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EVALUATION OF THE QUALITY AND EFFECTIVENESS OF SOME COMMERCIAL MICROBIAL PRODUCTS IN ENHANCING THE GROWTH AND YIELD OF SOYBEAN AND MAIZE

BY

GIDEON ASAMOAH

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BSc. LABORATORY TECHNOLOGY (HONS)

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MASTER OF SCIENCE

IN

SOIL SCIENCE

JULY, 2014

DECLARATION

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

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ABSTRACT

Field studies consisting of five treatments each for soybean and maize were conducted to assess the quality and effectiveness of some commercial products on soybean (Eco-Rhiz-Soya and Eco-T) as well as maize (Enrich and Eco-T). The treatments were arranged in a Randomised Complete Block Design with three replications. The quality of the commercial products was tested in the laboratory based on microbial density, pH and moisture content. The study showed that, although the products had microbial density lower than that labeled on them, the colony forming units (CFU's) determined met the Kenyan standards of biofertilizer specifications. The application of Eco-Rhiz-Soya + Eco-T significantly increased ($P<0.05$) soybean nodulation by 69% and 67% over the control at Kpongu and Akukayili respectively. Furthermore, shoot biomass yield increased by 45% and 58% respectively at Kpongu and Akukayili. Grain yield of soybean was increased by Eco-Rhiz-Soya + Eco-T by 19% and 43% over the control at Kpongu and Akukayili respectively. Moreover, soybean shoot N was significantly increased ($P<0.05$) by Eco-Rhiz-Soya + Eco-T at both sites of the study whereas shoot P uptake was only significantly increased at Akukayili. The soybean inoculation treatments had no significant effect on grain P uptake while grain N was significantly increased at Akukayili only.

With respect to maize, the treatments had no significant effect ($P>0.05$) on grain yield shoot biomass, shoot and grain N and P at both sites of the study. The products used for the maize experiments need to be evaluated further to assess their efficacy before concrete conclusions can be made on their effectiveness and subsequent recommendation for use by smallholder farmers.

DEDICATION

This work is dedicated to my mother, Ms. Janet Oforiwa and my Father, Mr. Eric Danquah for their support and encouragement.

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TABLE OF CONTENTS

LIST OF TABLES

APPENDICES

Page

CHAPTER ONE

1.0 INTRODUCTION

In order to obtain optimum growth of plants, the nutrients necessary for their growth must be available in sufficient and balanced quantities (Chen, 2006). The most important constraint limiting crop yield in developing nations and especially among resource poor farmers is soil fertility decline (Mokwunye *et al*., 1996). Unless the fertility is restored in these areas, farmers will gain little benefit from the use of improved varieties and even more productive agricultural technologies.

A number of interventions have been considered in the past but with limited success. The application of inorganic fertilizers though provides an option to overcome low soil infertility, extensive use of chemical fertilizers in long term cause decline in productivity and environmental quality (Rahim, 2002). The use of organic inputs to improve soil nutrient availability and use efficiency also has had limited success. In addition to the bulkiness of organic inputs, the amount of organics available for incorporation into the soil is limited. Again, they have alternative uses such as for fuel, livestock feeding, and construction. In recent years, biofertilizers have emerged as a promising component of integrating nutrient supply system in agriculture. They also offer environmentally friendly and sustainable agricultural practices (Bloemberg et al., 2000). Sustainable agriculture based on the use of microbial products is an effective option for overcoming problems of soil fertility (Chen, 2006).

Commercial microbial products are products containing living cells of different types of microorganisms which when, applied to seed, plant surface or soil, colonize the rhizosphere or the interior of the plant and promotes growth by converting

nutritionally important elements (nitrogen, phosphorus) from unavailable to available forms through biological processes such as nitrogen fixation and solubilization of rock phosphate (Rokhzadi et al., 2008). Beneficial microorganisms in biofertilizers accelerate and improve plant growth and protect plants from pests and diseases (El-yazeid et al., 2007). They are cost effective, eco-friendly and renewable sources of plant nutrients to supplement chemical fertilizers.

