allows the determination of sources of variation in the model. Regression analyses were carried out to determine the degree of relationship between and among variables and count data were transformed (Log) before running the analysis.

4.4 Results

4.4.1 Soil physical and chemical properties and MPN counts of the indigenious rhizobia

The soil of the experimental site was slightly acidic and low in all the soil nutrients measured (Table 4.1). The organic carbon (< 20 g kg^{-1}), total nitrogen (< 1 g kg^{-1}), exchangeable cations (< 5 cmol $_{(+)}$ kg^{-1}), effective cation exchange capacity (< 5 cmol $_{(+)}$ kg^{-1}) and extractable P (< 10 mg kg^{-1}) were low. The MPN count of indigenous rhizobia population at the study area was estimated as 5.12×10^1 cells g $soil^{-1}$.

Table 4.1 Physical and chemical properties and MPN counts of the experimental site

Soil property	Value
pH (1:2.5 H_2O)	5.5
Organic carbon (%)	0.9
Total N (g kg^{-1})	0.5
Extractable P (mg kg^{-1})	5.7
Exchangeable cations	
Ca ($cmol_{(+)}kg^{-1}$)	2.30
Mg ($cmol_{(+)}kg^{-1}$)	0.71
K ($cmol_{(+)}kg^{-1}$)	0.06
Na ($cmol_{(+)}kg^{-1}$)	0.08
Mn (mg kg^{-1})	4.09
Cu (mg kg^{-1})	9.02
Fe (mg kg^{-1})	19.00
Exchangeable acidity ($cmol_{(+)}kg^{-1}$)	0.73
Sand (%)	68
Silt (%)	24
Clay (%)	8
Texture	Sandy loam
MPN (cell g^{-1} soil)	5.12×10^1

MPN = Population estimate for indigenious soil rhizobia population

4.4.2 Effect of mineral nitrogen and Bradyrhizobium inoculation on nodulation and biomass yield

The application of nitrogen (25 kg N ha^{-1}) seven days after emergence resulted in 37% increase in nodule number over that of the control (Table 4.2). The uninoculated produced more nodules (44%) than the inoculated plants (Table 4.2), though not significant. There was no significant ($P > 0.05$) difference between 25 kg N ha^{-1} and the control in nodule dry weight, the Bradyrhizobium inoculation

produced 36% higher nodule dry weight than the uninoculated control which was significantly different.

Table 4.2 shows shoot biomass production of soybean at 50% flowering. Application of 25 kg N ha^{-1} resulted in a significantly higher soybean biomass yield of 3404 kg ha^{-1} over that of 0 kg N ha^{-1} (2157 kg ha^{-1}). However, the shoot biomass yield of the inoculated plots was not significantly ($P > 0.05$) different from the uninoculated control.

Table 4.2 Nodule number, nodule dry matter and shoot biomass yield of soybean as influenced by starter nitrogen and *Bradyrhizobium* inoculation

N_{25} = 25 kg N ha^{-1}

Treatment	Nodule number ha^{-1}	Nodule dry weight (kg ha^{-1})	Biomass yield (kg ha^{-1})
Fertilizer			
N_{25}	3127	182	3404
Control	2281	188	2157
SED	1133	27	353
Chi^2 pr	0.46	0.84	<0.001
Inoculation			
-Inoculation	3192	157	2820
+Inoculation (INO)	2216	213	2742
SED	1133	27	353
Chi^2 pr	0.39	0.04	0.83
CV (%)	9.5	24.0	22.0

4.4.3 Effect of nitrogen, Boost xtra and Bradyrhizobium inoculation on soybean grain yield and harvest index

Table 4.3 shows that the sole application of Boost xtra (BX), 25 and 50 kg N ha^{-1} produced grain yields which were significantly higher than that of the control. Furthermore, the addition of BX to the various levels of N resulted in significant increase in grain yield over that of the control except the combined use of BX and 25 kg N ha^{-1}. The BX + 75 kg N ha^{-1} gave the highest grain yield (3321 kg ha^{-1}) with corresponding increment of 17, 38 and 50% more than sole BX, 75 kg N ha^{-1} and control treatments respectively. The combined use of BX and 50 kg N ha^{-1} resulted in 3056 kg ha^{-1} which was significantly higher than the sole application of BX and 50 kg N ha^{-1}.

Table 4.3 shows the effect of nutrient application on harvest index of soybean. The untreated plot had higher harvest index value than the treated plots except the use of BX alone which produced the highest harvest index which significantly represented 10% increase over that of the control.

Table 4.3 Grain yield and harvest index of soybean as influenced by Boost xtra, nitrogen application and Bradyrhizobium inoculation

Treatment	Grain yield (kg ha^{-1})	Harvest index
Fertilizer		
BX	2849	0.56
N_{25}	2713	0.44
N_{50}	2955	0.47
N_{75}	2404	0.37
BX+N_{25}	2312	0.36
BX+N_{50}	3056	0.44
BX+N_{75}	3321	0.44
Control	2220	0.51
SED	404	0.04
Chi^2 pr	0.07	<0.001
Inoculation		
-Inoculation	2716	0.44
+Inoculation	2742	0.46
SED	202	0.02
Chi^2 pr	0.90	0.30
CV (%)	26.0	29.0

N_{25} = 25 kg N ha^{-1}, N_{50} = 50 kg N ha^{-1}, N_{75} = 75 kg N ha^{-1}

4.4.4 Soybean shoot biomass nitrogen and phosphorus contents and uptake as influenced by nitrogen fertilizer and Bradyrhizobium inoculation

Results of nitrogen content and uptake in shoot biomass at 50% flowering are presented in Table 4.4. The results indicate that application of 25 kg N ha^{-1} and sole *Bradyrhizobium japonicum* inoculant did not lead to significant increase over their respective control treatments.

Table 4.4 shows that the use of 25 kg N ha^{-1} did not result in significant increase in phosphorus content of shoot biomass at 50% flowering growth stage. Nonetheless, 25 kg N ha^{-1} showed 42% increase in phosphorus uptake over the control.

Table 4.4 Effect of nitrogen and Bradyrhizobium inoculation on nitrogen and phosphorus uptake in shoot biomass at 50% flowering growth stage

Treatment	N content (%)	N uptake (kg ha^{-1})	P content (%)	P uptake (kg ha^{-1})
Fertilizer				
N_{25}	1.43	48.7	0.18	6.06
Control	2.10	45.3	0.21	4.27
SED	0.48	8.71	0.03	0.65
Chi^2 pr	0.16	0.16	0.26	0.01
Inoculation				
-Inoculation	1.74	49.1	0.22	5.43
+Inoculation (INO)	1.79	49.1	0.19	4.90
SED	0.48	0.06	0.03	0.65
Chi^2 pr	0.91	0.77	0.62	0.41
CV (5%)	4.8	24.9	24.3	21.4

4.4.5 Agronomic efficiency of nitrogen in soybean shoot biomass

Agronomic efficiency of applied nitrogen was highest with the combination of BX and 25 kg N ha^{-1} followed by 25 kg N ha^{-1} and INO + 25 kg N ha^{-1} which recorded 24, 19 and 18 kg kg^{-1} respectively (Fig.4.1). Similarly, the combined use of BX + 50 kg N ha^{-1} led to agronomic efficiency of 14 kg kg^{-1}, while 25 kg N ha^{-1} + INO +BX resulted in an agronomic efficiency of 12 kg kg^{-1}.

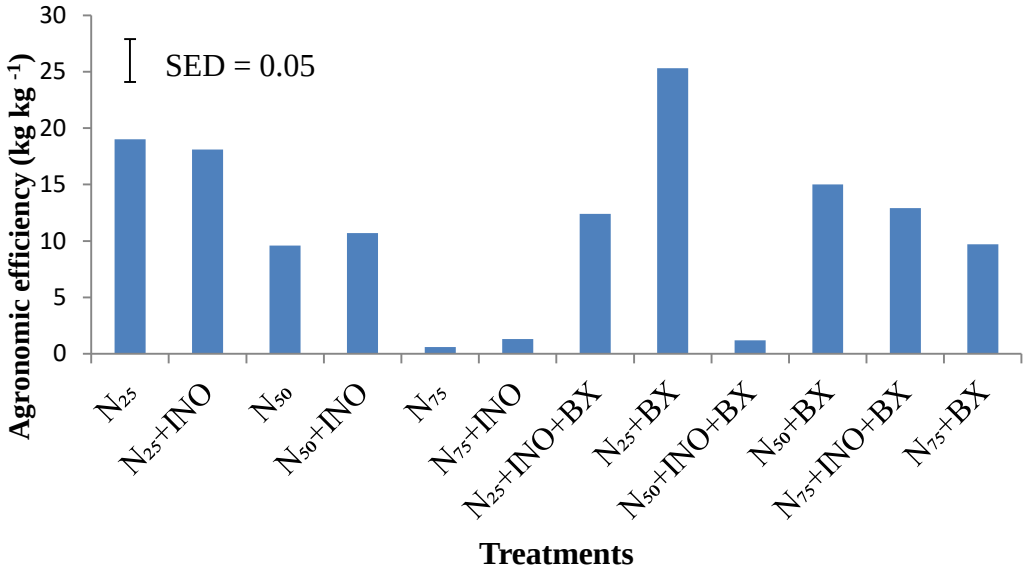

Figure 4.1 Agronomic efficiency of nitrogen in soybean shoot biomass as affected by treatments applied

4.4.6 Nutrient use efficiency (NUE) of soybean

The nutrient use efficiency of soybean with respect to the applied treatments is presented in Figure 4.2. Plots fertilized with 25 kg N ha^{-1} + Boost xtra gave the highest NUE value (300%) followed by Bradyrhizobium inoculant + 25 kg N ha^{-1} resulting in more than 200% N use efficiency. Conversely, the higher levels of N (50 kg N ha^{-1} and 75 kg N ha^{-1}) used were not as efficient as the lower level of N (25 kg N ha^{-1}) with and without inoculant or Boost xtra application.

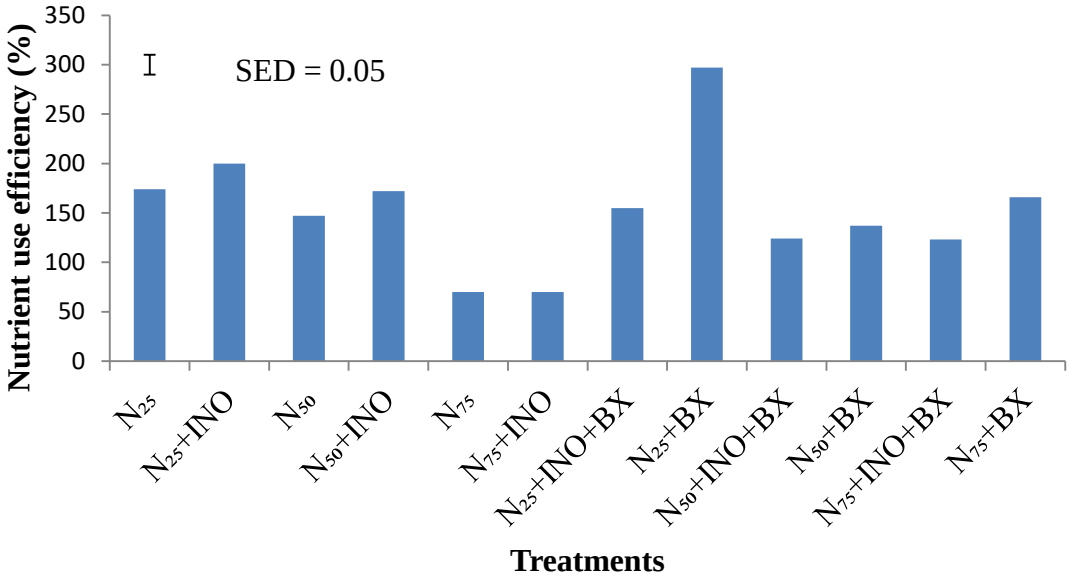

Figure 4.2 Effect of nitrogen, Boost xtra and Bradyrhizobium inocultion on nutrient use efficiency of soybean

4.5 Discussion

4.5.1 Influence of nitrogen and Bradyrhizobium inoculation on soybean nodulation and biomass yield at 50% flowering growth stage

4.5.1.1 Nodulation

Nodulation was improved by the use of 25 kg N ha^{-1} which was about 37% higher than control (Table 4.2). Chen *et al.* (1992); Gio, *et al*. (1993); Starling *et al.* (1998) and Eaglesham *et al.* (1981) reported that the use of starter nitrogen in soybean production enhances nodule formation. The finding in this study on nodule enhancement lends credence to the use of starter N for soybean production efficiency under low soil fertility status as was observed in the study area (Table 4.1). Chris *et al*. (1999) reported that at the initial vegetative growth stage, the root system is still poorly developed and the processes of nodule initiation and N_2 fixation are yet to be completed. The plant therefore depends on applied nitrogen for its survival at this

stage. According to Singleton *et al*. (1999), a starter N response will occur when the host plant shows poor nodulation or when ineffective rhizobial are present as was the case in the study site. The use of inoculant (*Bradyrhizobium japonicum*) did not significantly improve nodule number relative to the control, suggesting that the rhizobial populations' nodulating soybean were adequate in the study site. Thies *et al*. (1991) showed that the inoculation of eight leguminous crops growing in soils containing 10 to 100 indigenous rhizobia cells g^{-1} soil core increased the number of nodules per plant. In this study, the response to inoculation diminished as the indigenious rhizobia exceeded 50 cells g^{-1} soil. High population of the native rhizobia which resulted in more nodule formation in the control plants might have hindered soybean response to inoculation. However, this was contrary to the findings of other researchers, who showed larger responses to inoculation and higher nodule numbers per plant compared to the control (Okereke *et al*., 2004; Tahir *et al*., 2009; Bekere and Hailemariam, 2012). The findings of this study, notwithstanding the observation from earlier studies, suggest that inoculation does not enhance nodulation at all times. Okogun *et al*. (2004) reported that improved soybean varieties (TGx 1448-2E) did not respond to inoculation in terms of nodule production in Nigeria's moist Savanna zone possibly due to the soil fertility status at the commencement of the trial.

The fact that 25 kg N ha^{-1} as starter N did not significantly influence nodule dry weight despite the 37% nodule number advantage it had over the control, might be due to the soil pH (5.5) which did not favour efficient nodule development (Nyoki and Ndakidemi, 2014). Zhang *et al*. (2000) reported that mineral N affects several steps of the nodulation process by specifically reducing isoflavonoid concentration of soybean root systems. Conversely, the increase in nodule weight relative to the

control in response to inoculation is in line with the findings of Chemining'wa *et al.* (2007) who showed that there was no increase in nodule number during short and long - term rainy seasons of inoculant use but an increase in nodule dry weight during short-term rainy seasons was noticed in legumes such as common and lima beans which may be as a result of differences in inoculant type as well as environmental variation.

4.5.1.2 Influnce of nitrogen and *Bradyrhizobium* inoculation on shoot biomass yield

The application of starter nitrogen yielded 58% increase in shoot biomass over the control (Table 4.2). This appreciable increase could be attributed to the ability of starter N in averting the N hunger stage and therefore inducing effective photosynthesis especially at the early stage of the vegetative growth. The additional nodule number recorded from the use of starter nitrogen did not reflect in an increase in nodule dry weight but rather an increase in shoot biomass yield, an indication that nitrogen is indeed a major component of proteins and protoplasm and plays a vital role in achieving increased biomass development.

The inability of Bradyrhizobium inoculum alone to increase shoot biomass yield over the control could be attributed to the soil fertility status of the experimental field. Furthermore, the inability of inoculant to promote shoot biomass relative to the control could be as a result of an antagonistic effect between the indigenous strains and the introduced strains. According to Aliyu *et al*. (2013), the tendency of the native rhizobia to compete with the introduced strain cannot be underestimated. The findings of Bekere *et al*. (2012) also showed no positive relationship of seed inoculation and shoot dry weight as was the case in this study. On the contrary,

Theuri *et al*. (2003) and Solomon *et al*. (2012) observed significant effect on shoot dry weight and nodule number with respect to the use of commercial inoculants, possibly the strain used was different from that used in this study as well as differences in the environment.

4.5.2 Effect of Boost xtra, nitrogen and Bradyrhizobium inoculation on grain yield of soybean

The use of Boost xtra resulted in about 28% grain yield increase over the control, which is in line with the reports by Odeleye *et al*. (2007) and Hanway (1980) that the use of foliar fertilizer during seed filling increased soybean yield. Similarly, Ashour and Thalooth (1983) reported increased in seed yield as a result of foliar N during anthesis in soybean. Tayo (1981) reported that the application of essential nutrients was beneficial to the yield of cowpea, which implies that the application of foliar fertilizer at vegetative stage of soybean growth is essential, however, Odeleye (2007) indicated that NPK only without Mg was alright for soybean production.

The variation in grain yield at harvest (2404 - 2955 kg ha^{-1}) (Table 4.3) arising from the application of different nitrogen levels revealed that 25 kg N ha^{-1} might not sustain soybean growth till maturity for optimum grain yield. However, the 33% increase in yield obtained over the control from the application of 50 kg N ha^{-1} might not be economical especially for the smallholder farmers. Inoculation with Bradyrhizobium produced grain yield which was not significantly different from that of the control plot buttressing the fact that inoculation alone may not be enough to achieve optimum growth and yield of soybean in the study area. The fact that BX resulted in an increase in harvest index over the control and inoculation is an indication that the top dressing of macro and micro nutrients at mid - vegetative

stage of soybean is essential to effectively optimize its potential, hence the plant was able to partition effectively the absorbed nutrient through the leaves resulting in significant vegetative growth and grain yield.

4.5.3 Effect of nitrogen and Bradyrhizobium inoculation on N and P contents and uptake in shoot biomass at 50% flowering growth stage

There were no significant differences with regards to nitrogen concentration in the shoot biomass at 50% flowering, neither was there any with its uptake resulting from the use of starter nitrogen (25 kg N ha^{-1}) and inoculant. This implies that the assimilation rate of soybean was either low or soybean nitrogen demand was not satisfied by the available nitrogen (25 kg N ha^{-1}) in the soil at the time of harvest (50% flowering), which therefore connotes that top dressing of nutrients either as soil or foliar application is necessary especially in soils which are low in fertility. However, the fact that the use of 25 kg N ha^{-1} was able to enhance biomass P uptake (42%) shows that the ability of soybean to utilize any available nutrient in the soil has to be established on the availability of some level of nutrients in the soil at planting.