According to Giller (2001), microbial products have been proven to substantially enhance the productivity of specific crops. However, there appears to be proliferation of new biological products on the markets in sub-saharan Africa that claim a major impact in increasing crop productivity. In view of this, there is the need to rigorously test these new products in order 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 weaken their trust on the benefit of microbial products and other technologies for increasing agricultural productivity. Therefore, research aimed at scaling up quality standards as well as the performance of these products becomes very important especially in Ghana's agriculture where the use of commercial agricultural microbial products by smallholder farmers is still an emerging science.

The overall objective of this study therefore was to evaluate the quality and effectiveness of some commercial products on soybean and maize growth and yield in Kpongu in the Wa municipality and Akukayili in the Tolon districts of the Upper West and Northern regions of Ghana respectively.

The specific objectives were:

i. to evaluate the quality of some new commercial products identified for introduction into the Ghanaian market.

ii. to determine the effectiveness of the products purported to enhance the growth and yield of soybean and maize.

iii. to assess the ability of the products to enhance nitrogen and phosphorus uptake in soybean and maize

The above specific objectives were based on the null hypothesis that:

i. new commercial agricultural microbial products will not meet quality standards for inoculants specification.

ii. inoculation of soybean and maize with commercial microbial products will not lead to improved growth and yield of soybean and maize.

iii. nitrogen and phosphorus uptake in soybean and maize will not be improved following inoculation with microbial commercial products.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The need for inoculation

Although *rhizobia* seem to be widely distributed in the soil, soils in different places contain different strains of *rhizobia* and some of which may not be effective for nitrogen fixation. Some soils may have effective rhizobial strains, but contains higher number of ineffective strains (Herridge *et al*., 2002). In both cases, inoculation of the seed may be required. Inoculation of legume seed is a simple and practical means of ensuring effective nitrogen fixation.

The decision to inoculate is usually based on results from experimental plots. Date (1977) explained the experiments and trials necessary for recommending the use of rhizobial inoculant. The experiments included uninoculated control plants as well as those inoculated with effective *rhizobia* and N fertilizer control treatments. The uninoculated treatment evaluates the presence or absence of indigenous strains and, if indigenous strains are present, provides some assessment of their symbiotic effectiveness compared with plants inoculated with selected strains of *rhizobia*. The inoculated treatment assesses the ability of a known effective strain of *rhizobia* to colonize the rhizosphere and to compete for nodule forming sites with any indigenous strain that may be present. The nitrogen treatment is included to ensure that the legume has the potential to grow well when provided with adequate nitrogen and that growth is not limited by other factors such as phosphorus deficiency or low soil moisture availability.

2.2 Inoculant strains and carriers

The bacterial inoculant strain and its carrier formulation influence the field performance and survivability of the bacteria (Albareda *et al*., 2008). Inoculants are available in different forms including powder (usually in peat carrier), granule and liquid. Rhizobial cells contained in the inoculant are living organisms and continue to grow and multiply (Xavier *et al*., 2004). The formulation of the inoculant must be such that the *rhizobia* survive in sufficient quantities to ensure the minimum quantity of living cells required for successful nodulation at planting (Xavier *et al*., 2004). In a study comparing liquid and peat based inoculants, both were shown to adequately nodulate soybeans in the field (Tittabutr *et al*., 2007).

Most current inoculant products are supported in a liquid carrier due to the simplicity of production and application (Xavier *et al*., 2004). Bacterial survival in liquid carriers has been greatly improved with new formulations. Without the protection of peat or related carriers, liquid carriers have been less consistent in maintaining high bacterial cell counts in the inoculant in the past. Rhizobial cells in liquid inoculants tend to experience starvation stress or nutrient depletion at a greater degree in comparison to those in peat (Tittabutr *et al*., 2007). However, quality liquid formulations currently available will maintain adequate population densities for soybean inoculation for at least three months of storage (Albareda *et al*., 2008). Liquid additives in these inoculant product formulations improve performance and can be customized to the individual bacterial strain (Tittabutr *et al*., 2007). Additives also are able to protect *Bradyrhizobium japonicum* on the seed when exposed to high temperatures (Tittabutr *et al*., 2007). Even with recent improvements to liquid

formulations, peat carriers have proven to better protect *rhizobia* (Tittabutr *et al*., 2007).