4.5.4 Influence of nitrogen, Boost xtra and *Bradyrhizobium japonicum* on agronomic efficiency and nutrient use efficiency of nitrogen

The fact that 25 kg N ha^{-1} and BX + 25 kg N ha^{-1} gave high agronomic efficiencies for soybean growth shows that the more the available N the lesser the ability of soybean to use it because of its tendency to fix nitrogen. This observation is similar to the findings of Amanullah and Lal (2009) that showed a negative relationship between AE and increasing N rate. The more the nitrogen applied, the more the plant's tendency to attain a point of "luxury consumption". This point is described as

the critical level in plant growth (Ulrich *et al.,* 1967) when the concentration of a given nutrient (in this case nitrogen) leads to a decline within a specific plant part, after attaining the sufficiency range (Tan, 2005).

The lowest level of N used in combination with the other treatments resulted in high nutrient use efficiency. Halvorson *et al*. (2005) reported that N use efficiency often decreases with increasing levels of applied N which is similar to the findings in this study. Contrarily, Hartemink *et al*. (2000) reported increases in N use efficiency due to increased N application. The findings of this study can be attributed to the fact that starter N (25 kg N ha^{-1}) enhanced establishment of a good rooting system, before the commencement of nodule formation which was further supported by top dressing with Boost xtra (Figure 4.2), thus emphasizing the need for nitrogen, and indeed all the required nutrients for efficient soybean growth. Furthermore, inoculating with the right strain of rhizobia will also improve the potential of the introduced strain to withstand competition from the indigenous strain provided a suitable environment is available for the inoculant to carry out the symbiotic processes.

4.6 Conclusions

i. Starter nitrogen (25 kg N ha^{-1}) is essential for soybean establishment before full nodule commencement.

ii. There is need for the application of macro and micro nutrients at 50% flowering growth stage (before podding) to prolong the pre - senescence stage and complement the shortage in nutrients that may likely result from the nodule decay, which consequently might reduce the ability of soybean to fix nitrogen.

iii. The response of soybean to BX, inorganic nitrogen, inoculation with *Bradyrihizobium japonicum* and their combinations at different growth stages has proven that the soil of the study area has low inherent soil fertility.

CHAPTER FIVE

Effect of mineral and organic fertilizer on inoculated soybean production

5.0 Introduction

Soybean (*Glycine max* (L) Merril) is an economically important crop among grain legumes, mostly grown in a wide range of environments all over the world. Aside its nutritional value, it has the potential to restore soil fertility through N_2 fixation process (Giller, 2001). Nitrogen fixation is a process which demands an enabling environment for an efficient symbiotic relationship between the host plant and the rhizobia in the plant rhizosphere to maximize soybean potential effectively. Soybean according to Giller (2001), is a relatively host specific plant and does not nodulate when grown for the first time in many parts of Africa. It was reported that the native *Bradyrhizobium* populations often do not meet N demand of the tested TGx genotypes in many parts of Nigeria (Okereke and Eaglesham 1993, Sanginga *et al*., 1996), eastern and southern Africa (Mpepereki *et al*. 2000), a condition which might not be too different from what exists in Ghana. However, a lot of success stories have been reported about the use of inoculants in Brazil, USA, (Ferreira and Hungria. 2002), Argentina, (Melchorre *et al*., 2011) and Zimbabwe (Mpepereki *et al.,* 2000). Yet, soybean's full potential has not been adequately exploited in the context of Ghana's agriculture. This may in part be explained by the limited nutrients and organic matter in the soil at the time of sowing. Woomer *et al*. (1997) and Gentili *et al*. (2006) suggested that climatic or edaphic factors that are unfavorable for plant establishment and growth, especially at seedling stage will restrict nodulation and indirectly affect the plant's potential for yield increase. The edaphic instability of soils in Ghana centered on inherently low fertility status,

degradation and nutrient depletion resulting from continuous use of farmland with little or no soil amendments. In Ghana, it was reported that the current average fertilizer application rates is lower (7.2 kg ha^{-1}) relative to other countries, like Malawi and Kenya (IFDC, 2012, Fuentes *et al*., 2012). Bationo *et al*. (2006) attributed low adoption of mineral fertilizer to the wide gap between farmers' yields and crops' potential yields. The production of soybean in Ghana is far below its potential yield relative to other soybean producing countries such as Brazil and South Africa. Phosphorus in particular is highly required for soybean production because the process of symbiosis (N_2 fixation) is a high energy demanding process; it also enhances energy metabolism, synthesis of nucleic acids and membranes, photosynthesis, respiration and enzyme regulation (Raghothama, 1999). Certainly, the use of chemical fertilizers alone may not keep pace with time in sustaining soil health and consequently improving and increasing soybean production in Ghana. It therefore becomes imperative to source cheaper resources to meet the demand of soybean nutrient requirements so as to enhance growth, nodulation and yield of soybean.Tabo *et al*. (2007) suggested that in order to improve soil structure and enhance its capacity to store adequate moisture and nutrients, the addition and incorporation of organic manure to the soil is an option of immense potential. International fertilizer development center (2012) reported that in sub - Sahara Africa, high prices of commercial fertilizers and limited availability of quality organic inputs (manure, crop residues, etc.) have resulted in the overall low use of the organic inputs. Therefore, due to the limitations of inorganic and organic resources, it will be worthwhile to establish if a synergy will result from the combined use of these resources and at the same time optimize soybean potential.

5.1 Objective

The objective of this study is to evaluate the effect of phosphorus, Fertisoil and Bradyrhizobium inoculation on nodulation, growth and grain yield of soybean.

5.2 Literature review

5.2.1 Response of soybean to phosphorus fertilizer application

Despite the substantial amount of total phosphorus in tropical soils, phosphorus deficiency is one of the most important soil fertility problems in tropical agriculture (Nyemba, 1986; Mengel and Kirkiby, 1987). According to Yahiya *et al*. (1995), phosphorus application to legumes can increase leaf area, number and weight of nodules on roots and acetylene reduction rate of the nodules. Furthermore, phosphorus is a major factor in many plant processes such as storage and transfer of energy, stimulation of root growth, flowering, fruiting and seed formation, nodule development and N_2 fixation (Mclaren and Cameron, 1996). Soybean requires phosphorus for attaining high yield especially under low available soil P status. However, its response to phosphorus fertilizer application is dependent on the crop environment and management factors. Also, Mallarino and Reuben (2005) observed that soybean response to P is dependent on soil available P while Ferguson *et al*. (2006) reported that P concentration above 12 ppm might hinder seed yield. Many studies have confirmed that the use of P is essential for increasing soybean seed yield (Haradagatti *et al*., 1996 and Nimje and Potkile, 1998; Ogoke 2004) while other researchers have suggested that phosphorus in combination with rhizobia inoculant increase nodulation N - fixation of legumes (Bhuiyan *et al*., 2008; Hoque and Haq, 1994). However, little information is available on the combination of Fertisoil and phosphorus on inoculated seed in the northern region of Ghana.

5.2.2 Response of soybean to organic manure application

The use of organic inputs as external nutrient sources has been advocated as a logical alternative to expensive fertilizers in Africa (Reinjitjes *et al*., 1992; Ganeshamurthy and Reddy, 2000). It is also an important part in establishing the intrinsic properties of a soil, which makes plant growth possible. Organic matter content is important for the proper management of soil fertility as it improves water - holding capacity of the soil and drought – resistance of many crops. Moreover, it permits better aeration, enhances nutrient absorption and release and reduce leaching and erosion (Sekhon and Meelu, 1994; Waters-Bayer, 1992). Composting is one of the options of rebranding organic manure. It helps to concentrate nutrients and kill disease causing organisms, slow down the release of nitrogen that might otherwise percolate into ground-water, and eliminate aesthetically objectionable odours (Kurihara, 1984). Other studies have reported positive responses in physiological and morphological growth of soybean with regards to the use of organic manure (CIAT, 1992; Mutitu *et al*., 1989) especially when combined with some other nutrient resources such as inoculants or minimum level of inorganic fertilizer (Otieno *et al*., 2009). Therefore, it is worth evaluating these resources in the northern region of Ghana to ascertain if soybean potential will be optimized using those treatment combinations.

5.3 Materials and Methods

5.3.1 Experimental site

A field trial was carried out at Cheshegu in the Northern region of Ghana, during the 2012 cropping season. Cheshegu is located on latitude 09^{o} 27’ 17.3’’ N, and longitude 00^{o} 57’ 23.0’’ W at an elevation of 187 m above mean sea level. The

annual rainfall is 1200 mm with mean average minimum and maximum temperatures of 26 ^{o}C and 39 ^{o}C, respectively.

5.3.2 Soil sampling, preparation and chemical analyses

Soil samples (Changnalili soil series classified as Gleyic luvisol (FAO/UNESCO, 1988)) were collected from the experimental field at a depth of 0 – 20 cm. The initial soil chemical characteristics of the study site were determined using standard procedures as indicated in sections 3.1 – 3.4.

5.3.3 Treatments, experimental design and layout

The field experiment consisted of the sole and combined applications of Fertisoil, inorganic fertilizer and Legumefix (*Bradyrhizobium japonicum*) to soybean growth, nodulation and yield. Fertisoil (0 and 3 tons ha^{-1}), NPK with nitrogen as ammonium sulphate at the rate of 25 kg ha^{-1}, phosphorus as triple super phosphate at the rate of 30, 60 and 90 kg P_2O_5 ha^{-1}, potassium as muriate of potash at the rate of 30 kg K_2O ha^{-1} and two levels of inoculum (uninoculated and inoculated) were used as treatments. The soil was amended with Fertisoil two weeks before planting while phosphorus, nitrogen and potassium were applied basally prior to planting. The experiment was a split - plot replicated four times. The treatment combinations (FS and P levels) were the main - plot factors while the inoculant was the sub - plot factor. The inoculation was carried out as was previously described under section 3.3.4.

The treatments used were: T_1=Fertisoil (FS), T_2=FS + *Bradyrhizobium japonicum* inoculant, T_3=30 kg P ha^{-1}, T_4=30 kg P ha^{-1} + INO, T_5=60 kg P ha^{-1}, T_6=60 kg P ha^{-1} + INO, T_7=30 kg P ha^{-1} + FS, T_8=*Bradyrhizobium japonicum* (INO), T_9=30 kg P ha^{-1} + FS + INO, T_{10}=60 kg P ha^{-1} + FS + INO, T_{11}=90 kg P ha^{-1}, T_{12}=90 kg P ha^{-1} + FS,

T_{13}=90 kg P ha^{-1} + FS + INO, T_{14}=90 kg P ha^{-1} + INO, T_{15}=Control and T_{16}=60 kg P ha^{-1} + FS. Fertisoil, a compost made from poultry waste and neem plant biomass was applied two weeks before planting.

5.4 Results

5.4.1 Initial soil characteristics of the experimental site

The initial soil physical and chemical analysis of the study area is characterized as generally low in soil fertility and slightly acidic (pH 5.5) (Table 5.1). Physical analysis showed that the soil is sandy loam. Other measured parameters were all lower than the required rate for most crop cultivation according to the classification by Landon (1991). The organic carbon (< 20 g kg^{-1}), total nitrogen (< 1 g kg^{-1}), exchangeable cations (< 5 c mol $_{(+)}$ kg^{-1}), effective cation exchange capacity (< 5 $cmol_{(+)}kg^{-1}$) and extractable P (< 10 mg kg^{-1}) were low. The soil Most Probable Number count for the native rhizobia was 5.5×10^1 cells g^{-1} soil at planting (Table 5.1).

Table 5.1 Physical and chemical properties of the soil experimental field before planting

Property	Value
pH (1:2.5 H_2O)	5.5
Organic carbon (%)	0.5
Total N (%)	0.2
Extractable P (mg kg^{-1})	3.5
Ca ($cmol_{(+)}kg^{-1}$)	1.68
Mg ($cmol_{(+)}kg^{-1}$)	0.30
K ($cmol_{(+)}kg^{-1}$)	0.18
Na ($cmol_{(+)}kg^{-1}$)	0.23
Mn (mg kg^{-1})	5.33
Cu (mg kg^{-1})	6.50
Fe (mg kg^{-1})	13.23
Exchangeable acidity ($cmol_{(+)}kg^{-1}$)	0.34
Sand (%)	61
Silt (%)	29
Clay (%)	10
Texture	Sandy loam
MPN (cell g^{-1} soil)	5.5×10^{1}

MPN = Population estimate for indigenious soil rhizobia population

5.4.2 Effect of Fertisoil, phosphorus and Bradyrhizobium inoculation on soybean nodulation

Table 5.2 shows the response of soybean nodulation to Fertisoil, phosphorus application, and Bradyrhizobium inoculation. The application of Fertisoil, phosphorus and *Bradyrhizobium japonicum* and their combinations did not result in significant increase in nodule number. On the contrary, data on nodule dry weight indicates that Fertisoil significantly ($P < 0.05$) improved nodule development

resulting in 51% dry weight increase over the control. Inoculated soybean produced significantly ($P < 0.05$) higher nodule dry weight than the control resulting in 407% over the uninoculated treatments.

Table 5.2 Nodule number and nodule dry weight as influenced by Fertisoil, phosphorus and Bradyrhizobium inoculation

Treatment	Nodule number ha^{-1}	Nodule dry weight (kg ha^{-1})
Fertilizer		
Fertisoil (FS)	2465	69.25
P_{30}	3090	34.88
P_{60}	2084	25.88
P_{90}	1910	34.63
P_{30} + FS	1825	48.75
P_{60} + FS	2823	43.50
P_{90} + FS	3317	45.00
Control	3447	45.75
SED	8044	13.77
Chi^2 pr	0.26	0.10
Inoculation		
-Inoculation	2882	14.31
+Inoculation	2358	72.59
SED	4022	6.88
Chi^2 pr	0.19	< 0.001
CV (%)	15.1	29.0

IN0 = Inoculation, FS = Fertisoil, P_{30} = 30 kg P ha^{-1}, P_{60} = 60 kg P ha^{-1}, P_{90} = 90 kg P ha^{-1}

5.4.3 Effect of Fertisoil, phosphorus and *Bradyrhizobium* inoculation on soybean biomass, grain yield and harvest index

5.4.3.1 Biomass yield

Table 5.3 shows the effect of Fertisoil, phosphorus and inoculant application on shoot biomass yield. The amendment of soil with Fertisoil produced biomass yield of 6250 kg ha^{-1} which was significantly different from the uninoculated control (3324 kg ha^{-1}) obtained from the un-amended control. The response to P fertilizer application to increase biomass yield can be ranked as 30 > 60 > 90 kg P_20_5 ha^{-1} though the differences between them were not significant but were significantly higher than that of the control. The combination of Fertisoil with the various P levels resulted in 28, 40 and 61% increase in shoot biomass yield over sole P level of 30, 60 and 90 kg P ha^{-1} respectively.

5.4.3.2 Grain yield

The response of soybean grain yield to the application of Fertisoil, P and *Bradyrhizobium japonicum* inoculant is shown in Table 5.3. The treatment plots amended with fertisoil produced significantly ($P < 0.05$) higher soybean grain yield relative to the control. The response of soybean grain yield to fertilizer (P levels) application can be ranked as 90 > 30 > 60 kg P ha^{-1} which represented 22, 20 and 13% increase, respectively over the grain yield of the control. Furthermore, the amendment of soil with combined Fertisoil and varying P levels resulted in marginal increase in grain yield over the yields from treatment plots that received sole application of 90, 30 and 60 kg P ha^{-1} respectively which were also significantly higher ($P < 0.05$) than the control and the use of Fertisoil only. The inoculation of soybean seed with *Bradyrhizobium japonicum* did not significantly increase soybean

grain yield. The amendment of soil with increasing levels of P and the use of *Bradyrhizobium* inoculant did not significantly increase the harvest index of soybean (Table 5.3).

Table 5.3 Grain yield as influenced by Fertisoil, phosphorus and *Bradyrhizobium japonicum* inoculum

Treatment	Biomass yield (kg ha^{-1})	Grain yield (kg ha^{-1})	Harvest index
Fertilizer			
FS	6250	3308	0.30
P_{30}	4775	3367	0.32
P_{60}	4752	3146	0.33
P_{90}	4214	3418	0.34
P_{30} + FS	6106	3524	0.32
P_{60} + FS	6649	3583	0.28
P_{90} + FS	6798	3588	0.26
Control	3324	2796	0.45
SED	633	236	0.05
Chi^2 pr	<0.001	0.013	0.007
Inoculation			
-Inoculation	5177	3324	0.33
+Inoculation	5540	3358	0.32
SED	316	118	0.03
Chi^2 pr	0.25	0.77	0.56
CV (%)	23.6	14.1	10.13

5.4.4 Treatment effect on shoot biomass nitrogen content and uptake at 50% flowering stage

The use of Bradyrhizobium inoculant led to 7% increase in N content over the uninoculated treatment, while the addition of FS to inoculated treatments resulted in an increase of 21% over the use of Bradyrhizobium inoculant only (Table 5.4). The

30 kg P ha^{-1} × INO and 60 kg P ha^{-1} × INO resulted in increased shoot N content at flowering over (30 kg P ha^{-1} + FS) × INO and (60 kg P ha^{-1} + FS) × INO respectively (Table 5.4) which were at par with the treatment that produced the highest N content value (FS × INO). The various treatment combinations increased N content in shoot biomass but resulted in N contents which were not significantly higher than that of inoculated treatment. However, the combined application of 30 kg P ha^{-1}, FS and INO, and 90 kg P ha^{-1} × INO produced shoot N contents that were significantly higher than that of the inoculation only (Table 5.4).

Table 5.4 shows the influence of sole nutrient application and their combinations on nitrogen uptake by soybean plant at 50% flowering. Soybean on Fertisoil amended soil resulted in a significant nitrogen uptake (31%) relative to the control. The application of 30, 60 and 90 kg P ha^{-1} increased soybean biomass nitrogen uptake by 43, 32 and 20%, respectively over that of the control. The combination of Fertisoil and the P levels also resulted in significant increases in N uptake of biomass. Similarly, N uptake of the inoculated treatment was significantly higher than that of uninoculated.