Companies have developed inoculants with superior bacterial strains for vigorous nodulation. However, if planting into a field with indigenous rhizobial populations, the introduced bacteria must be competitive against the resident bacterial in the soil (Berg *et al*., 1988). The number of root nodules per plant is directly correlated with the years since the last soybean crop. The more the number of years since the last soybean crop, the fewer the numbers of root nodules per plant (Larson and Siemann, 1998). Despite new inoculants on the market that boast of improved bacterial strains, many times the naturalized *rhizobia* in the soil out competes the newer strains for infection of soybean roots. For improved competiveness with resident bacterial populations, in-furrow inoculants have proven to be superior (Lopez-Garcia *et al*., 2009). The competiveness of naturalized *rhizobia* may be the reason for lack of separation in the performance of inoculant products (Furseth *et al*. 2012). According to the authors, soybean yield or oil and protein contents did not differ between inoculant products and the non-inoculated controls in soil with persisting *B. japonicum* populations. Therefore, if the strains in the inoculant cannot compete with the naturalized populations, there is no benefit of inoculating the seed with superior rhizobia strains.

The minimum bacterial density for achieving adequate nodulation is 10^3 rhizobia per seed (Hiltbold *et al*., 1980). Nodulation in soil free of *B. japonicum* is directly related to the number of bacteria applied per seed. Lopez-Garcia *et al*. (2009) found that certain formulations or carriers out-perform others. In-furrow inoculants yielded slightly more than seed-applied inoculant on ground new to soybean in one study

(Berg *et al*., 1988). On the other hand, Schulz and Thelen (2008) reported that liquid inoculants provided a significant yield advantage over other products in areas new to soybean production. Finally, inoculant product brand did not affect yields in a study that compared several products (Schulz and Thelen, 2008). With the ongoing inconsistencies in research results, inoculant product development will continue to be an area of research activity.

2.3 Importance of inoculant quality

Following the identification of the most effective rhizobial strain and the best inoculant carrier, it is imperative to maximize the numbers of *rhizobia* that colonize the seedling rhizosphere. This can be achieved by having large numbers of viable *rhizobia* in the inoculant itself (i.e. high quality inoculant), using higher-than-normal rates of inoculation or by minimizing the death of *rhizobia* between the time the seed or soil is inoculated and nodulation occurs (Kuykendall *et al*., 1982). All three strategies have merit, although using high-quality inoculants is more advantgeous (Brockwell *et al*. 1995).

Roughley *et al* (1993), in a field study of narrow – leafed lupin reported that increasing the numbers of *rhizobia* applied to the seed from 1.9 x 10^4 to 1.9 x 10^6 increased nodule number from 8 to 26/plant; nodule dry weight from 65 to 393 mg/plant; % plants nodulated from 89 to 98%; shoot dry matter from 7.8 to 9 t/ha and most importantly, grain yield from 1.9 to 2.1 t/ha.

In similar studies of soybean, Brockwell *et al*. (1985) highlighted the strong, linear relationships between rhizosphere populations of *rhizobia* and nodulation, plant

growth (shoot DM) and grain yield. Highest yields were only achieved when rhizosphere populations were >1× 10^5/plant. Hume and Blair (1992) reported that soybean yields in land that had not grown soybean before were increased by an average of 24% when rhizobial numbers on the seed were increased from 10^5 to 10^6.

In the narrow-leafed lupin study of Roughley *et al*., (1993), the survival of the inoculum through the various stages of inoculation, sowing and immediate post-sowing in the soil was quantified. Results indicated that 95% of the *rhizobia* died between inoculations and sowing and, of those surviving, 83% died after 23 h in the soil. Thus, only 1% of the original rhizobial cells had survived the first 24 h. Their results reinforce the need for the highest quality inoculants coupled with inoculation and sowing procedures that aid inoculant survival.

Hiltbold *et al*. (1980), on the other hand, examined commercial inoculants for quality and efficacy. In that study, rhizobial numbers in the commercial inoculants varied widely, from less than 10^3 /g to about 10^9/g. Nodulation of the soybean was directly related to numbers, with no nodulation produced by products supplying less than 10^3 *rhizobia*/seed, and abundant nodulation by products providing 10^5–10^6 /seed. Effects of inoculant quality on grain yield were similar. Yield increased linearly with increasing rhizobial numbers on the seed, in turn related to inoculant quality (Hiltbold *et al*. 1980).

Another important consideration, with respect to inoculant efficacy is the age of the inoculant. Non- sterile inoculants will contain large numbers of contaminants and they depress numbers of *rhizobia* with time (Date and Roughley, 1977). Even in sterile carriers, numbers of viable rhizobia will decrease over time, although not at the same rate as in non- sterile carriers. Boonkerd (1991) reported differences

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

2.4 Factors limiting the quality of inoculants

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

2.4.1 Temperature

Nearly all inoculants are best stored in a refrigerator. The exceptions are the inoculants for fine stem stylos, centrosema, desmodium and lablab, which should be stored between 20°C and 30°C (Deaker *et al*., 2012). Optimum long-term storage conditions for rhizobial survival, has assumed that temperature and moisture status are important for rhizobial viability over time (Deaker *et al*., 2012). At farm supply dealerships and on individual farms, legume inoculants may be stored in uninsulated steel sheds in which temperatures can fluctuate widely and can rise far above the constant low temperatures considered optimal for maintenance of rhizobial cultures (Sparrow and Ham, 1983).

Peat is the most commonlly used carrier for commercial legume inoculants in North America and elsewhere (Date 1972; Sparrow and Ham, 1983). Finely ground peat has a high water-holding capacity and provides a nutritive medium for growth of *rhizobia*. It also offers effective protection against adverse environmental conditions during inoculant distribution and storage and after application on seed (Kremer and Peterson, 1983; Materon and Weaver, 1984). Peats can sustain high numbers of *rhizobia* to the tune of 10^8 g^{-1}; when incubated at temperatures between 28°C and 30°C (van Schreven, 1970). In tropical and subtropical regions of the world, where inoculants are often exposed to extremely hot and dry conditions, survival of *rhizobia* is generally better in peat than in alternative organic carrier materials because peat provides superior protection against desiccation (Vincent, 1965; Van Schreven, 1970; Biederbeck *et al*., 1992).

2.4.2 Desiccation

Desiccation is one of the major factors causing poor rhizobial survival on legume seeds. *Rhizobia* are susceptible to drying on surfaces particularly where individual cells are exposed to low ambient relative humidity (Deaker *et al*., 2006). This has been demonstrated on various surfaces such as glass (Vincent *et al*., 1962), sand (Bushby and Marshall, 1977) and membrane filters (Mary *et al*., 1985; Deaker *et al*., 2006).

Several investigations have focused on effects of suspending medium on the recovery of cells after dehydration. Sugars, sugar alcohols, amino acids, synthetic and natural polymers and clay minerals have improved recovery of dried bacterial cells. Sucrose was superior to sorbitol, lysine, amino acids, and milk and yeast mannitol broth for preserving *rhizobia* during freeze-drying (Deaker *et al*., 2006). Annear (1962) found that peptone mixed with glucose or sorbitol was effective for the preservation of vacuum dried bacteria on cellulose fibers at room temperature. He concluded that a drying medium must be assessed with regard to the protection it affords during drying storage, the simplicity and definability of its composition and the range of organisms with which it is effective. In addition clays (montmorillonite, bentonite, illite, vermiculite) can also provide protection against desiccation and thus extend survival of inoculants (Biederbeck *et al*., 1992). This is particularly true for the fast-growing *rhizobia* (e.g., *Rhizobium meliloti*, *Rhizobium leguminosarum*), which are more susceptible to desiccation than the slow-growing *Rhizobium lupine* and *R. japonicum* (Beck *et al*., 1993). Consequently, clay-base inoculants have recently gained some popularity in North America.