Table 5.4 Effect of treatments on shoot biomass nitrogen content and uptake at 50% flowering growth stage

Treatment	N content (%)	N uptake (kg ha^{-1})
Fertilizer		
FS	2.21	108.5
P_{30}	2.53	118.2
P_{60}	2.60	109.3
P_{90}	2.41	99.5
P_{30} + FS	2.19	123.2
P_{60} + FS	2.44	161.5
P_{90} + FS	2.25	132.3
Control	2.40	82.7
SED	0.25	20.12
Chi^2 pr	0.61	0.008
Inoculation		
-Inoculation	2.30	107.9
+Inoculation	2.46	125.9
SED	0.12	10.06
Chi^2 pr	0.19	0.07
Fertilizer × Inoculation		
FS × INO	2.85	133.3
Control × INO	2.36	104.0
P_{30} × INO	2.55	120.7
(P_{30} + FS) × INO	1.87	117.0
P_{60} × INO	2.73	119.3
(P_{60} + FS) × INO	2.66	167.7
P_{90} × INO	2.14	81.0
(P_{90} + FS) × INO	2.51	164.3
SED	0.35	28.45
Chi^2 pr	0.003	0.20
CV (%)	9.10	29.8

5.4.5 Treatment effects on phosphorus content and uptake in shoot biomass at 50% flowering growth stage

Table 5.5 shows that the application of sole P and FS did not significantly increase P content in shoot at flowering relative to the control. Only the use of 90 kg P ha^{-1} + FS resulted in increased P content in biomass relative to only Fertisoil treatment. The response to the different levels of P applied on P uptake was not significantly different from each other but could be ranked as $P_{30} > P_{60} > P_{90}$ representing 44, 36 and 20% increase over the control respectively. Phosphorus uptake was increased by 31% over the control when Fertisoil was added. The use of inoculant resulted in more P accumulation than obtained from the control treatment.

Table 5.5 Effect of nutrients applied on shoot biomass phosphorus and uptake at 50% flowering growth stage

Treatment	P content (%)	P uptake (kg ha^{-1})
Fertilizer		
FS	0.26	12.83
P_{30}	0.29	14.17
P_{60}	0.30	13.33
P_{90}	0.30	11.83
P_{30} + FS	0.28	15.83
P_{60} + FS	0.28	19.00
P_{90} + FS	0.31	18.67
Control	0.29	9.83
SED	0.04	3.42
Chi^2 pr	0.93	0.09
Inoculation		
-Inoculation	0.28	13.46
+Inoculation	0.30	15.42
SED	0.02	1.71
Chi^2 pr	0.39	0.25
CV (%)	15.6	21

5.4.6 Agronomic efficiency of phosphorus

Figure 5.1 shows that among the various treatment combinations the agronomic efficiency was highest for 30 kg P ha^{-1} + FS and INO and 30 kg P ha^{-1} + INO treatments. This was followed by FS + 30 kg P ha^{-1}. Furtheremore, the sole application of 30 kg P ha^{-1} gave a higher agronomic efficiency relative to 60 and 90 kg P ha^{-1} treatments.

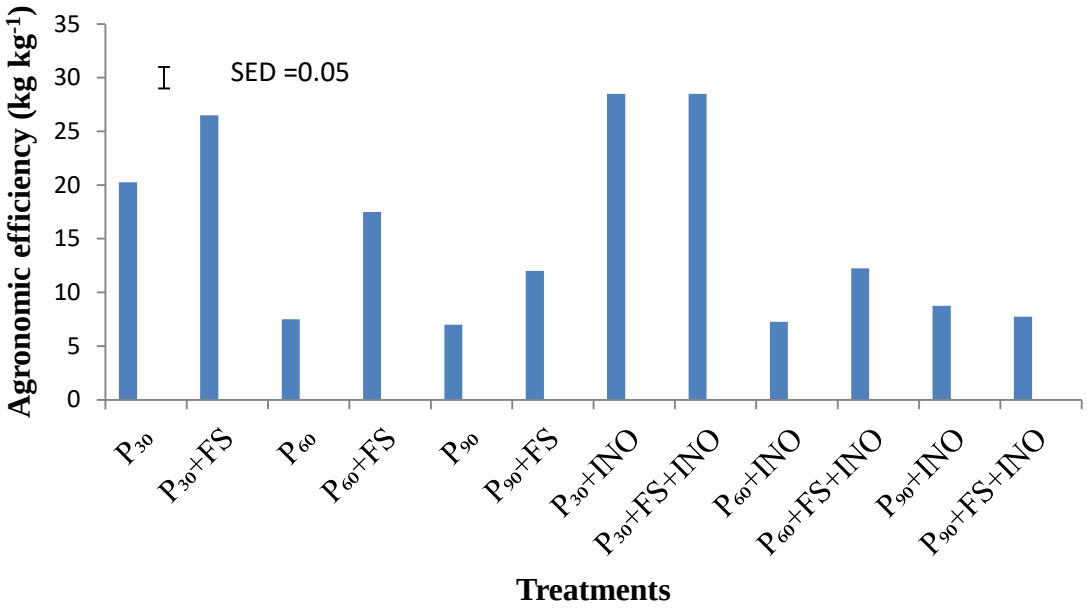

Figure 5.1 Agronomic efficiency of phosphorus in soybean as influenced by treatments applied

5.4.7 Nutrient use efficiency of soybean

The trend of soybean response to the applied treatment combinations with regards to nutrient use efficiency (NUE) was similar to that obtained from the AE. The lowest level of P (30 kg P ha^{-1}) and its combination with FS and / or inoculant resulted in the highest nutrient use efficiency value (Fig. 5.2) followed by 30 kg P ha^{-1}. However, the highest level of P (90 kg P ha^{-1}) and its combination with the other inputs resulted in the lowest NUE.

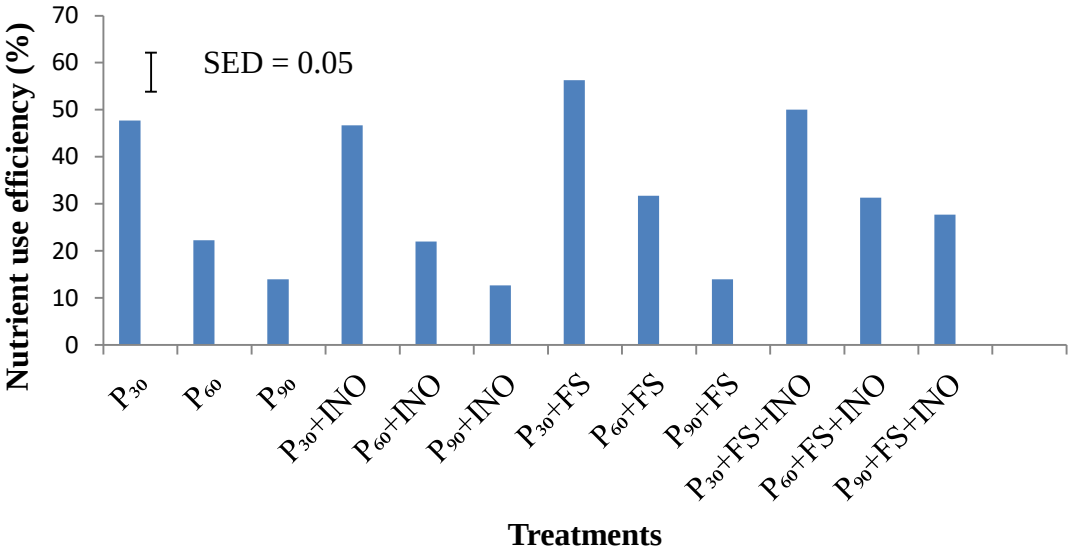

Figure 5.2 Nutrient use efficiency of phosphorus in soybean as influenced by treatments applied

5.5 Discussion

5.5.1 Effect of Fertisoil, phosphorus and *Bradyrhizobium japonicum* inoculation on nodulation and shoot biomass of soybean

5.5.1.1 Nodule number

The fact that sole application of Fertisoil produced nodule numbers which are comparable to the control (Table 5.2) implies that the release of nutrients in organic manure is a slow process, coupled with the slightly acidic nature of the soil at the study site. Nyoki and Ndakidemi (2014) reported that inoculation is not usually favoured below pH 5.5. The application of the various levels of P did not produce nodule numbers that were significantly higher than the control plots. This finding is similar to the reports of Bekere *et al*. (2012) and Bekere and Hailemariam (2012) who showed that no differences in nodule number were produced when different phosphorus levels were used in Ethiopia. However, Ogoke *et al*. (2006) reported an

increase in nodule number in response to the application of phosphorus in the moist savanna of West Africa.

The inability of inoculated soybean to produce significant nodule number compared to the control plot could be attributed to the inherent low nutrient characteristics of the soil in the study area (Table 5.1). This is however contrary to earlier reports of Okereke *et al*. (2004) and Tahir *et al*. (2009) who recorded increase in nodule numbers with the use of inoculant. These observations underscore the report by Okogun *et al*. (2004) that improved soybean varieties (TGx 1448-2E) did not always respond to inoculation in terms of nodule production in Nigeria's moist savanna zone which might be the case in Ghana. The combined application of phosphorus and Fertisoil resulted in increased nodule numbers which was possibly due to the decomposition of Fertisoil by microorganisms, thereby secreting different organic acids (Arora and Gaur, 1979) like carboxylic acid and thus lowering the rhizosphere pH (He and Zhu, 1988). Such organic acids have the possibility of enhancing the dissociation of the bound forms of phosphate like $Ca_3(P0_4)_2$ thereby making more phosphorus available for nodule formation. It also helps in improving soil water retention.

Despite the fact that the nodule numbers produced by Fertisoil amended plots were not significantly ($P > 0.05$) higher than the un-amended plots, the dry weight of the nodules produced was about 51% higher than the control and consequently with biomass P uptake of 12.83 kg ha^{-1} (Tables 5.3 and 5.6) which is a confirmation of the fact that more P is made available for nodule development and plant uptake with the use of organic manure in a slightly acid soil (pH 5.5). Bocchi and Tano (1994) reported that the positive response of legumes to manure can be attributed to the quantity of manure N already available for the plants, amount of N that becomes

available after mineralization during the season and the release and availability of phosphorus, potassium and microelements. There was also a significant ($P < 0.05$) increase in nodule dry weight when inoculation was complemented with Fertisoil which could be attributed to a synergy (positive interaction) that might have resulted by combining the two inputs, thereby enhancing the nodule weight of soybean.

5.5.1.2 Biomass yield

The sharp increase (50%) in shoot biomass accumulation resulting from the use of Fertisoil can be attributed to increased microbial population (Mabood *et al*., 2005) and organic colloids during Fertisoil decomposition (Son *et al*., 1981 and Botha *et al*., 2004) moisture/water relations. Similarly, the biomass yields obtained from the plots treated with the different P levels (30, 60 and 90 kg P ha^{-1}) were higher than that of the control. This implies that omission of P from soybean nutrition could drastically reduce shoot dry matter yield of soybean as suggested by Bekere *et al*. (2012) and, Bekere and Hailemariam (2012). The inoculation of soybean seed with *Bradyrhizobium japonicum* did not reflect in a significant ($P > 0.05$) increase in biomass yield over control.

5.5.1.3 Grain yield

The amendment of soil with Fertisoil produced 3308 kg ha^{-1} which was 18% higher than that of the control. The application of phosphorus at 60 kg P ha^{-1} gave the least grain yield among the different P levels which was 13% higher than the control. It can thus be said that phosphorus is involved in several energy transformation processes and biochemical reactions including nitrogen fixation, root development, stalk and stem strength in legume. Shahid *et al*. (2009) reported that large amounts of phosphorus are required for legume growth, nitrogen fixation and seed filling.

This study revealed that the application rates higher than 30 kg P ha^{-1} may not necessarily lead to corresponding increase in yield. The application of inoculant did not produce a significantly different grain yield from the control which is in agreement with Otieno *et al*. (2007) who reported that there was no significant effect on yield improvement by inoculation. This however is in disagreement with reports of Sable *et al.* (1998) and Shahid *et al*. (2009) that grain yield increased due to the use of inoculants.

5.5.2 Nitrogen uptake in soybean at 50% flowering

The amendment of soil with Fertisoil led to a distinct N uptake resulting in more than 100% increase in N uptake relative to the control treatment. This can be attributed to the achievement of a balanced nutrient situation from the mineralization process associated with organic manure. The application of the various P levels also contributed to N uptake in soybean, which could possibly be attributed to the importance of phosphorus to flowering, fruiting and root growth of leguminous plants because of its contribution to improving the nitrogenase activities in nitrogen fixation. The inoculation of soybean with *Bradyrhizobium japonicum* had a significantly higher N uptake than the control which had more nodule numbers. This observation could be due to the observed tiny nodules produced from the native rhizobia and the bigger few nodules formed from the introduced strain reflecting that soybean response to the introduced strain was positive and resulted in efficient N uptake.

5.5.2.1 Phosphorus uptake of soybean at flowering

The uptake of phosphorus varied among the treatments used (Table 5.5). The biomass harvested from the Fertisoil amended plots had the highest P uptake which

was significantly different from the control. This could be attributed to substrates produced from microorganisms during decomposition resulting in the dissociation of the bound form of phosphate ($Ca_3\ PO_4)_2$ (He and Zhu, 1988). This finding confirms to that of Whalen and Chang (2001) who reported that effectiveness of inorganic phosphorus fertilizers was increased by the addition of organic manure claiming that the transport of organic and inorganic phosphorus dissolved through the soil profile is favored by irrigation. Inoculated soybean enhanced shoot P uptake relative to uninoculated treatment. This agrees with the findings of Khair *et al* (2002) that inoculation slightly increased phosphorus uptake efficiency in soybean.

5.5.2.2 Agronomic efficiency and nutrient use efficiency

The agronomic and nutrient use efficiencies showed that the lowest level of P (30 kg P ha^{-1}) relatively responded better to shoot biomass which was in agreement with the findings of Bationo and Buerkert (2001) that small amounts of applied fertilizer optimized nutrient use efficiency. Similarly, the combination of 30 kg P ha^{-1} with FS and inoculation further enhanced soybean response to applied phosphorus. This observation can be attributed to the ability of the inputs to supplement each other thereby resulting in positive influence on soybean nutrient uptake and use.

5.6 Conclusions

i. The results of this experiment revealed that the amendment of soil with Fertisol (3 t ha^{-1}) gave 18% increase in grain yield relative to the control signifying its important role for soybean yield in the study area.

ii. The response of soybean to the different phosphorus levels is an indication of the need for phosphorus in soybean growth and yield.

iii. The yield of the lowest rate of P used (30 kg P ha^{-1}) was comparable to the highest level of P which was 26% over the control.

iv. Sole application of *Bradyrhizobium japonicum* inoculum could not significantly enhance soybean yield.

CHAPTER SIX

Effect of integrated nutrient management on soybean production

6.0 Introduction

One of the most important challenges facing food security today is how to sustain soil for productive farming activities, considering the inherent low soil quality in the tropics. There is the need for the possibility of using affordable nutrient resources for increasing food production while protecting the environment. With increase in human population, the stress on available land for farmers' use is becoming alarming, making it difficult to sustain food security. However, long term food security requires a balance between increasing crop production, maintaining soil health and environmental sustainability (Milkha, 2004).

Commercial fertilizers such as inorganic, organic, bio-fertilizer and foliar fertilizers have been in use for enhancing food production. However, the sole and combined use of these resources have not really been given much attention by researchers with respect to the production of soybean especially in the Northern region of Ghana knowing that if farmers are given the option to use fertilizer for their farm crops, they would rather use it for cereal instead of soybean (Camara and Heinemann, 2006). This is because farmers are aware of the fact that legumes in general have an inbuilt tendency of enhancing cereal crops grown in rotation or intercropped with them (Brophy and Heichel, 1989; Ta *et al*., 1989). It has been affirmed that for the production of soybean to be efficient with regards to growth, nodulation, grain yield and seed quality, it requires an optimum amount of nutrient especially in a depleted soil. Aulakh *et al*. (2005) reported that the application of unbalanced nutrients led to declining nutrient - use efficiency making fertilizer consumption uneconomical. On

the other hand, nutrient mining has occurred in many soils due to lack of accessible inorganic fertilizer sources and where fewer or no organic residues are returned to the soils. The use of inorganic fertilizer is indeed confronted with so many challenges such as high cost, lack of credit facilities, delivery delays and low variable returns (Heisey and Mwangi, 1996; Larson and Frisvold, 1996). Furthermore, the long-term use of inorganic fertilizers without organic supplements damages soil physical, chemical and biological properties and causes environmental pollution. Although, Albiach *et al*. (2000) reported that organic manures act not only as a source of nutrients and organic matter, but also increase size, biodiversity and activity of the microbial population in soil, influence structure, nutrients turnover and many other changes related to physical, chemical and biological parameters of the soil. However, Ewusi-Mensah (2009) attributed the low use of organic manure in the Upper East region of Ghana to their low nutrient and the bulky quantities needed for effective crop production; this situation is no different in Northern region of Ghana.

The hot climate in the Northern region of Ghana has resulted in soils that are inherently poor in organic matter and water - holding capacity. In such soils, macro and micronutrients, and organic matter are limitations leading to low productivity of soybean. Therefore, research interventions that address the factors limiting soybean production potential in the study area are paramount. Such options from the perspective of this study must aim at improving soil fertility based on soil nutrient - supply capacity, and judicious use of inorganic and organic fertilizers so as to achieve balanced nutrient - management system.

Integrated nutrient management is also important for marginal farmers who cannot afford to supply crop nutrients through costly chemical fertilizers and bulky organic manure. Alternative approach which considers the use of such resources in their minimum rates and in combination with of bio-fertilizer is now been advocated. Thus, to avail smallholder farmers the choice of best fit option, this study was carried out to develop an integrated nutrient management option to harness economically - viable and sustainable soybean production.

6.1 Objective

To assess the contribution of *Bradyhizobia japonicum* inoculation, inorganic and organic fertilizer on nodulation, growth and grain yield of soybean.

6.2 Literature review

6.2.1 Soybean production in Ghana

In Ghana, soybean is cultivated mainly in the Northern, Upper West, Upper East, Central and Volta regions. Among these geographical regions, the largest production occurs in Northern Ghana, which lies within the Guinea savanna and Sudan agro-ecological zones (Lawson *et al*., 2008). The average yield for Northern Ghana (Northern, Upper West and Upper East regions) is about 2.5 tons/ha on the farmers' field (Awuku, 1991) compared to that of USA which is 4.6 tons/ha (Richard *et al*., 1984).