2.4.3 Moisture content

Moisture content plays a crucial role in the growth rate of *Rhizobia* in inoculants. However, microbial inoculants have a very short shelf life. The biological activity of the PGPR may decline rapidly if the handling and storage is not done in the correct manner (Caballero- Mellado, 2005). The use of carrier materials for the microbial inoculants proves to be beneficial to protect the bacteria and has long been practiced (Fuentes-Ramirez). Among various types of carrier materials, peats are the most frequently utilized. This is because peats were able to support high number of *rhizobia* and maintain its survivability due to high moisture holding capacity and large surface area. The usage of peat however, poses problems typically in the tropics as it is not readily available in many countries (Bashan, 1998).

2.4.4 Nature and preparation of carrier

Biofertilizers are usually prepared as carrier-based inoculants containing effective microorganisms. Incorporation of microorganisms in carrier material enables easy-handling, long-term storage and high effectiveness of biofertilizers (Somasegaran and Hoben, 1994). Various types of material are used as carrier for seed or soil inoculation. For preparation of seed inoculant, the carrier material is milled to fine powder with particle size of 10-40 μm. According to the Somasegaran and Hoben, (1994), the properties of a good carrier material for seed inoculation are: non-toxic to inoculant bacterial strain, high moisture absorption capacity, easy to process and free of lump-forming materials, easy to sterilize by autoclaving or Gamma-irradiation, available in adequate amounts, inexpensive, has good adhesion to seeds, and of good pH buffering capacity. Non - toxicity to the host plant is another important property.

However other essential criteria for carrier selection relating to survival of the inoculant bacteria should be considered. Seeds are not always sown immediately after seed coating with the inoculant bacteria. The bacteria have to survive on seed surface against drying condition until placed into the soil (Nápoles *et al*., 2000). Optimum long-term storage conditions for rhizobial survival on inoculated seed are not well defined. As with inoculants, it is assumed that temperature and moisture states are important for rhizobial viability over time. Polymers also play a role in reducing exposure of cells to environmental stress but protective properties vary with every polymer. In addition, contaminants in inoculant carriers are known to suppress growth of *rhizobia* during inoculants production (Nápoles *et al*., 2000).

2.5 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 (Okogun and Sanginga, 2003; Fening and Danso, 2002; Okereke *et al*., 2000). 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.

Annually, 3×10^{14} g of N_2 is fixed as NH_4^+ (Rees *et al*., 2005). The amounts of energy required to break the triple, double and the single bonds of N_2 molecule are 225, 100, and 39 kcal mol^{-1} respectively (Howard and Rees, 1994). Before assimilation, N_2 must be fixed and converted into biologically usable forms. The most common forms of fixed N_2 are NH_4^+ and NO_3^-.

2.5.1 Factors affecting BNF

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

and N fixation is severely affected than the plant growth (FAO, 1984). Highly alkaline (pH > 8) soils tend to be high in sodium (Na^+), chloride (Cl^-), bicarbonate (HCO_3^-) and borate (BO_3^-) which reduces N fixation (Bordeleau and Prévost, 1994). Uddin *et al*. (2008) revealed that nodule number and size were significantly inhibited by the application of N fertilizer (urea). Symbiotic N fixation varies according to the carbon allocation to the nodules, in relation to endogenous factors, current photosynthesis, crop growth rate and other competing sinks for carbon (Voisin *et al*., 2003).

2.6 *Trichoderma* Fungi

Trichoderma species are fungi that are present in nearly all soils and other diverse habitats. *Trichoderma* species include *T. harzianum*, *T. viride*, *T. koningii*, *T. hamatum* and other species (McAllister *et al*., 1994). In soil, they are frequently the most prevalent culturable fungi. Some strains are highly rhizosphere competent, i.e., able to colonize and grow on roots as they develop. The most strongly rhizosphere competent strains can be added to soil or seeds. Once they come into contact with roots, they colonize the root surface or cortex (Ghahfarokhy *et al*., 2011). In addition to colonizing roots, *Trichoderma* species attack, parasitize and otherwise gain nutrition from other fungi. Since Trichoderma species grow and proliferate best when there are abundant healthy roots, they have evolved numerous mechanisms for both attack of other fungi and for enhancing plant and root growth (Elad and Kapat, 1999). Several new general methods for both biocontrol and for causing enhancement of plant growth have recently been demonstrated and it is now clear that there must be hundreds of separate genes and gene products involved in these

processes. The recent list of mechanisms include mycoparasitism, antibiosis, tolerance to stress through enhanced root and plant development, solubilization and sequestration of inorganic nutrients, induced resistance and inactivation of the pathogen's enzymes (Geremia *et al*., 1993; Chang *et al*., 1986; Harman *et al*., 2004a)