The total annual tonnage of processed soybeans in Ghana is estimated to range between 26,100 and 35,200 MT, and 14, 707 MT of soybeans/grains and oil were exported between 1997 and 2005 from Ghana (Anonymous, 2006). Soybean is one of the most important annual grain legumes in the world, and it is considered as a

highly nutritive crop (Rothore, 2005). The seed of soybean consists of 40% protein, 18% fat, 6% ash and 29% carbohydrates (Antarlina *et al.*, 1999). At the household level, farmers in Northern Ghana use soybean in the preparation of "dawadawa", soups, "koko" (porridge), milk etc., as it is considered a source of inexpensive dietary protein, mineral and vitamin for both rural and urban dwellers. Despite the numerous uses of soybean, the crop has been faced with several challenges that result in low productivity (both quantitative and qualitative) among which include low yields as a result of bad farming practices that can be traced to inherent low soil fertility status and improper timing of fertilizer application and mode of application.

6.2.2 Integrated nutrient management

In the recent times, the use of integrated nutrient management (INM) approach is widely being advocated by researchers for crop production and soil fertility sustenance. The advantages derived from the combination of different resources such as organic manure, inorganic fertilizer and bio - fertilizers are tremendous, especially with the cultivation of grain crops. This has been attributed to the synergy which results from the combination of such resources. Each of such resources have its limiting factors hindering the efficiency of each at one time or the other, which when combined will be complemented for by the other resources, consequently resulting in an improvement in the efficiency of one another. The use of INM in soybean cultivation is not a usual practice among farmers because it is believed that legumes (soybean) have the ability to fix its own nitrogen from the atmosphere, so farmers in the study area do not usually use fertilizer for soybean cultivation. However, there is need for fertilizer application to enhance the growth and yield of soybean especially with the present soil fertility condition of the study area. Published reports on the use

of INM geared towards increase in yield, improvement in seed quality and soil sustainability is guaranteed when all other conditions have been satisfied in crop production. Wakene *et al*. (2001) reported that there is an indication that the combination of FYM and mineral fertilizer will improve the organic or farm yard manure (FYM) potentials. Several reports have reported the importance of organic nutrient sources particularly when integrated with mineral fertilizers in improving crop yields and land productivity under Ethiopian conditions (Asfaw *et al*; 1997 and Heluf., 2002).

6.3 Materials and Methods

6.3.1 Experimental sites

Three separate field experiments located at Cheshegu, Ghulahgu and Akukayili were conducted during the 2013 planting season. Ghulahgu is located on latitude 07^{o} 24' 14.3'' N and longitude 00^{o} 37' 20.0'' W at an elevation of 167 m above mean sea level whiles Akukayili and Cheshegu are located on latitude 09^{o} 23'22.4'' N and longitude 01^{o} 00' 12.1'' W, at an elevation of 195 m above sea level and on latitude 09^{o} 27' 17.3'' N, and longitude 00^{o} 57' 23.0'' W at an elevation of 187 m above mean sea level, respectively. The annual rainfall during the cropping season was 1200 mm with mean minimum and maximum temperature of 26 ^{o}C and 39 ^{o}C, respectively. The soils of Akukayili, Cheshegu and Ghulahgu are classified by FAO, (1988) as Ferric Luvisoil (Tingolis series), Gleyic Luvisoil (Chagnalili series) and Lixic Pinthosol (Nyankpala series), respectively.

6.3.2 Treatments and experimental design

Sole and combined applications of organic fertilizer (Fertisoil), inorganic fertilizer ($N_{25}P_{30}$ + Boost xtra) and Legumefix (*Bradyrhizobium japonicum*) were used as

treatments; T_1=Fertisoil (FS), T_2=Boost xtra (BX), T_3=25: 30 kg ha^{-1} (NP), T_4=Control, T_5=FS + BX, T_6=FS + $N_{25}P_{30}$, T_7=BX + $N_{25}P_{30}$, T_8=FS + BX + $N_{25}P_{30}$, T_9=*Bradyrhizobium japonicum* (lNO), T_{10}=INO + $N_{25}P_{30}$, T_{11}=FS + BX + INO, T_{12}=I + BX, T_{13}=INO + FS, T_{14}=INO + $N_{25}P_{30}$ + FS + BX, I T_{15}=NO + $N_{25}P_{30}$ + BX and T_{16}= lNO + $N_{25}P_{30}$ + FS. Plot size measuring 3 × 4.5 m^2 were made with 1 m, 2 m and 3 m spacings between plots, rows and blocks respectively. Each block was divided into eight plots and laid in a split - plot design (to avoid *Bradyrhizobium japonicum* inoculum being transferred to the uninoculated plots). The treatment combinations of Fertisoil, Boost xtra and $N_{25}P_{30}$ were the main plot factors while *Bradyrhizobium japonicum* inoculant was the sub – plot factor. Each treatment was replicated four times. Boost xtra was applied from 50% flowering growth stage to advanced podding stage giving a total rate of 600 mls ha^{-1} corresponding to 20% NPK, 1.5% MgO, 0.15%, 0.075% Mn, Fe and Zn, 0.0012% Co and Mo.

6.3.3 Fertilizer application

Ammonium sulphate (25 kg N ha^{-1}), triple super phosphate (30 kg P_2O_5 ha^{-1}) and muriate of potash (30 kg K_2O ha^{-1}) were applied seven days after sowing. Fertilizer treatments were applied by drilling beside each row at planting. Boost xtra (foliar fertilizer) was applied at the recommended rate of 4 L ha^{-1} and sprayed as foliar fertilizer using knapsack at two weeks intervals from late vegetative to advanced podding stage. Fertisoil was applied two weeks before sowing at a rate of 3 t ha^{-1}. Soybean seed inoculation was done as described in section 4.3.5.

6.3.4 Determination of chlorophyll content in leaves

Total chlorophyll content was measured using the SPAD meter by selecting five matured plants randomly within a plot and taking the SPAD measurements and the

average reading was recorded. The soil – plant Analyses Development (SPAD) unit of Minolta Camera Co. developed SPAD – 502 chlorophyll meters, a hand – held, self – calibrating, convenient and nondestructive light weight device used to calculate the amount of chlorophyll present in plant leaves. The meter records optical density measurement at two wavelengths, converts them into digital signals and then into a SPAD value (Minolta, 1989).

6.3.5 Determination of value cost ratio

This is the ratio between the value of the additional crop yield obtained from fertilizer use and the cost of fertilizer used. The gross rate of returns from the applied fertilizer represented by VCR was calculated according to Roy *et al.*, (2006) formular as:

$$\text{VCR} = \frac{X-Y}{Z}$$

where:

X = value of crop produced from fertilized plots

Y = value of crop produced from unfertilized plots

Z = cost of fertilizer

6.3.6 Other experimental procedures

Soil and plant analysis, harvesting schedules and statistical analysis were carried out as described in sections 3.1.2 – 3.1.8 and 3.1.11 – 3.1.13.

6.4 Results

6.4.1 Initial soil characteristics

Soil chemical properties determined in the study area before the commencement of the experiment showed that generally the soil fertility status is below the optimum recommended rate for most plants (pH 5.5, 5.8 and 4.7 for Akukayili, Cheshegu and Ghulahgu respectively). Across locations, the organic carbon (< 20 g kg^{-1}), total nitrogen (< 1 g kg^{-1}), exchangeable cations (< 5 cmol $_{(+)}$ kg^{-1}), effective cation exchange capacity (< 5 cmol $_{(+)}$ kg^{-1}) and extractable P (< 10 mg kg^{-1}) were low. The physical analysis showed that the soil is sandy loam at Akukayili and Cheshegu but silty loam at Ghulahgu (Table 6.1). The Most Probable Number of Bradyrhizobia determined shows that the native rhizobia populations are 6.3×10^1, 5.5×10^1 and 5.8×10^1 for Akukayili, Cheshegu and Ghulahgu respectively.

Table 6.1 Initial physical and chemical properties of the experimental field

Soil property	Akukayili	Cheshegu	Ghulahgu
pH (1:2.5 H_20)	5.5	5.8	4.7
Organic carbon (%)	0.7	0.7	0.5
Total N (%)	0.3	0.3	0.2
Extractable P (mg kg^{-1})	7.4	6.3	2.3
Ca ($cmol_{(+)}kg^{-1}$)	2.92	3.50	2.56
Mg ($cmol_{(+)}kg^{-1}$)	0.65	0.43	0.50
K ($cmol_{(+)}kg^{-1}$)	0.09	0.06	0.30
Na ($cmol_{(+)}kg^{-1}$)	0.05	0.18	0.36
Mn (mg kg^{-1})	5.00	3.00	4.35
Cu (mg kg^{-1})	4.02	5.00	3.46
Fe (mg kg^{-1})	10.00	12.20	14.00
Exchangeable acidity ($cmol_{(+)}kg^{-1}$)	0.58	0.25	0.30
Bulk density (g cm^3)	1.38	1.40	1.46
Moisture content (%)	40.00	39.00	45.00
Sand (%)	70	69	44
Silt (%)	20	21	54
Clay (%)	10	10	2
Texture	Sandy loam	Sandy loam	Silty loam
MPN (cells g^{-1} soil)	6.3×10^1	5.5×10^1	5.8×10^1

6.4.2 Evaluation of soybean nodule number and dry weight to treatments used

At Akukayili the amendment of soil with Fertisoil, Boost xtra and inorganic fertilizer ($N_{25}P_{30}$) did not significantly increase nodule number over that of the control (Table 6.2). The combined application of FS + BX did not lead to significant increase of nodule number over the control. However, FS + $N_{25}P_{30}$ and BX + $N_{25}P_{30}$ increased nodule number over their sole applications and the control. There was also a marginal nodule number increase from the use of FS + BX + $N_{25}P_{30}$ over the control.

The application of *Bradyrhizobium japonicum* inoculant resulted in a significant (P = 0.05) increase over the uninoculated control (16%) (Table 6.2).

Table 6.2 shows the response of soybean nodule dry weight to sole and combined application of the inputs used. The application of boost xtra at 50% flowering resulted in 21% increase in nodule dry weight even though this was not significantly different from the control. Soil amendment with fertisoil gave an increase of 53, 27 and 13% over the control, Boost xtra and $N_{25}P_{30}$, respectively. The FS + BX, FS + N_{25} P_{30} and FS + BX + N_{25} P_{30} treatment combinations also led to a corresponding increase of 72, 111 and 106% over the control, respectively.

At Cheshegu, the effect of sole treatments as well as their combinations on nodule number and nodule dry weight was not significantly different relative to the control (Table 6.3). Also, responses of nodule numbers to the combination of inoculation with the other treatment combinations were generally not significant. However, the use of (FS + BX) × INO resulted in higher nodule dry weight than other treatment combinations but was not significantly different from the weight recorded by ($N_{25}P_{30}$ + FS + BX) × INO treatment.

At Ghulahgu, soybean nodule number was significantly increased by the use of sole Fertisoil and $N_{25}P_{30,}$ FS + BX and $N_{25}P_{30}$ + FS + BX, compared to the control treatment (Table 6.4). The use of *Bradyrhizobium japonicum* inoculant also resulted in a significant (P < 0.05) nodule number production which was 35% higher than that of the uninoculated soybean. The highest nodule dry weight was produced by FS which was 88% higher than that of the control treatment. However, nodule dry weight was not significantly increased by the application of BX and $N_{25}P_{30}$ relative to the control. The use of $N_{25}P_{30}$ + FS + BX resulted in 14, 16 and 30% increase in

nodule dry weight over sole BX, $N_{25}P_{30}$ and control, respectively. Similarly, the use of *Bradyrhizobium japonicum* inoculant resulted in 190% nodule dry weight relative to that of the uninoculated control (Table 6.4).

Table 6.2 Effect of treatments and their combinations on nodule number and nodule dry weight at Akukayili

Treatment	Nodule number (ha^{-1})	Nodule dry weight (kg ha^{-1})
Type of fertilizer		
BX	6200	16.00
FS	6119	20.25
$N_{25}P_{30}$	6116	17.88
FS + BX	6204	22.75
BX + $N_{25}P_{30}$	6404	21.63
FS + $N_{25}P_{30}$	6507	28.00
FS + BX + $N_{25}P_{30}$	6400	27.25
Control	6008	13.25
SED	2685	3.50
Chi^2 pr	0.56	<0.001
Inoculation		
-Inoculation	5789	18.53
+Inoculation	6701	23.22
SED	1343	1.75
Chi^2 pr	<0.001	0.01
CV (%)	8.3	25.0

Table 6.3 Effect of treatments and their combinations on nodule number and nodule dry weight at Cheshegu

Treatment	Nodule number ha^{-1}	Nodule dry weight (kg ha^{-1})
Type of fertilizer		
BX	5765	21.00
FS	5694	17.50
$N_{25}P_{30}$	5718	24.12
FS + BX	5928	24.62
$N_{25}P_{30}$ + BX	5974	31.88
$N_{25}P_{30}$ + FS	5929	25.12
$N_{25}P_{30}$ + FS + BX	6190	35.00
Control	6212	33.75
SED	4822	9.63
Chi^2 pr	0.94	0.54
Inoculation		
-Inoculation	5893	26.12
+Inoculation	5959	27.12
SED	2411	4.82
Chi^2 pr	0.78	0.86
Fertilizer × Inoculation		
BX × INO	6302	27.00
FS × INO	5663	16.00
$N_{25}P_{30}$ × INO	5429	20.50
(FS + BX) × INO	6287	39.75
($N_{25}P_{30}$ + BX) × INO	5684	20.00
($N_{25}P_{30}$ + FS) × INO	5675	19.00
($N_{25}P_{30}$ + FS + BX) × INO	6329	25.25
Control × INO	6307	49.50
SED	6819	13.62
Chi^2 pr	0.57	0.02
CV (%)	16.0	31.8

Table 6.4 Effect of treatments and their combinations on nodule number and nodule dry weight at Ghulahgu

Treatment	Nodule number ha^{-1}	Nodule dry weight (kg ha^{-1})
Type of fertilizer		
BX	5123	216.5
FS	5858	356.6
$N_{25}P_{30}$	5519	213.3
FS + BX	5344	180.0
$N_{25}P_{30}$ + BX	5277	213.4
$N_{25}P_{30}$ + FS	5173	230.1
$N_{25}P_{30}$ + FS + BX	5429	246.6
Control	5237	189.9
SED	2675	58.19
Chi^2 pr	0.14	0.09
Inoculation		
- Inoculation	4563	118.3
+Inoculation	6178	343.3
SED	1337	29.09
Chi^2 pr	<0.001	<0.001
CV (%)	10.0	16.2

6.4.3 Evaluation of soybean leaf chlorophyll and shoot biomass yield to treatments used

Table 6.5 shows soybean leaf chlorophyll and shoot biomass response at 50% flowering to nutrient sources and their combinations at Akukayili. Fertisoil application led to 16, 18 and 20% increase in leaf chlorophlly content over that of BX, $N_{25}P_{30}$ and control, respectively. The combined application of FS + BX led to 17% more chlorophyll accumulation over that of sole BX application, while FS + $N_{25}P_{30}$ yielded 20% more than $N_{25}P_{30}$ treatment. However, both treatment combinations were not significantly different from that of sole FS. The combination of FS, BX and $N_{25}P_{30}$ resulted in 17% increase in chlorophyll accumulation relative to that of BX + $N_{25}P_{30}$ but was not different from that of FS + $N_{25}P_{30}$.

Data collected on mean biomass yield at Akukayili is as shown in Table 6.6. Sole application of BX resulted in shoot biomass yield which was significantly lower than FS, $N_{25}P_{30}$ and the control. Amendment of soil with Fertisoil enhanced biomass yield significantly ($P = 0.05$) given an additional weight which represented 89, 40 and 141% for BX, $N_{25}P_{30}$ and control, respectively. Similarly, FS + $N_{25}P_{30}$ application led to 17, 65 and 183% increase over biomass weight of sole FS, $N_{25}P_{30}$ and control, respectively. The use of Bradyrhizobium inoculant significantly ($P < 0.05$) increased biomass yield relative to the control.

Table 6.6 shows that the sole application of BX, FS and $N_{25}P_{30}$ contributed significantly to chlorophyll accumulation over the control at Cheshegu. The combination of these inputs (BX, FS and $N_{25}P_{30}$) shows that all the combinations significantly enhanced chlorophyll accumulation except $N_{25}P_{30}$ + BX which produced the least recorded value. The use of Bradyrhizobium inoculant only did not

significantly increase chlorophyll accumulation over the uninoculated control at 50% flowering stage. The biomass yields of BX and FS were not significantly different from each other (Table 6.6). Similarly, shoot biomass of plants treated with $N_{25}P_{30}$ were not significantly different from that of the control. Inoculation with Bradyrhizobium inoculant did not lead to significantly higher biomass accumulation relative to the control but its combination with BX led to 48% increase over that of sole application of inoculum, combined application of FS × INO led to 103% while $N_{25}P_{30}$ × INO yielded 17% more biomass than sole application of inoculum. All the treatment combinations with inoculation performed better in shoot biomass accumulation over that of sole application of inoculum except $N_{25}P_{30}$ × INO.

The response of soybean shoot biomass and leaf chlorophyll accumulation to treatment combinations at 50% flowering in Ghulahgu is as shown in Table 6.7. The sole use of BX, FS, $N_{25}P_{30}$ and their combinations resulted in more chlorophyll accumulation over that of the control. Furthermore, the leaf chlorophyll accumulation of $N_{25}P_{30}$ + FS plants was significantly ($P < 0.05$) different from BX, $N_{25}P_{30}$ and $N_{25}P_{30}$ + BX. The highest biomass yield (4473 kg ha^{-1}) at Ghulahgu was produced by $N_{25}P_{30}$ + FS + BX which was however not significantly different from other treatment combinations but significantly different from the control (2762 kg ha^{-1}).

Table 6.5 Effect of treatments and their combinations on leaf chlorophyll and shoot biomass yield at 50% flowering growth stage at Akukayili

Treatment	Chlorophyll	Biomass yield (kg ha^{-1})
Type of fertilizer		
BX	35.64	1645
FS	41.24	3103
$N_{25}P_{30}$	34.93	2216
FS + BX	41.79	2535
BX + $N_{25}P_{30}$	34.03	2046
FS + $N_{25}P_{30}$	41.15	3646
FS + BX + $N_{25}P_{30}$	40.04	2665
Control	34.39	1289
SED	1.30	445
Chi^2 pr	< 0.001	< 0.001
Inoculation		
- Inoculation	38.08	2120
+Inoculation	37.72	2666
SED	0.65	222
Chi^2 pr	0.57	0.01
Fertilizer × Inoculation		
BX × INO	34.48	1616
FS × INO	40.53	3997
$N_{25}P_{30}$ × INO	36.15	2622
(FS + BX) × INO	39.93	2684
(BX + $N_{25}P_{30}$) × INO	34.95	2553
(FS + $N_{25}P_{30}$) × INO	42.8	3882
(FS + BX + $N_{25}P_{30}$) × INO	39.58	2754
Control × INO	33.33	1221
SED	1.83	629
Chi^2 pr	0.06	0.40
CV (%)	6.80	25.0

Table 6.6 Effect of treatments and their combinations on leaf chlorophyll and shoot biomass yield at 50% flowering at Cheshegu

Treatment	Chlorophyll	Biomass yield (kg ha^{-1})
Type of fertilizer		
BX	35.87	2151
FS	38.05	2295
$N_{25}P_{30}$	37.53	1783
FS + BX	40.00	2389
$N_{25}P_{30}$ + BX	33.12	2091
$N_{25}P_{30}$ + FS	40.02	2183
$N_{25}P_{30}$ + FS + BX	39.46	2088
Control	36.01	1531
SED	1.13	362
Chi^2 pr	< 0.003	0.31
Inoculation		
- Inoculation	40.00	2197
+Inoculation	38.00	1931
SED	0.56	181
Chi^2 pr	0.43	0.14
Fertilizer × Inoculation		
BX × INO	33.30	1935
FS × INO	39.20	2652
$N_{25}P_{30}$ × INO	35.12	1528
(FS + BX) × INO	38.80	1901
($N_{25}P_{30}$ + BX) × INO	34.83	2276
($N_{25}P_{30}$ + FS) × INO	41.20	1837
($N_{25}P_{30}$ + FS + BX) × INO	38.30	2008
Control × INO	31.10	1308
SED	1.65	511.7
Chi^2 pr	0.06	0.31
CV (%)	6.8	17.3

Table 6.7 Effect of treatments and their combinations on leaf chlorophyll and shoot biomass yield at 50% flowering at Ghulahgu

Treatment	Chlorophyll	Biomass yield (kg ha^{-1})
Type of fertilizer		
BX	40.69	3862
FS	44.62	3717
$N_{25}P_{30}$	41.81	3414
FS + BX	44.60	3163
$N_{25}P_{30}$ + BX	43.65	4046
$N_{25}P_{30}$ + FS	45.23	4101
$N_{25}P_{30}$ + FS + BX	45.50	4473
Control	39.69	2762
SED	1.31	717
Chi^2 pr	< 0.001	0.30
Inoculation		
-Inoculation	42.20	4392
+Inoculation	44.24	2992
SED	0.66	359
Chi^2 pr	0.002	< 0.001
Fertilizer × Inoculation		
BX × INO	41.95	3043
FS × INO	45.35	2895
$N_{25}P_{30}$ × INO	42.47	2945
(FS + BX) × INO	45.27	2927
($N_{25}P_{30}$ + BX) × INO	45.07	3213
($N_{25}P_{30}$ + FS) × INO	45.25	2975
($N_{25}P_{30}$ + FS + BX) × INO	47.12	2619
Control × INO	41.45	3319
SED	1.85	1014
Chi^2 pr	0.90	0.07
CV (%)	6.1	28.9

6.4.4. Evaluation of soybean grain yield and harvest index to treatments used

Grain yield of soybean responded differently to the various treatment combinations (Table 6.8). Percentage increases of 24, 163 and 68% were recorded for BX, Fertisoil and $N_{25}P_{30}$, respectively over the control. The FS + BX treatment recorded grain yield which was statistically similar to that of FS treatment but was 118 and 169% higher than that of BX and control, respectively. The application of $N_{25}P_{30}$ + Fertisoil gave 30, 103 and 241% grain yield increase over that of FS, $N_{25}P_{30}$ and control, respectively. Furthermore, FS + BX + $N_{25}P_{30}$ gave 18, 166 and 218% grain yield increase over that of FS + BX, BX + $N_{25}P_{30}$ and the control respectively. The use of *Bradyrhizobium japonicum* inoculant led to 13% increase over that of the uninoculated control.

The result of harvest index is as shown in Table 6.8. The application of BX and $N_{25}P_{30}$ did not result in a significantly higher harvest index over that of the control. Sole application of Fertisoil gave a significant increase in harvest index which was 12% over that of the control treatment. The combination of Fertisoil + Boost xtra resulted in an increase in harvest index over that of the sole use of BX and control. Furthermore, application of FS + BX + $N_{25}P_{30}$ produced HI significantly different from that of the control, but not significantly different from the combination of FS and BX (Table 6.8). The sole use of Bradyrhizobium inoculum produced lower harvest index than that of the uninoculated control.

The grain yields produced from the sole use of the various treatments at Cheshegu were generally not significantly different from each other except that of $N_{25}P_{30}$ which produced lower grain yield relative to that of BX and FS (Table 6.9). The treatments FS + BX, $N_{25}P_{30}$ + BX and FS + $N_{25}P_{30}$ produced marginal increase in

grain yield over that of the control. However, the use of *Bradyrhizobium japonicum* inoculum resulted in a significant grain yield increase which was 21% higher than that of the uninoculated control. There were statistically significant ($P < 0.05$) differences resulting from the interaction effects, where the highest yield (3124 kg ha^{-1}) was produced from the plot treated with the four treatment (INO × (FS+ BX + $N_{25}P_{30}$) which was significantly different from the yield obtained from the other combinations and *Bradyrhizobium japonicum* inoculation only. The combination of the four inputs (INO × (FS + BX + $N_{25}P_{30}$) led to 13, 17 and 8% increase in grain yield relative to INO × BX, INO × FS and ($N_{25}P_{30}$ + BX) × INO respectively.

The sole use of BX, FS and $N_{25}P_{30}$ at Cheshegu resulted in harvest index not significantly different from each other (Table 6.9). The use of Bradyrhizobium inoculum caused 15% increase in harvest index over that of the control. Furthermore, the various treatments combinations with the addition of inoculation produced HI which was not significantly higher than that from inoculation alone.

The response of soybean grain yield to the treatment combinations at Ghulahgu is as shown in Table 6.10. The application of sole FS led to a significantly higher grain yield compared to that of the control. The use of FS and $N_{25}P_{30}$ led to 109 and 71% increase in grain yield over that of the control. The combinations of these treatments (BX, FS and $N_{25}P_{30}$) generally led to an increase in grain yield relative to the sole applications and the control. However, the combination of $N_{25}P_{30,}$ FS and BX resulted in a significantly higher grain yield (4008 kg ha^{-1}) relative to that of the combination $N_{25}P_{30}$ + BX, sole use of BX and control represented by 47, 149 and 166%, respectively. The sole use of *Bradyrhizobium japonicum* inoculum generally increased grain yield by 6% relative to other control. The harvest index data at Ghulahgu is as shown in Table 6.10. It was observed that all the sole treatments (BX,

FS and $N_{25}P_{30}$) and their combinations resulted in a significantly higher harvest index over that of the control except that of BX only. Similarly, the harvest index of Bradyrhizobium inoculated plants was also significantly higher than that of the uninoculated control.

Table 6.8 Effect of treatments and their combinations on grain yield and harvest index at Akukayili

Treatment	Grain yield (kg ha^{-1})	Harvest index
Type of fertilizer		
BX	1187	0.43
FS	2527	0.48
$N_{25}P_{30}$	1614	0.43
FS + BX	2582	0.51
BX + $N_{25}P_{30}$	1484	0.44
FS + $N_{25}P_{30}$	3272	0.48
FS + BX + $N_{25}P_{30}$	3054	0.52
Control	960	0.43
SED	242	0.05
Chi^2 pr	< 0.001	0.20
Inoculation		
- Inoculation	1955	0.48
+Inoculation	2215	0.45
SED	121	0.02
Chi^2 pr	0.03	0.36
Fertilizer × Inoculation		
BX × INO	1089	0.39
FS × INO	2502	0.42
$N_{25}P_{30}$ × INO	1932	0.43
(FS + BX) × INO	2803	0.51
(BX + $N_{25}P_{30}$) × INO	1603	0.40
(FS + $N_{25}P_{30}$) × INO	3334	0.47
(FS + BX + $N_{25}P_{30}$) × INO	3468	0.56
Control × INO	985	0.45
SED	342	0.06
Chi^2 pr	0.39	0.48
CV (%)	21.6	8.9

Table 6.9 Effect of treatments and their combinations on grain yield and harvest index at Cheshegu

Treatment	Grain yield (kg ha^{-1})	Harvest index
Type of fertilizer		
BX	2461	0.53
FS	2627	0.53
$N_{25}P_{30}$	1972	0.52
FS + BX	2081	0.48
$N_{25}P_{30}$ + BX	2158	0.49
$N_{25}P_{30}$ + FS	1982	0.48
$N_{25}P_{30}$ + FS + BX	2034	0.46
Control	1914	0.57
SED	305	0.04
Chi^2 pr	0.20	0.10
Inoculation		
-Inoculation	1951	0.47
+Inoculation	2356	0.54
SED	153	0.02
Chi^2 pr	0.008	< 0.001
Fertilizer × Inoculation		
BX × INO	2776	0.59
FS × INO	2674	0.50
$N_{25}P_{30}$ × INO	1812	0.53
(FS + BX) × INO	1902	0.50
($N_{25}P_{30}$ + BX) × INO	2885	0.56
($N_{25}P_{30}$ + FS) × INO	2006	0.51
($N_{25}P_{30}$ + FS + BX) × INO	3124	0.61
Control × INO	1668	0.55
SED	432	0.05
Chi^2 pr	< 0.001	< 0.001
CV (%)	28.30	10.00

Table 6.10 Effect of treatments and their combinations on grain yield and harvest index at Ghulahgu

Treatment	Grain yield (kg ha^{-1})	Harvest index (%)
Type of fertilizer		
BX	1608	0.31
FS	3151	0.48
$N_{25}P_{30}$	2573	0.44
FS + BX	3662	0.54
$N_{25}P_{30}$ + BX	2726	0.42
$N_{25}P_{30}$ + FS	3918	0.50
$N_{25}P_{30}$ + FS + BX	4008	0.51
Control	1507	0.36
SED	349	0.45
Chi^2 pr	< 0.001	< 0.001
Inoculation		
-Inoculation	2808	0.40
+Inoculation	2981	0.49
SED	175	0.02
Chi^2 pr	0.32	< 0.001
Fertilizer × Inoculation		
BX × INO	1491	0.35
FS × INO	3524	0.55
$N_{25}P_{30}$ × INO	2893	0.51
(FS + BX) × INO	3238	0.53
($N_{25}P_{30}$ + BX) × INO	2766	0.46
($N_{25}P_{30}$ + FS) × INO	4294	0.59
($N_{25}P_{30}$ + FS + BX) × INO	3975	0.60
Control × INO	1664	0.33
SED	494	0.07
Chi^2 pr	0.26	0.04
CV (%)	24.1	8.00

6.4.5 Soybean shoot biomass nitrogen content and uptake at 50% flowering growth stage at Akukayili

Percent nitrogen and uptake in soybean biomass of BX and $N_{25}P_{30}$ did not significantly contribute to increased biomass N content at the 50% flowering growth

stage (Table 6.11). However, the sole use of Fertisoil amendment led to an increase of 27% in shoot biomass N content. The combination of these treatments (BX, FS and $N_{25}P_{30}$) resulted in significant increase in biomass N content except BX + $N_{25}P_{30}$. Furthermore, (FS + BX + $N_{25}P_{30}$) × INO led to 14% increase over the *Bradyrhizobium japonicum* inoculation only, while (FS + BX) × INO and (FS + $N_{25}P_{30}$) × INO resulted in significantly higher N content in shoot biomass at flowering.

Shoot nitrogen uptake at Akukayili was significantly influenced by the use of sole inputs (BX, FS and $N_{25}P_{30}$) and their combinations (Table 6.11). However, BX, $N_{25}P_{30}$ and BX + $N_{25}P_{30}$ applications did not lead to significant increase in biomass N uptake at Akukayili. The use of *Bradyrhizobium japonicum* inoculant resulted in 22% increase in N uptake over that of the uninoculated control.

At Cheshegu all nutrient inputs did not increase biomass N content of soybean at 50% flowering except BX and FS + BX (Table 6.12). Nitrogen uptake was significantly influenced by treatments relative to the control; however, the use of *Bradyrhizobium japonicum* inoculants led to 15% increase in N uptake over the uninoculated control.

At Ghulahgu soybean responded significantly to the application of the various combinations of test nutrient sources except BX. Fertisoil application led to a high (34%) biomass N content while FS + BX and $N_{25}P_{30}$ + FS + BX application resulted in an increased N content (28 and 31%) relative to control. The use of *Bradyrhizobium japonicum* inoculant alone did not significantly ($P > 0.05$) increase soybean biomass N uptake.

Table 6.13 shows the uptake of N with respect to the applied treatments, Boost xtra, N_{25} P_{30} and FS which gave 18, 26 and 68% increase in N uptake by soybean biomass over that of the control. Likewise all the treatment combinations led to an increase in N uptake except BX + $N_{25}P_{30}$.

Table 6.11 Treatment effects on nutrient content and uptake in soybean biomass yield at 50% flowering at Akukayili

Treatment	N content (%)	N uptake (kg ha^{-1})
Type of fertilizer		
BX	1.99	30.98
FS	2.35	69.69
$N_{25}P_{30}$	1.76	35.77
FS + BX	2.35	55.56
BX + $N_{25}P_{30}$	1.85	37.27
FS + $N_{25}P_{30}$	2.58	69.23
FS + BX + $N_{25}P_{30}$	1.88	47.71
Control	1.84	24.45
SED	0.27	16.71
Chi^2 pr	0.01	0.04
Inoculation		
-Inoculation	2.05	41.67
+Inoculation	2.10	51.00
SED	0.14	8.36
Chi^2 pr	0.76	0.26
Fertilizer × Inoculation		
BX × INO	1.72	26.81
FS × INO	2.5	94.76
$N_{25}P_{30}$ × INO	1.89	43.29
(FS + BX) × INO	2.59	66.55
(BX + $N_{25}P_{30}$) × INO	1.80	47.84
(FS + $N_{25}P_{30}$) × INO	2.84	65.56
(FS + BX + $N_{25}P_{30}$) × INO	1.23	35.50
Control × INO	2.19	27.65
SED	0.38	23.64
Chi^2 pr	0.003	0.46
CV (%)	22.4	25.0

Table 6.12 Treatment effects on nitrogen content and uptake in soybean biomass yield at 50% flowering at Cheshegu

Treatment	N content (%)	N uptake (kg ha^{-1})
Type of fertilizer		
BX	3.10	66.68
FS	2.74	59.83
$N_{25}P_{30}$	2.52	49.10
FS + BX	3.08	56.81
$N_{25}P_{30}$ + BX	2.46	52.70
$N_{25}P_{30}$ + FS	2.38	52.05
$N_{25}P_{30}$ + FS + BX	2.67	43.82
Control	2.99	45.70
SED	0.45	14.42
Chi^2 pr	0.57	0.87
Inoculation		
-Inoculation	2.78	50.59
+Inoculation	2.71	57.88
SED	0.22	7.14
Chi^2 pr	0.77	0.31
Fertilizer × Inoculation		
BX × INO	3.09	68.47
FS × INO	2.52	58.58
$N_{25}P_{30}$ × INO	2.59	50.39
(FS + BX) × INO	3.04	58.99
($N_{25}P_{30}$ + BX) × INO	2.61	54.65
($N_{25}P_{30}$ + FS) × INO	2.35	58.73
($N_{25}P_{30}$ + FS + BX) × INO	2.58	55.74
Control ×INO	2.90	55.74
SED	0.63	20.29
Chi^2 pr	1.00	0.69
CV (%)	28.0	24.3

Table 6.13 Treatment effects on nitrogen content and uptake in soybean biomass yield at 50% flowering at Ghulahgu

Treatment	N content (%)	N uptake (kg ha^{-1})
Type of fertilizer		
BX	1.84	61.78
FS	2.57	88.14
$N_{25}P_{30}$	2.37	66.17
FS + BX	2.46	82.02
$N_{25}P_{30}$ + BX	2.20	82.16
$N_{25}P_{30}$ + FS	2.35	95.89
$N_{25}P_{30}$ + FS + BX	2.51	114.49
Control	1.92	52.54
SED	0.27	25.76
Chi^2 pr	0.06	0.30
Inoculation		
-Inoculation	2.23	90.99
+Inoculation	2.33	69.81
SED	1.35	12.88
Chi^2 pr	0.48	0.10
Fertilizer × Inoculation		
BX × INO	2.03	53.95
FS × INO	2.63	79.59
$N_{25}P_{30}$ × INO	2.30	50.69
(FS + BX) × INO	2.21	68.75
($N_{25}P_{30}$ + BX) × INO	2.33	97.05
($N_{25}P_{30}$ + FS) × INO	2.33	74.18
($N_{25}P_{30}$ + FS + BX) × INO	2.54	66.85
Control × INO	2.23	67.43
SED	0.38	36.42
Chi^2 pr	0.59	0.29
CV (%)	11.20	25.50

6.4.6 Soybean shoot biomass phosphorus content and uptake at 50% flowering

Fertisoil application increased the P content of soybean by 53% relative to that of the control while Boost xtra, $N_{25}P_{30}$ and *Bradyrhizobia japonicum* inoculant did not result in any significant increases. The combined application of FS and BX led to 35% increase in P content over that of the control. $N_{25}P_{30}$ + FS and $N_{25}P_{30}$ + FS +

BX led to 59 and 17% increase in N content over the control. Treatments FS × INO, INO × (FS + $N_{25}P_{30}$), and (FS + BX) × INO resulted in more than 50% increase in P content in shoot biomass at flowering stage than the sole application of *Bradyrhizobium japonicum* inoculum. The combination of any two of the treatments was more supportive to the accumulation of P in soybean biomass than the combination of any three of the treatments, even though all the combinations significantly ($P < 0.05$) supported P concentration in soybean biomass.