2.6.1 *Trichoderma* and nutrient/water uptake

It has been documented that several strains of *Trichoderma* have been developed as biocontrol agents against fungal diseases of plants through and those various mechanisms such antibiosis, parasitism, inducing host - plant resistance and competition (Harman *et al*., 2004). But what is not well known is the ability of *Trichoderma* to promote the uptake of nutrients and water to enhance overall plant growth (Yedidia *et al*., 2001). In most instances, increased plant growth and yields were attributed to the reduction in plant disease, but *Trichoderma harzianum* has been reported to increase plant growth independent of any plant disease (Baker *et al*., 1984). Rudresh (2005) found increased growth, P uptake and yield in *Trichoderma* - inoculated treatments compared with fertilized control and rock phosphate control treatments suggesting that *Trichoderma spp*. solubilize insoluble rock phosphate and supply P in a soluble form to plants in addition to acting as a biological control agents and possibly producing growth - promoting substances. Among the *Trichoderma* isolates, *T. harzianum* was found to be the best isolate whose performance was just behind the standard phosphate - solubilizing bacterium. Kleifeld (1992) reported similar observations of an increase in growth and yield parameters by inoculation with *Trichoderma* species. Some studies have also shown that *Trichoderma spp*. can stimulate the growth of a number of vegetable and

bedding plant crops. Baker (1988) and Ousley *et al*. (1994) concluded that specific *Trichoderma* strains have the potential to consistently increase plant growth. Naseby (2000) has found that inoculation with *Trichoderma* strains significantly increased fresh shoot weight by 15%, significantly increased the root weights by 22%, and significantly greater wet root weights up to 62%.

2.7 *Herbaspirillum*

Herbaspirillum is an endophyte which colonises sugarcane, rice, maize, sorghum and other cereals (James et al., 2000). It can fix 31 – 54% of total rice plant (30-day-old rice seedlings) N from the atmosphere (Baldani *et al*., 2000). The estimated N fixed by *Herbaspirillum* was 33 – 58 mg under aseptic conditions (Reis *et al*., 2000). In a greenhouse study, inoculation with *Herbaspirillum* increased rice yield significantly (at 5% probability level) up to 7.5 g/plant (Mirza *et al*., 2000). Inoculation with *Herbaspirillum seropedicae* in field conditions can increase shoot and root length, 1000 - grain weight and grain yield of rice (Arangarasan *et al*., 1998). Inoculation with *Herbaspirillum* can also enhance seed germination significantly (Pereira *et al*., 1988). Mirza *et al*. (2000) quantified the BNF by different strains of *Herbaspirillum* in both basmati and super basmati rice. The %Ndfa (N_2 derived from the atmosphere) values were 19.5 – 38.7 and 38.1 – 58.2 in basmati and super basmati, respectively. Thus, *Herbaspirillum* can fix 19 – 58% of the N required by rice crop depending on *Herbaspirillum* strain and rice variety. *Herbasprillum seropedicae* also acts as an endophytic diazotroph of wheat plants (Kennedy and Islam, 2001), colonising wheat roots internally between the cells in a fashion similar to *Azospirillum brasilense*. Its

application can increase straw and grain yields, %Ndfa and %N recovery in wheat plant under field conditions (El-Mohandes, 1999).

Herbasprillum seropedicae is also found in roots and stems of sugarcane plants while *Herbaspirillum rubrisubalbicans* is an obligate endophyte of roots, stems and leaves (Reis *et al*., 2000). These diazotrophs can increase leaf N content and cane yield significantly, but cannot substitute for urea - N completely (Muthukumarasamy *et al*., 1999). The population of these bacteria is not affected by chemical N fertilisation even at applications of 300 kg N/ha under field conditions (Reis *et al*., 2000). *Herbaspirillum* and *Azospirillum* have both been applied to sorghum (Baldani *et al*., 1986b; Pereira *et al*., 1988) with positive results.

Herbaspirilla can also colonise maize plants endophytically and fix N_2, as with sugarcane and wheat (James, 2000). Riggs *et al*. (2001) concluded from the results of extensive greenhouse and field experiments using non – sterilised soils that there were beneficial effects of maize seed inoculation with *H. seropedicae* on maize with increased yield in greenhouse conditions by 49 – 82% with applied fertilizer N compared to an increase of only 16% without fertilizer N. This indicates *H. seropedicae* can improve the ability of maize plant to use fertilizer N more efficiently. In field experiments, the increases in yields due to *H. seropedicae* inoculation were up to 19.5%.

2.8 *Bacillus spp*

Bacillus is the most abundant genus in the rhizosphere, and the plant growth promoting rhizobacteria (PGPR) activity of some of these strains has been known for many years, resulting in a broad knowledge of the mechanisms involved (Gutiérrez

Mañero *et al*., 2001). There are a number of metabolites that are released by these strains (Charest *et al*., 2005), which strongly affect the environment by increasing nutrient availability of the plants (Barriuso and Solano, 2008). Naturally present in the immediate vicinity of plant roots, *B. subtilis* is able to maintain stable contact with higher plants and promote their growth. In a micropropagated plant system, bacterial inoculation at the beginning of the acclimatisation phase can be observed from the perspective of the establishment of the soil microbiota rhizosphere. *Bacillus licheniformis* when inoculated on tomato and pepper showed considerable colonisation and can be used as a biofertiliser without altering normal management in greenhouses (García *et al*., 2004). Jaizme-Vega *et al*. (2004) evaluated the effect of a rhizobacteria consortium of Bacillus spp. on the first developmental stages of two micropropagated bananas and concluded that this bacterial consortium can be described as a prospective way to increase plant health and survival rates in commercial nurseries. *Bacillus* is also found to have potential to increase the yield, growth and nutrition of raspberry plant under organic growing conditions (Orhan *et al*., 2006). Bacillus megatorium is very consistent in improving different root parameters such as rooting performance, root length and dry matter content of root (Kaymak *et al*., 2008). The phosphate solubilizing bacteria (PSB) *Bacillus megaterium* var. phosphaticum and potassium solubilising bacteria (KSB), *Bacillus mucilaginosus* when inoculated in nutrient limited soil showed that rock materials (P and K rocks) and both bacterial strains consistently increased mineral availability, uptake and plant growth of pepper and cucumber, suggesting its potential use as fertilizer (Han *et al*., 2006).

2.9 Summary of literature review

Both chemical and organic fertilizers exhibit different characteristics in terms of nutrient release. These fertilizers also have their advantages and disadvantages with regard to crop growth and soil fertility. Due to the solubility and availability of nutrients in chemical fertilizers, they are commonly used because the effect is usually direct and fast. Organic fertilizers on the other hand do not have standardized nutritional constituents and also their rate of nutrient release to meet crop growth is too slow and may sometimes lead to a nutrient deficiency in plants. Owing to the problem associated with the use of both chemical and organic fertilizers, biofertilizers can be used as substitute since they do not cause pollution like chemical fertilizer and give faster effect compared to organic fertilizer.

However, the use of microbial fertilizers in practice has not achieved much of the expected results. This might be as a result of the usage of poor quality and ineffective inoculants which may not elicit the desired effect as stipulated by manufacturers. As a result of this, assessing the quality and effectiveness of new commercial products (inoculants) would be necessary in order to assure small holder farmers who are the end users of these products of their performance.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental sites

All laboratory procedures were carried out at the Soil Microbiology Laboratories of Kwame Nkrumah University of Science and Technology, Kumasi and Soil Research Institute, Kwadaso.