The use of the sole treatment (BX, FS and $N_{25}P_{30}$) inputs led to an increase in P uptake in biomass at 50% flowering growth stage which represented 36, 74 and 271% for BX, $N_{25}P_{30}$ and FS, respectively (Tables 6.14 – 6.16). However, P uptake from BX and $N_{25}P_{30}$ treatment plots were not significantly different from that of the control. Furthermore, the combination of the various inputs (BX, FS and $N_{25}P_{30}$) in twos and in threes, led to an increase in P uptake, also, the sole use of Bradyrhizobium inoculant increased P uptake relative to the uninoculated control.

The use of BX did not significantly increase P content in shoot biomass while $N_{25}P_{30}$ and FS applications resulted in 58 and 32% increase in P content respectively over the control (Table 6.15). Inoculation with *Bradyrhizobium japonicum* increased P content in biomass by 8% relative to the control. All the combinations with *Bradyrhizobium japonicum* inoculum except BX × INO increased P content relative to sole use of *Bradyrhizobium japonicum* inoculant.

At Ghulahgu, sole application of $N_{25}P_{30}$ and FS resulted in 26 and 20% increase in biomass P content over that of the control while their combination ($N_{25}P_{30}$ and FS) resulted in 33, 26 and 60% more than FS, $N_{25}P_{30}$ and control respectively (Table 6.16). Also the other treatment combinations led to an increase in biomass P content

at 50 % flowering growth stage than the sole applications (BX, FS and $N_{25}P_{30}$) and control. The use of *Bradyrhizobium japonicum* inoculum led to a slight increase in P content in shoot biomass at 50% flowering growth stage.

Shoot biomass phosphorus uptake shown on Table 6.16 indicates that both sole ((BX, FS and $N_{25}P_{30}$) and treatment combinations increased P uptake in shoot biomass at 50% flowering growth stage with and without *Bradyrhizobium japonicum* inoculation except the treatments of *Bradyrhizobium japonicum* and the combination of BX and INO (Table 6.16). In fact, $N_{25}P_{30}$ + FS + BX yielded as high as 198% increase in P uptake over that of control while plants amended with $N_{25}P_{30}$ + FS had 144% more P uptake than that of the control. On the whole, the various treatment combinations led to increase in P uptake more than the sole applications.

Table 6.14 Treatment effects on shoot biomass P content and uptake at Akukayili

Treatment	P content (%)	P uptake (kg ha^{-1})
Type of fertilizer		
BX	0.18	3.09
FS	0.26	8.43
$N_{25}P_{30}$	0.19	3.95
FS + BX	0.23	5.54
$N_{25}P_{30}$ + BX	0.18	3.48
$N_{25}P_{30}$ + FS	0.27	7.15
$N_{25}P_{30}$ + FS + BX	0.20	5.00
Control	0.17	2.27
SED	0.03	2.18
Chi^2 pr	<0.001	0.07
Inoculation		
-Inoculation	0.21	4.364
+Inoculation	0.21	5.357
SED	0.01	1.09
Chi^2 pr	0.67	0.36
Fertilizer × Inoculation		
BX × INO	0.20	3.08
FS × INO	0.29	11.99
$N_{25}P_{30}$ × INO	0.19	4.41
(FS + BX) × INO	0.27	6.78
($N_{25}P_{30}$ + BX) × INO	0.16	4.17
($N_{25}P_{30}$ + FS) × INO	0.28	6.33
($N_{25}P_{30}$ + FS + BX) × INO	0.13	3.85
Control × INO	0.18	2.26
SED	0.04	3.08
Chi^2 pr	0.01	0.51
CV (%)	11.23	15.10

Table 6.15 Treatment effects on shoot biomass P content and uptake at Cheshegu

Treatment	P content (%)	P uptake (kg ha^{-1})
Type of fertilizer		
BX	0.16	4.27
FS	0.25	5.47
$N_{25}P_{30}$	0.30	4.62
FS + BX	0.25	6.01
$N_{25}P_{30}$ + BX	0.24	3.81
$N_{25}P_{30}$ + FS	0.33	5.31
$N_{25}P_{30}$ + FS + BX	0.28	4.33
Control	0.19	5.95
SED	0.05	1.74
Chi^2 pr	0.01	0.87
Inoculation		
-Inoculation	0.24	4.82
+Inoculation	0.26	5.12
SED	0.02	0.87
Chi^2 pr	0.54	0.74
Fertilizer × Inoculation		
BX × INO	0.17	5.36
FS × INO	0.30	5.44
$N_{25}P_{30}$ × INO	0.28	4.57
(FS + BX) × INO	0.25	5.93
($N_{25}P_{30}$ + BX) × INO	0.24	4.38
($N_{25}P_{30}$ + FS) × INO	0.33	5.59
($N_{25}P_{30}$ + FS + BX) × INO	0.30	5.39
Control × INO	0.20	4.27
SED	0.06	2.46
Chi^2 pr	0.95	0.83
CV (%)	12.0	15.2

Table 6.16 Effect of treatments on shoot biomass P content and uptake at Ghulahgu

Treatment	P content (%)	P uptake (kg ha^{-1})
Type of fertilizer		
BX	0.13	4.71
FS	0.18	6.09
$N_{25}P_{30}$	0.19	5.20
FS + BX	0.22	7.35
$N_{25}P_{30}$ + BX	0.22	8.12
$N_{25}P_{30}$ + FS	0.24	9.56
$N_{25}P_{30}$ + FS + BX	0.25	11.68
Control	0.15	3.92
SED	0.04	2.95
Chi^2 pr	0.02	0.13
Inoculation		
-Inoculation	0.19	7.81
+Inoculation	0.20	6.35
SED	0.02	1.47
Chi^2 pr	0.48	0.32
Fertilizer × Inoculation		
BX × INO	0.14	4.04
FS × INO	0.17	5.22
$N_{25}P_{30}$ × INO	0.18	4.09
(FS + BX) × INO	0.22	7.42
($N_{25}P_{30}$ + BX) × INO	0.23	9.73
($N_{25}P_{30}$ + FS) × INO	0.27	8.73
($N_{25}P_{30}$ + FS + BX) × INO	0.28	7.31
Control × INO	0.14	4.21
SED	0.06	4.17
Chi^2 pr	0.96	0.69
CV (%)	24.4	17.5

6.4.7. Effect of treatments on grain N content and uptake at harvest maturity

Grain N content at maturity was not significantly increased at Akukayili with the use of the inputs (Table 6.17). The uptake of nitrogen in soybean grain by plants treated with Fertisoil led to an increase of 19% over the control. Furthermore, the use of FS + BX + $N_{25}P_{30}$ resulted in 19% increase over that of the control.

Sole BX, FS and $N_{25}P_{30}$ and their combinations significantly increased N content marginally in most cases at Cheshegu (Table 6.18). However, $N_{25}P_{30}$ + BX resulted in increased N content which represented 31% more than that of the control. The use of *Bradyrhizobium* inoculant did not result in significant increase in N content over the uninoculated control.

The results of N uptake showed that FS resulted in 32% increase over that of the control. The combined treatments of BX, FS and $N_{25}P_{30}$ also contributed to N uptake in grain at harvest maturity than the sole applications and control.

The percent grain N content at Ghulahgu is as shown in Table 6.19. The treatment combinations of BX, FS and $N_{25}P_{30}$ resulted in higher percent N in grain than their sole applications and control. Bradyrhizobium inoculant did not significantly increase grain N content. The combination of all other treatments with inoculation resulted in increased grain N content except the use of (FS + BX) × INO. The grain N uptake was influenced by the sole and combined use of BX, FS and $N_{25}P_{30}$ which led to an increase in grain N uptake over that of the control at Ghulahgu (Table 6.19). *Bradyrhizobium japonicum* inoculation resulted in a marginal increase of 7% over that of the uninoculated control.

Table 6.17 Effect of treatments on grain N content and uptake at Akukayili

Treatment	N content (%)	N uptake (kg ha^{-1})
Type of fertilizer		
BX	4.25	78.5
FS	5.50	125.3
$N_{25}P_{30}$	4.76	90.2
FS + BX	4.95	107.9
BX + $N_{25}P_{30}$	4.88	85.2
FS + $N_{25}P_{30}$	4.91	81.2
FS + BX + $N_{25}P_{30}$	5.52	155.8
Control	5.90	105.1
SED	0.71	30.48
Chi^2 pr	0.37	0.16
Inoculation		
-Inoculation	5.56	100.8
+Inoculation	4.61	106.8
SED	0.35	15.24
Chi^2 pr	0.01	0.71
Fertilizer × Inoculation		
BX × INO	4.26	96.4
FS × INO	5.00	105
$N_{25}P_{30}$ × INO	4.20	76.4
(FS + BX) × INO	4.34	92.2
(BX + $N_{25}P_{30}$) × INO	4.35	107.5
(FS + $N_{25}P_{30}$) × INO	5.27	121.6
(FS + BX + $N_{25}P_{30}$) × INO	4.80	154.4
Control × INO	4.61	98.7
SED	1.00	43.11
Chi^2 pr	0.46	0.42
CV (%)	8.23	25.0

Table 6.18 Effect of treatments and their combinations on grain N content and uptake at Cheshegu

Treatment	N content (%)	N uptake (kg ha^{-1})
Type of fertilizer		
BX	4.89	89.4
FS	4.95	109.2
$N_{25}P_{30}$	4.41	84.7
FS + BX	4.24	88
$N_{25}P_{30}$ + BX	5.69	120.2
$N_{25}P_{30}$ + FS	4.92	121.5
$N_{25}P_{30}$ + FS + BX	5.07	111.2
Control	4.35	82.5
SED	0.78	37.43
Chi^2 pr	0.64	0.91
Inoculation		
-Inoculation	5.31	112.9
+Inoculation	4.32	88.7
SED	0.40	18.7
Chi^2 pr	0.01	0.20
Fertilizer × Inoculation		
BX × INO	4.59	73.8
FS × INO	3.78	68.8
$N_{25}P_{30}$ × INO	3.99	88.0
(FS + BX) × INO	3.30	57.6
($N_{25}P_{30}$ + BX) × INO	5.11	116.3
($N_{25}P_{30}$ + FS) × INO	5.13	123.8
($N_{25}P_{30}$ + FS + BX) × INO	5.00	102.6
Control × INO	3.73	79.0
SED	1.10	52.93
Chi^2 pr	0.72	0.93
CV (%)	9.12	23.5

Table 6.19 Effect of treatments and their combinations on grain N content and uptake at Ghulahgu

Treatment	N content (%)	N uptake (kg ha^{-1})
Type of fertilizer		
BX	5.74	226.8
FS	5.89	192.3
$N_{25}P_{30}$	5.65	204.4
FS + BX	5.40	180.6
$N_{25}P_{30}$ + BX	6.65	231.4
$N_{25}P_{30}$ + FS	6.01	247.2
$N_{25}P_{30}$ + FS + BX	6.78	206.6
Control	5.70	134.0
SED	0.43	41.86
Chi^2 pr	0.01	0.19
Inoculation		
-Inoculation	6.161	196.6
+Inoculation	5.788	209.3
SED	0.22	20.93
Chi^2 pr	0.09	0.54
Fertilizer × Inoculation		
BX × INO	6.00	216.1
FS × INO	6.10	196.9
$N_{25}P_{30}$ × INO	6.18	244.6
(FS + BX) × INO	5.20	203.6
($N_{25}P_{30}$ + BX) × INO	6.21	254.7
($N_{25}P_{30}$ + FS) × INO	5.42	245.1
($N_{25}P_{30}$ + FS + BX) × INO	6.00	166.2
Control × INO	5.29	146.8
SED	0.61	59.2
Chi^2 pr	0.03	0.67
CV (%)	14 .0	21.3

6.4.8 Effect of treatments on grain P content and uptake at Akukayili

The grain phosphorus content and uptake result are as shown in Table 6.20. The sole and combined application of BX, FS and $N_{25}P_{30}$ and *Bradyrhizobium japonicum* inoculant were not significantly different from that of the control in terms of percent P or P uptake. Similarly, the use of sole BX, FS and $N_{25}P_{30}$ and their combined applications did not significantly contribute to P uptake at Akukayili. However, the combination of FS + BX + $N_{25}P_{30}$ significantly increased grain phosphorus content at harvest maturity. Grain P uptake was slightly increased (7%) by the use of *Bradyrhizobium japonicum* inoculum over the uninoculated control.

Grain phosphorus content at Cheshegu as influenced by the treatments is given in Table 6.21. The grain P content was not influenced significantly by the sole application of BX, FS, $N_{25}P_{30}$ and *B. japonicum* inoculant. However, the use of these treatment (BX, FS and $N_{25}P_{30}$) combinations increased grain P content over that of the control and inoculation respectively.

Table 6.21 shows the effect of treatment combinations on grain P uptake at Cheshegu. The sole use of the applied treatments (BX, FS, $N_{25}P_{30}$ and INO) was not significantly different from that of the control in terms of increasing P uptake.

At Ghulahgu, BX, FS and $N_{25}P_{30}$ treatments and their combinations did not significantly result in grain P content increase, however, the application of FS × INO and $N_{25}P_{30}$ × INO significantly increased the grain P content which represented 11% increase than sole application of the treatments. Table 6.22 shows that P uptake was significantly influenced with regards to the applied treatments except *B. japonicum* inoculation and ($N_{25}P_{30}$ + BX + FS) × INO treatments. The $N_{25}P_{30}$ + FS treatment gave the highest increase although not significantly different from the P uptake

recorded from BX, $N_{25}P_{30}$ × INO, BX × INO, ($N_{25}P_{30}$ + FS) × INO and ($N_{25}P_{30}$ + BX) × INO treatments.

Table 6.20 Effect of treatments on grain P content and uptake at Akukayili

Treatment	P content (%)	P uptake (kg ha^{-1})
Type of Fertilizer		
BX	0.17	3.09
FS	0.21	4.80
$N_{25}P_{30}$	0.19	3.56
FS + BX	0.20	4.19
BX + $N_{25}P_{30}$	0.22	4.08
FS + $N_{25}P_{30}$	0.20	3.08
FS + BX + $N_{25}P_{30}$	0.22	6.15
Control	0.23	4.03
SED	0.03	1.24
Chi^2 pr	0.67	0.24
Inoculation		
-Inoculation	0.22	3.99
+Inoculation	0.18	4.26
SED	0.01	0.62
Chi^2 pr	0.02	0.66
Fertilizer × Inoculation		
BX × INO	0.17	3.78
FS × INO	0.18	3.63
$N_{25}P_{30}$ × INO	0.16	3.00
(FS + BX) × INO	0.18	3.71
(BX + $N_{25}P_{30}$) × INO	0.22	5.62
(FS + $N_{25}P_{30}$) × INO	0.19	4.38
(FS + BX + $N_{25}P_{30}$) × INO	0.20	6.26
Control × INO	0.17	3.70
SED	0.05	1.75
Chi^2 pr	0.73	0.30
CV (%)	11.23	15.10

Table 6.21 Effect of treatments on grain P content and uptake at Cheshegu

Treatment	P content (%)	P uptake (kg ha^{-1})
Type of fertilizer		
BX	0.17	3.08
FS	0.17	3.53
$N_{25}P_{30}$	0.15	2.81
FS + BX	0.14	2.95
$N_{25}P_{30}$ + BX	0.19	3.51
$N_{25}P_{30}$ + FS	0.17	4.22
$N_{25}P_{30}$ + FS + BX	0.20	4.25
Control	0.17	3.16
SED	0.03	1.14
Chi^2 pr	0.40	0.86
Inoculation		
-Inoculation	0.18	3.76
+Inoculation	0.16	3.12
SED	0.01	0.57
Chi^2 pr	0.02	0.26
Fertilizer × Inoculation		
BX × INO	0.16	2.61
FS × INO	0.14	2.58
$N_{25}P_{30}$ × INO	0.12	2.54
(FS + BX) × INO	0.12	2.10
($N_{25}P_{30}$ + BX) × INO	0.18	3.64
($N_{25}P_{30}$ + FS) × INO	0.18	4.33
($N_{25}P_{30}$ + FS + BX) × INO	0.19	3.88
Control × INO	0.15	3.24
SED	0.04	1.62
Chi^2 pr	0.72	0.97
CV (%)		

Table 6.22 Effect of treatments on grain P content and uptake at Ghulahgu

Treatment	P content (%)	P uptake (kg ha^{-1})
Type of fertilizer		
BX	0.20	8.06
FS	0.20	6.80
$N_{25}P_{30}$	0.20	6.97
FS + BX	0.18	6.06
$N_{25}P_{30}$ + BX	0.23	7.52
$N_{25}P_{30}$ + FS	0.20	8.28
$N_{25}P_{30}$ + FS + BX	0.21	6.52
Control	0.20	4.68
SED	0.02	1.38
Chi^2 pr	0.47	0.19
Inoculation		
-Inoculation	0.22	6.78
+Inoculation	0.19	7.00
SED	0.01	0.69
Chi^2 pr	0.03	0.80
Fertilizer × Inoculation		
BX × INO	0.20	7.00
FS × INO	0.21	6.80
$N_{25}P_{30}$ × INO	0.21	8.10
(FS + BX) × INO	0.17	6.85
($N_{25}P_{30}$ + BX) × INO	0.20	8.11
($N_{25}P_{30}$ + FS) × INO	0.18	8.04
($N_{25}P_{30}$ + FS + BX) × INO	0.20	5.49
Control × INO	0.19	5.24
SED	0.03	1.95
Chi^2 pr	0.43	0.66
CV (%)	9.10	23.4

6.4.9 Economic evaluation of the commercial fertilizers used

The value cost ratio of the treatments applied at Akukayili is as shown in Figure 6.1 The following treatments, FS, BX + FS, $N_{25}P_{30}$ + FS, INO + FS, INO + (BX + FS), INO + (BX + FS + N_{25} P_{30}), and IN0 + (FS + $N_{25}P_{30}$) resulted in VCR of more than 2, an indication of a positive return on investment. FS and INO + (FS + $N_{25}P_{30}$) recorded a VCR of more than 3 while other treatments and their combinations recorded below 2. The use of sole *B. japonicum* inoculum and BX + N_{25} P_{30} was extremely low with VCR < 1.

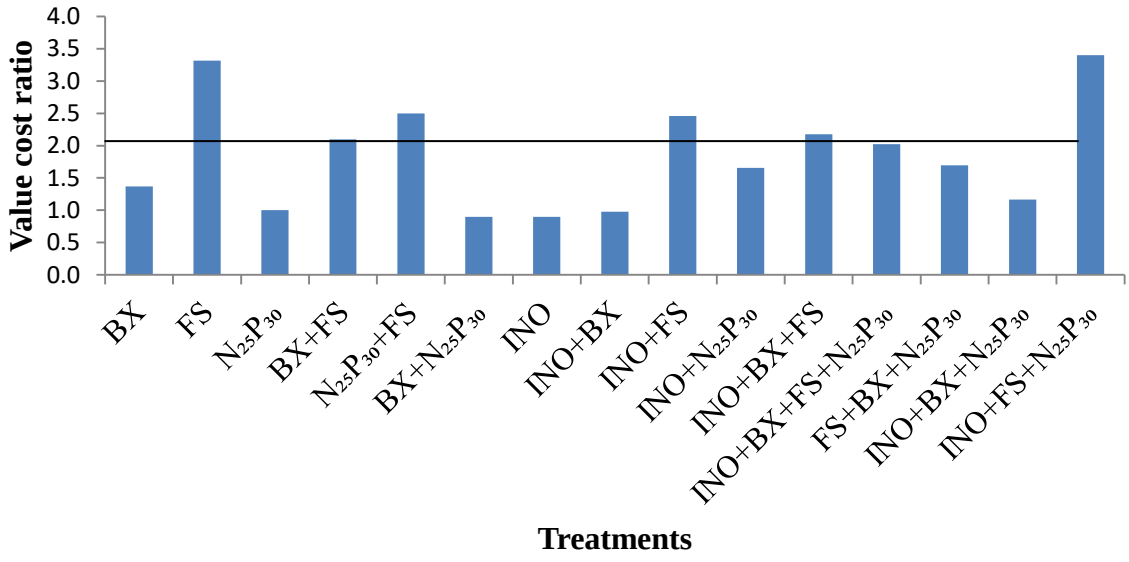

Figure 6.1 Value cost ratio of treatments used at Akukayili

The value cost ratio of the various treatments used at Cheshegu is as shown in Figure 6.2. None of the treatments combination gave a value cost ratio of 2. However the use of inoculation + (FS + $N_{25}P_{30}$) resulted in the highest value cost ratio of 1.4 while $N_{25}P_{30}$ + BX, INO + BX and INO + FS were extremely low, less than 1.

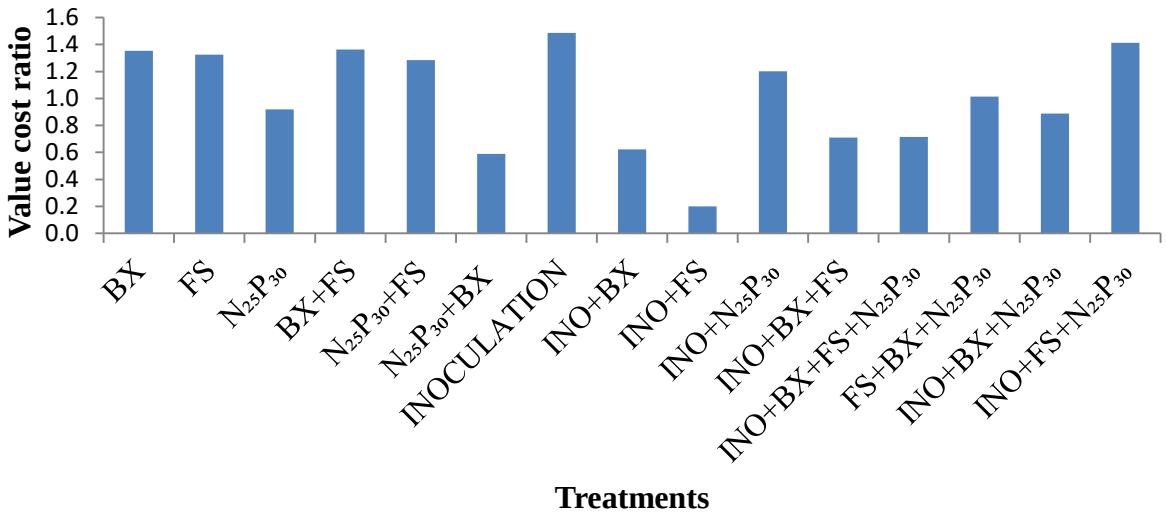

Figure 6.2 Value cost ratio of treatments used at Cheshegu

At Ghulahgu, the application of Boost xtra at vegetative stage (five WAP) resulted in the highest value cost ratio (5.0) (Figure 6.3). Similarly, most of the treatments used attained value cost ratio above 2. The use of BX + FS, BX + $N_{25}P_{30}$, INO + (BX + FS + $N_{25}P_{30}$) and FS + BX + $N_{25}P_{30}$ gave value cost ratio below the threshold of 2.

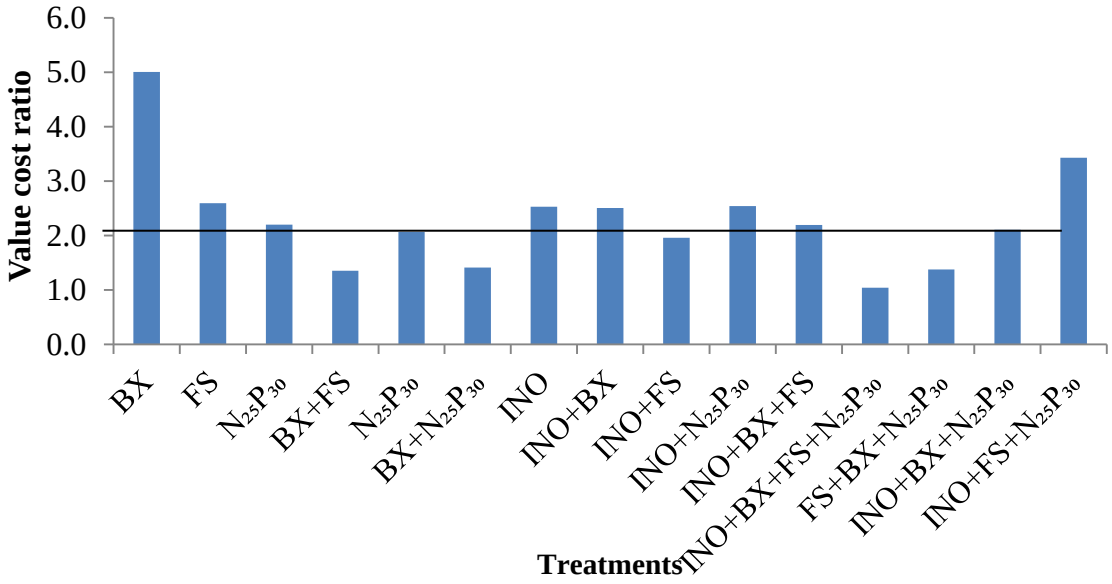

Figure 6.3 Value cost ratio of treatments used at Ghulahgu

6.5 Discussion

6.5.1 Soil characteristics of the study area

The soils of the study area clearly exhibited signs of low fertility soil as described by Landon (1991) for effective crop growth. The organic carbon (< 20%), total nitrogen (< 1 g kg^{-1}), exchangeable cations (< 5 $cmol_{(+)}kg^{-1}$), effective cation exchange capacity (< 5 $cmol_{(+)}kg^{-1}$) and extractable P (< 10 mg kg^{-1}) were all too low for effective crop growth. Furthermore, the soils were all slightly acidic in nature. The low organic carbon in the top soil might have been as a result of low rate of biomass production and C input and mineralization (Lal *et al*., 2009). Furthermore, the Ferric Luvisoil and Gleyic Luvisoil of Akukayili and Cheshegu were sandy loam in texture with more than 50% sand content with bulk densities of 1.37 and 1.40 g cm^{-3}, respectively. The most probable number count of rhizobia was moderate across the study locations according to the rating given by Thies (1991).

6.5.2 Effect of treatments on soybean nodulation

6.5.2.1. Nodule number

Nodule number was not significantly increased with the sole use of Fertisoil, Boost xtra (Foliar fertilizer) and inorganic fertilizer ($N_{25}P_{30}$) at Akukayili and Cheshegu, even though at Ghulahgu, marginal increases were recorded with the application of FS and $N_{25}P_{30}$ resulting in 12 and 5% increase respectively relative to that of the control (Table 6.4). This observation may imply that perhaps the quantity of the applied nutrients from Fertisoil and $N_{25}P_{30}$ were not sufficient for effective nodule initialization at Akukayili and Cheshegu, while the timing of application of Boost xtra might have been done after nodule initialization and formation stages. The sole application of Bradyrhizobium inoculant significantly supported nodule number

production at the experimental sites except at Cheshegu (Tables 6.2, 6.3 and 6.4). This confirms the findings of Subasinghe *et al*. (2001) who attributed the performance of the introduced strain to the inability of the indigenous rhizobia to produce appreciable number of nodules, hence necessitating the need for inoculation. Revellin *et al*. (2000) and Abbasi *et al.* (2008) also reported that larger responses to inoculation and higher number of nodules per plant was observed as compared to uninoculated treatments in a field that had no soybean cropping history. First of all, this study has revealed that the combinations of organic, inorganic and bio - fertilizer are needed for optimizing soybean nodule formation which invariably positively influence soybean N fixation if all necessary conditions are satisfied. For instance, in the case of FS + $N_{25}P_{30}$, possibly the decomposition of Fertisoil made available nutrients required for plant growth initialization, which when supplemented with the addition of N_{25} P_{30}, resulted in more nodule number relative to the sole FS and $N_{25}P_{30}$ (Tables 6.2 and 6.3). Giller *et al*. (1997) reported that, to increase and sustain crop production, replenishment of soil P must be supported with soil N.

The non responsiveness of soybean nodules to applied treatments observed at Cheshegu might be attributed to the soil nutrient status which was low at the onset of the experiment (Table 6.2). Secondly, the erratic pattern of rainfall that was recorded during the course of conducting the trial could also hinder the response of soybean to the applied treatments at this location (Appendix 1). Senescence of bacteriods according to Ramos *et al*. (2003) may result if a plant is exposed to moisture stress for more than 10 days, leading to adverse effect on nodule functions. Hungria and Franco (1993) reported that total nitrogenase activity was reduced drastically as a result of high temperature while Sanginga and Woomer (2009) and Christianson and

Vlek (1991) also reported that lack of oxygen and insufficient moisture availability could hinder the efficiency of N and P uptake.

6.5.2.2 Effect of treatments on nodule dry weight

The nodule dry weight at 50% flowering growth stage revealed that the sole application of nutrients supported nodule dry weight at Ghulahgu (Table 6.4). However, FS + BX, BX + $N_{25}P_{30}$, FS + $N_{25}P_{30}$ and FS + BX + $N_{25}P_{30}$ produced higher nodule dry weight at Akukayili and Ghulahgu, though at Cheshegu the reverse was the case (Tables 6.2, 6.3, 6.4). In the case where inoculation was in combination with Fertisoil, the positive response observed could be attributed to rhizobia inoculation which has been confirmed to increase available macro elements by releasing dead cells which may contain macro elements or biomolecules that can solubilize unavailable to available nutrients (Makoi *et al*. 2013, Cechin *et al*. 2004, Abd-alla 1994, and Halder 1993). Furthermore, Fertisoil might have also aided in increasing the organic matter level of the soil thereby making the rhizosphere conducive for effective root development and subsequently efficient nodule development.

6.5.3 Effect of treatments on shoot biomass yield at 50% flowering stage

Fertisoil was able to produce shoot biomass yield that was comparable to the biomass yield recorded from combined treatments across locations (Tables 6.5 - 6.7). This could possibly mean that Fertisoil contains high amount of organic matter which improves soil structure and in turn soil porosity and gives room for better root growth and hence better nutrient uptake. At Akukayili, the use of *Bradyrhizobium japonicum* inoculation increased biomass yield which is in line with the findings of Theuri *et al*. (2003) who reported that commercial inoculants are more effective than

indigenous strains in their response to shoot biomass and nodule number. However, Abayomi *et al*. (2008) concluded that plants which received inorganic nitrogen performed better than those that depended on biological nitrogen fixation, an observation which was the case at Akukayili and Ghulahgu (Table 6.5 and 6.7). Similarly, at Cheshegu, the combined treatments performed relatively better than sole applications (BX, FS and $N_{25}P_{30}$) while at Ghulahgu the sole and combined use of the various treatments led to an increase in biomass yield, but the combinations yielded more than the sole treatments. The emphasis is on the benefit of combining more than one nutrient at a time to aid the ability of these inputs to complement the limitations of one another. Asuming - Brempong *et al*. (2013) reported that plant dry weight increased with the increasing rate of applied phosphorus whiles Bationo *et al*. (2002) also reported that the application of fertilizers could triple biomass yield of cowpea. However, this study has shown that the use of Fertisoil alone enhanced soybean shoot biomass yield at Akukayili and Ghulahgu.

6.5.4 Effect of treatments on grain yield

Seed yield is one of the morphological characteristics of many plant species (Chmielewski and Ruit, 2002). This study has shown that the sole application of the treatments (BX, FS and $N_{25}P_{30}$) had their relative contributory effect on the grain yield over their respective controls at the different study sites. However, FS + BX, FS + $N_{25}P_{30}$, FS + BX + $N_{25}P_{30}$ treatments appeared to be the best options for farmers to increase grain yield significantly in the study area. The contribution of *Bradyrhizobium japonicum* inoculant to grain yield was minimal across the study locations. This observation can be attributed to the fact that indigenous rhizobia might have out competed the introduced rhizobia which nodulated the legume as was reported by Chemining'wa *et al*. (2004). Furthermore, Otieno *et al*. (2009) reported

that in many cases rhizobia inoculation increases the number of nodules and nodule dry weight per plant, but the increase in nodulation is neither translated into shoot biomass and root dry matter accumulation nor to the grain yield as was the case in this study. Such low response from the sole inoculation (BX, FS and $N_{25}P_{30}$) may be due to restriction of nodulation, reduced functioning of nodules and subsequently low yields as asserted by Sanginga *et al*. (2001). This may also be attributed to the competition for survival by the above and below ground organs. Having in mind that the nodules, root and biomass are all sink competitors for photosynthase, translocation from the shoot to the underground portion of the plant during plant establishment and vegetative stages of growth makes it difficult for the sole use of *Bradyrhizobium japonicum* inoculant to increase grain yield substantially. Moreso, there has been a suggestion that the use of inoculation only is not likely to sustain plant growth and grain yield. This corroborates the findings of Panchali (2011) that persistent rhizobium strains might not be able to effectively penetrate the plant root and consequently N fixation in their full capacity due to adverse environmental factors. Furthermore, the soil pH which was slightly acidic could have influenced the effective performance of the introduced strain; as previously asserted by Nyoki and Ndakidemi (2014).

The contribution of Fertisoil consistently enhanced soybean growth across the study locations. This might be attributed to the countless contributory characteristics of organic manure leading to better root growth and enhanced nutrient uptake. In addition, readily decomposed organic manure is said to improve crop tolerance to root rots and hence yield. The application of foliar fertilizer at vegetative growth stage did not result in a significant increase in yield and this buttressed the need for a certain level of nutrients in the soil for the plants to start with. Kesser *et al*. (1992)

and Carsky *et al*. (2001) reported that mineral nutrient is required for plant initial establishment before nodulation commences. The combined use of Fertisoil, foliar fertilizer, and *Bradyrhizobium japonicum* inoculum resulted in a significant soybean yield increases at Akukayili, Cheshegu, and Ghulahgu. Furthermore, BX + FS also increased grain yield at Akukayili and Cheshegu, indicating that each essential nutrient has specific physiological and biochemical role to play (Graham *et al*., 1988) and minimal nutrient concentrations are required by legumes and rhizobia to sustain metabolic function at appreciable rates for effective growth. Lourduraj (2000) has also reported that the combined application of' inorganic and organic manures significantly enhanced the growth attributes and yield of' soybean as compared to the sole application of either of them.

6.5.5 Effect of treatments on harvest index

The amount of carbohydrate translocated from the vegetative part of soybean increased with the size of the reproductive sink, which is a function of the relative growth rate of the plant (Nyemba 1986). The findings of this study have demonstrated that harvest index was significantly increased by the amendment of soil with Fertisoil across the study locations except at Cheshegu (Tables 6.8 - 6.10). Similarly, the application of *Bradyrhizobium japonicum* inoculum also enhanced harvest index at the study locations except at Akukayili. The combination of FS, *Bradyrhizobium japonicum* inoculum and inorganic fertilizer increased harvest index indicating that more dry matter was converted into seed when external inputs were made available for the plant at the right time needed. Also it is an indication of how much of the biological development of the plant is translated into economic value. This can also be attributed to the various constituents of the treatment combinations resulting in high biological, grain and quality of grain yield obtained. This is similar

to Khaim *et al*. (2013) report that the combination of bio - fertilizer and organic fertilizer enhanced biological and grain yield hence resulting in high harvest index compare to the control. Samia *et al*. (2012) also reported an increase of 19.86% in soybean harvest index relative to the un - amended control.

6.5.6 Nutrient N concentration and uptake in biomass at 50% flowering growth stage

The percent nitrogen content recorded at Akukayili and Ghulahgu were positively influenced by Fertisoil (Tables 6.11 and 6.13) leading to an increase of 27 and 34% respectively in the partitioning of nitrogen into soybean shoot biomass at 50% flowering growth stage. Also, there was an increase in response to BX + FS (27%) and FS + $N_{25}P_{30}$ (40%) at Akukayili, and response to BX (4%), FS + BX (3%) at Cheshegu. While at Ghulahgu only BX was not positively responded to. Furthermore the sowing of inoculated soybean in soil amended with Fertisoil and its addition with FS + BX and FS + $N_{25}P_{30}$ also contributed to increase the nitrogen accumulated by biomass at 50% flowering in Akukayili. This invariably can be attributed to the various micro and macro nutrients of the different treatments used, which basically are required for the establishment of the plants rooting system, vegetative growth and assimilation of nutrients by the plant if made available to the plant. Hence it can be inferred that the uptake of nitrogen by soybean can be more enhanced by the application of combined nutrients than the sole application, emphasizing the fact that the limitation of either one of the nutrients can be supplemented by the combined treatment. Verma *et al.* (2006) also found a significantly higher NPK uptake by maize-wheat cropping system by the application of 100% NPK + FYM 10 t ha^{-1} and Kumar and Sharma (2004) reported a maximum nutrient uptake in cabbage and tomato with FYM + 150 % NPK. For example, the combined application of INO and

FS consistently boosted N uptake at Akukayili and Ghulahgu implying that organic matter decomposition created a stimulated rhizosphere effect through the well established rooting system and consequently resulting in a positive influence in enhancing the efficiency of rhizobium – legume symbiosis. Furthermore, the introduced strain of *Bradyrhizobium japonicum* contributed to biological nitrogen fixation as asserted by Nyoli and Ndakidemi (2014) that inoculation with rhizobium perhaps can increase N fixation.

6.5.7 Nutrient P concentration and uptake in biomass at 50% flowering growth stage

The use of Fertisoil, and all the treatment combinations except BX + $N_{25}P_{30}$ contributed positively to phosphorus concentration in soybean biomass at Akukayili. At Cheshegu and Ghulahgu, only BX application did not amount to an increase in P content in biomass at 50% flowering growth stage (Tables 6.14, 6.15, 6.16).

The fact that the use of Fertisoil increased P concentration and uptake could possibly be linked to the slightly acidic nature of soils and perharps the decomposition of Fertisoil which which led to the dissolution of P fixed in the soil, making it readily available for plant assimilation and translocation in the plant parts. Doe (2006) reported that the soil pH could create an opportunity for P fixation. Furthermore, the ability of FS, FS + BX + N_{25} P_{30}, FS + N_{25} P_{30} and BX + N_{25} P_{30} treatments to have caused an increase in biomass P uptake at Akukayili and Ghulahgu can also be attributed to the fact that the soil is limiting in macro and micro nutrients (Table 6.1). Furthermore, the increased P uptake of soybean biomass due to the use of *Bradyrhizobium japonicum* inoculation is worth considering, Galal *et al*. (2001)

reported that there is strong evidence that soil bacteria are capable of transforming soil P to forms that are available to plants.

However, at Cheshegu all through the trial, responses to the applied nutrients did not result in higher concentration or uptake of N and P. (Tables 6.12 and 6.15). This could be attributed to the soil fertilizer status compared relative to the other sites (Table 6.1), coupled with the rainfall pattern experienced during the planting season (Appendix 1). Sanginga and Woomer (2009) and Christianson and Vlek (1991) however attributed the hindrance in the efficient use of applied N and plant P uptake to insufficient moisture availability. The relationship between the biomass yield and P uptake reveals that the partitioning of P in soybean depends on its biomass and might be attributed to the shoot biomass ability to photosynthesize effectively.

6.5.8. Grain N uptake as influenced by the treatments at harvest maturity

The increase in N uptake observed across the locations in response to the amendment of soil with Fertisoil and the addition to other nutrient constituents signifies that organic manure indeed is beneficial for optimizing soybean yield potential. This finding is similar to the findings of Tran *et al.* (2001) who reported that the incorporation of organic waste enhanced N uptake in soybean grain. Many researchers also had the same view about the increase in N grain with the use of organic manure (Sharma and Mittra, 1988; Thanikachalam and Rangarajan, 1992). Similarly, the combination of the other treatments with FS also led to an increase in soybean nitrogen uptake and seed quality, hence suggesting that more emphasis should be placed on balanced management systems for improved and sustained soybean production.

6.5.9 Grain P uptake as influenced by the treatments and their combinations at harvest maturity

Nutrient uptake is an indication of the availability and accessibility of soil nutrients in plant, grain nutrient uptake gives a reflection of both quantity and quality of the grain produced. The combination of BX + $N_{25}P_{30}$, FS + BX and FS + BX + $N_{25}P_{30}$ treatment at Ghulahgu and FS + BX + $N_{25}P_{30}$ treatments across locations (Tables 6.20, 6.21 and 6.22) enhanced P content in grain yield at harvest across location, emphasizing the need for macro and micro nutrients in soybean production. The combination of $N_{25}P_{30}$ and FS also enhanced grain P uptake which agrees with Tran *et al*., 2001 and Sharma and Mittra, 1991) findings who reported that the use of Farm yard manure + inorganic fertilizer resulted in increase in P uptake. Tran *et al*., (2001) also reported that inorganic fertilizer supplies the needed energy and nutrient for the decomposition of complex organic matter and convert them to mineralized organic colloids which might add to the soil organic matter reserves and rapid multiplication of the microbial population Tran *et al*. (2001) further explained, hence making P available for plant uptake and translocation into the seed at seed filling. He also reported that the organic acids released during decomposition of organic materials influences the pH forming stable complexes or chalates with cations responsible from P fixation and in turn increases P availability. The use of organic manure (Ghosh *et al*., 1981, Son and Ramaswami, 1997) significantly reduced the fixation of added as well as native P, making P more available to plant.

6.5.10 Value cost ratio

Value cost ratio is an important step in the evaluation of the financial incentives to be derived from the use of fertilizer treatments. Variations were observed in the

profitability of the various treatments investigated across locations. At Akukayili, FS and 25 kg N ha^{-1} + FS + INO were the most economically viable fertilizer treatment with VCR > 3 which according to FAO (2006) connotes the best for farmers with low technology, with no credit availability or limited capital. Other treatment combinations like FS + INO, $N_{25}P_{30}$ + FS and $N_{25}P_{30}$ + INO also holds profitable potential for farmers. Abebe *et al*., (2013) reported that the integrated use of N and P with FYM resulted in better economic advantage with an additional yield of soybean. However, at Cheshegu both sole and combined applications did not depict profitability that puts risk and high cost of production into consideration. None of the treatments applied at Cheshegu reached the acceptable threshold (VCR = 2) which is the minimum requirement for a farmer to adopt a fertilizer recommendation. This might also be attributed to the erratic rain condition experienced during the course of carrying out the experiment (Appendix 1). However, BX, FS, BX + FS, $N_{25}P_{30}$ + FS and $N_{25}P_{30}$ + INO led to VCR value above 1, meaning that the use of these treatments will help farmers attain a point of positive net return but not profitable. However, at Ghulahgu, the use of BX gave the highest VCR (5) which is also an economically viable treatment that can be considered by farmers. Similarly, the combination of INO × (FS + 25 kg N ha^{-1}) was a profitable treatment. Abdul khaliq *et al*. (2006) reported that the use of organic manure + ½ mineral inorganic nutrients saves mineral N fertilizer by almost 50% compared to only NPK.

6.6 Conclusion

The use of integrated nutrient management has proved to be the best option for soybean production in the study area. The use of FS + BX, FS + $N_{25}P_{30}$, FS + BX + $N_{25}P_{30}$ gave grain yields which were relatively higher than that of the control.

CHAPTER SEVEN

7.0 Summary, Conclusion and Recommendations

7.1 Summary of main findings

i. This study has confirmed that the use of starter N (25 kg N ha^{-1}) for soybean production is necessary in the study area because of the low inherent soil fertility.

ii. The study has established that the supply of additional nitrogen at 50% flowering growth stage resulted in increased grain yield suggesting that the N requirement of soybean cannot be fully met through biological nitrogen fixation. However, application rates greater than 50 kg N ha^{-1} may lead to luxury consumption.

iii. The interactive effects observed on nodule number and biomass yield is an indication that there may be the need for the combination of N and INO for effective nodule and biomass development and increased grain yield.

iv. Boost xtra significantly enhanced soybean grain yield emphasizing the need for introduction of new blends of macronutrient based fertilizer that contain micronutrients.

v. The results of this study have shown that Fertisoil and/or phosphorus increased nodule number.

vi. Phosphorus applied at 30 kg P ha^{-1} is enough for soybean growth and development in the study area.

7.2 General conclusions

i. The results showed that the use of starter N (25 kg N ha) before nodule commencement is essential for soybean growth in the study area.

ii. The results clearly demonstrated that Fertisoil applied at 3 t/ha had a positive influence on virtually all the measured parameters either as a sole treatment or in combination with either of the other evaluated treatments. This confirmed the null hypotheis of this research that the use of Fertisoil will enhance soybean production.

iii. This study has further buttressed the importance and benefit of combined use of organic (Fertisoil) and inorganic (Boost xtra) nutrient sources in sustaining soybean growth and yield. However, inoculation with *Bradyrhizobium japonicum* did not significantly increase soybean grain yield exept when in combination with organic or inorganic nutrient sources.

iv. There is need to supplement soybean with micro nutrients (Boost xtra) at 50% flowering growth stage. Suprisingly, the highest VCR (5) across all the study locations was produced by sole Boost xtra at Ghulahgu followed by INO+FS+$N_{25}P_{30}$ which recorded a VCR > 4. This may suggest that the use of Boost xtra holds profitable potential for farmers in the study area.

7.3 Recommendations

1. The residual effect of Fertisoil on subsequent crops can be evaluated in the study area in prospective studies.
2. The treatments considered in this study should be assessed under long - term trials to ascertain some of the conclusions outlined in this study.

3. It is also worthwhile to identify the strain of rhizobia in soils of the study site so as to evaluate the effectiveness of an introduced strain.

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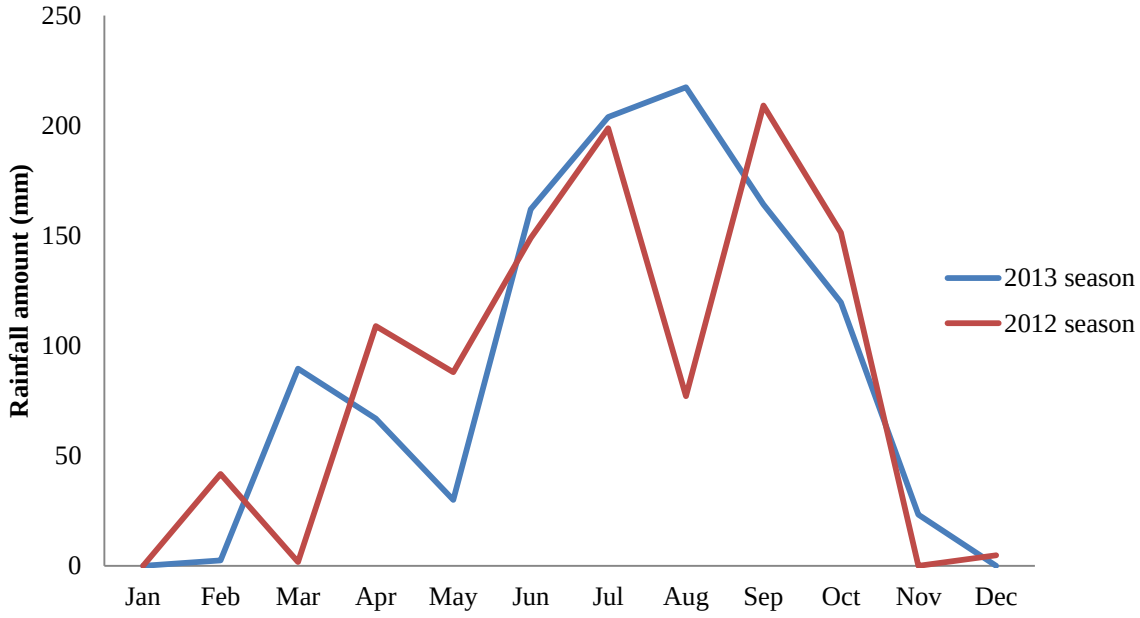
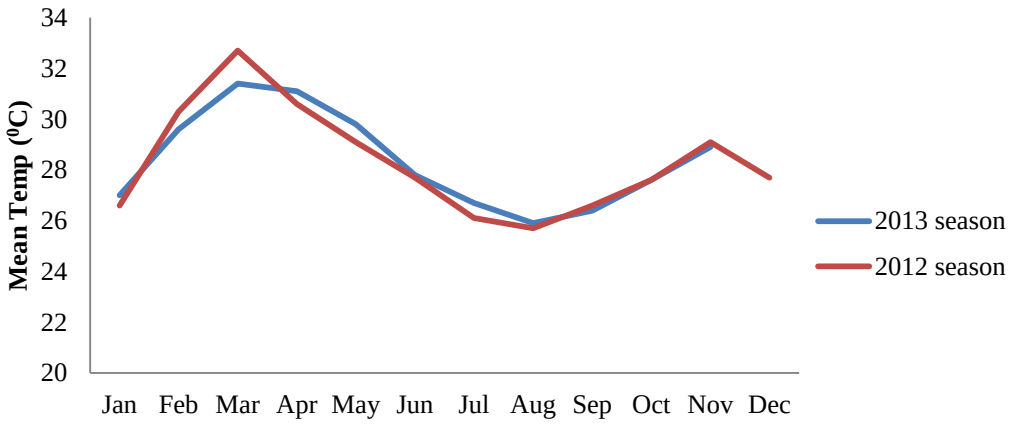
APPENDICES



Appendix 1. Rainfall pattern in 2012 and 2013 at the study sites



Appendix 2. Monthly distribution of mean temperature during the 2 cropping seasons

Appendix 3. Table for value cost ratio of treatments used at Akukayili

Treatment	Grain yield (kg ha^{-1})	Actual yield	price yield	Amt. of seed /ha	Price of seed /ha	Labour cost	Fertilizer cost	Total denomenator	VCR
Control	934			47.17	141.51				
BX	1284	350	560	47.17	141.51	505	132	778.51	1.4
FS	2551	1617	2587.2	47.17	141.51	485	300	926.51	3.3
$N_{25}P_{30}$	1296	362	579.2	47.17	141.51	485	435	1061.51	1.0
BX + FS	2362	1428	2284.8	47.17	141.51	990	432	1563.51	2.1
$N_{25}P_{30}$ + FS	3210	2276	3641.6	47.17	141.51	970	735	1846.51	2.5
BX + $N_{25}P_{30}$	1365	431	689.6	47.17	141.51	990	735	1866.51	0.9
INO	985	51	81.6	47.17	141.51	485	3.3	629.81	0.9
INO + BX	1089	155	248	47.17	141.51	990	135.3	1266.81	1.0
INO + FS	2502	1568	2508.8	47.17	141.51	970	303.3	1414.81	2.5
INO + $N_{25}P_{30}$	1932	998	1596.8	47.17	141.51	970	438.3	1549.81	1.7
INO +BX + FS	2803	1869	2990.4	47.17	141.51	1475	435.3	2051.81	2.2
INO +BX + FS + $N_{25}P_{30}$	3468	2534	4054.4	47.17	141.51	1960	870.3	2971.81	2.0
FS + BX + $N_{25}P_{30}$	2640	1706	2729.6	47.17	141.51	1475	867	2483.51	1.7
INO + BX + $N_{25}P_{30}$	1603	669	1070.4	47.17	141.51	1475	570.3	2186.81	1.2
INO + FS + $N_{25}P_{30}$	3334	2400	3840	47.17	141.51	970	303.3	1414.81	3.4

Appendix 4. Table for value cost ratio of treatments used at Cheshegu

Treatment	grain yield (kg ha^{-1})	Actual yield	price yield	Amt. of seed /ha	Price of seed /ha	Labour cost	Fertilizer cost	Total den.	VCR
Control	1722			47.17	141.51				
BX	2065	343	548.8	47.17	141.51	505	132	778.51	1.4
FS	2186	464	742.4	47.17	141.51	485	300	926.51	1.3
$N_{25}P_{30}$	2029	307	491.2	47.17	141.51	485	435	1061.51	0.9
BX + FS	2435	713	1140.8	47.17	141.51	990	432	1563.51	1.4
$N_{25}P_{30}$ + FS	2599	877	1403.2	47.17	141.51	970	735	1846.51	1.3
$N_{25}P_{30}$ + BX	1790	68	108.8	47.17	141.51	990	735	1866.51	0.6
INOCULATION	2004	282	451.2	47.17	141.51	485	3.3	629.81	1.5
INO × BX	1596	-126	-201.6	47.17	141.51	990	135.3	1266.81	0.6
INO × FS	1292	-430	-688	47.17	141.51	970	303.3	1414.81	0.2
INO × $N_{25}P_{30}$	2279	557	891.2	47.17	141.51	970	438.3	1549.81	1.2
INO × BX + FS	1710	-12	-19.2	47.17	141.51	1475	435.3	2051.81	0.7
INO × BX + FS + $N_{25}P_{30}$	1824	102	163.2	47.17	141.51	1960	870.3	2971.81	0.7
FS + BX + $N_{25}P_{30}$	2375	653	1044.8	47.17	141.51	1475	867	2483.51	1.0
INO × BX + $N_{25}P_{30}$	2015	293	468.8	47.17	141.51	1475	570.3	2186.81	0.9
INO × FS + $N_{25}P_{30}$	2365	643	1028.8	47.17	141.51	970	303.3	1414.81	1.4

Appendix 5. Table for value cost ratio of treatments used at Ghulahgu

Treatment	Grain yield kg ha-1	Actual yield	price yield	Amt. of seed /ha	Price of seed /ha	Labour cost	Fertilizer cost	Total den	VCR
Control	2090			47.17	141.51				
BX	4208	2118	3388.8	47.17	141.51	505	132	778.51	5.00
FS	3290	1200	1920	47.17	141.51	485	300	926.51	2.60
$N_{25}P_{30}$	3246	1156	1849.6	47.17	141.51	485	435	1061.51	2.20
BX + FS	2796	706	1129.6	47.17	141.51	990	432	1563.51	1.36
$N_{25}P_{30}$	3864	1774	2838.4	47.17	141.51	970	735	1846.51	2.06
BX + $N_{25}P_{30}$	3118	1028	1644.8	47.17	141.51	990	735	1866.51	1.41
INO	2783	693	1108.8	47.17	141.51	485	3.3	629.81	2.53
INO + BX	3457	1367	2187.2	47.17	141.51	990	135.3	1266.81	2.51
INO + FS	3217	1127	1803.2	47.17	141.51	970	303.3	1414.81	1.96
INO + $N_{25}P_{30}$	3943	1853	2964.8	47.17	141.51	970	438.3	1549.81	2.54
INO + BX + FS	3982	1892	3027.2	47.17	141.51	1475	435.3	2051.81	2.19
INO + BX + FS + $N_{25}P_{30}$	2797	707	1131.2	47.17	141.51	1960	870.3	2971.81	1.04
FS + BX + $N_{25}P_{30}$	3310	1220	1952	47.17	141.51	1475	867	2483.51	1.38
INO + BX + $N_{25}P_{30}$	4049	1959	3134.4	47.17	141.51	1475	570.3	2186.81	2.11
INO + FS + $N_{25}P_{30}$	4515	2425	3880	47.17	141.51	970	303.3	1414.81	3.43