The field work was carried out at Kpongu in the Wa metropolis in the Upper West Region and Akukayili in the Tolon District in the Northern region. Akukayili lies on altitude 183 m, latitude 09º 25N and longitude 0º 58W. Rainfall pattern is unimodal with mean annual rainfall of 1000 - 1200 mm fairly distributed from April-November. The area has high temperatures during the day and cool temperatures at night with mean monthly minimum of 23.4 ºC and of 34.5 ºC, and a minimum maximum relative humidity of 46% and maximum of 476.8% respectively. The soils are sandy loam and are classified as Ferric luvisols (FAO, 1998).

Kpongu is located in the Guinea savanna zone of Ghana stretching from Latitude 9°35′N to 11°00′N and from Longitude 01° 25E to 02° 50E, and at an altitude of 200 - 350 m above sea level. It is characterized by erratic and poorly distributed unimodal rainfall, averaging about 1000 mm per annum (MSDG, 1997). The soils are loamy sand in texture and classified as Ferric cambisols (FAO, 2001). The soils are inherently low in natural fertility and have a low moisture retention capacity.

3.2 Field work

3.2.1 Source of planting materials

The soybean and maize seeds used for the study were obtained from the Savannah Agricultural Research Institute, Nyankpala, near Tamale, Ghana. The varieties of the test crops used were 'Jenguma' and 'Obaatampa' for soybean and maize respectively.

3.2.2 Biofertilizer products used

- Eco-Rhiz-Soya (a rhizobial inoculant containing the Bradyrhizobium japonicum strain WB74 for fixing atmospheric nitrogen in the root noodles of soybean. This was imported from Plant Health Products (Pty) Ltd from South Africa).
- Eco- T (is a bio-pesticide containing the fungal species Trichoderma harzianum strain kd for control of pathogens that cause root diseases. This was also imported from Plant Health Products (Pty) Ltd from South Africa).
- Enrich (is a plant growth promoting inoculant containing the bacterial species Herbaspirillum and Bacillus for nitrogen fixation in cereals and phosphate solubilization respectively. This product was imported from AgriBioServices Ltd, UK).

3.2.3. Land preparation, inoculant application and planting

The fields were ploughed and manually levelled with a hoe after which the field layout was done. Plot sizes measuring 4.5 m x 4.5 m were demarcated. The Eco-

Rhiz-Soya was applied by dissolving 12.5 g of it in 50 ml of water after which 5 ml of the resulting solution was added to 1 kg of seed in a sealed container. This was then vigorously shaken to ensure that all the seeds were adequately wetted with the solution. The Eco-T product was applied by moistening 1 kg of each seeds with water, after which 1.5 g of the product was added and shaken in a sealed container to ensure uniform coating of all the seeds with the product. The Eco-Rhiz-Soya and Eco-T treatment combination was applied by dissolving 12.5 g of Eco-Rhiz-Soya and 2.5 g of Eco-T in 50 ml of water after which 5 ml was used to inoculate 1 kg of seeds and shaken to achieve uniform seed coating. The inoculated seeds were then allowed to dry under shade for about 30 minutes with intermittent mixing before planting.

The Enrich product was applied by mixing thoroughly the activator solution and the freeze dried bacterial cells in 15 litres of non-chlorinated water in a Knapsap sprayer after which the resulting solution was sprayed on the young maize plants having four to six leaves for treatments requiring Enrich. Soybean and the maize were planted at three seeds per hill at a planting distance of 50 cm x 5 cm and 80 x 40 cm, respectively. The maize was thinned to two seedlings per hill whiles the soybean to 1 seedling per hill two weeks after planting. During planting, it was ensured that the control plots were planted before the inoculated plots. Moreover, hands were washed with soap and water after planting triplicate plots having the same inoculant treatment to avoid the incidence of cross contamination.

3.2.4 Treatments and experimental design

Two separate field experiments using soybean and maize with five different treatments each were conducted. The treatments for the soybean trial were: Control

(Uninoculated), Eco-T, Eco-Rhiz-Soya, Eco-T + Eco-Rhiz-Soya and Urea (100 kg N/ha); and the treatments for the maize trial were: Control (Uninoculated), Eco-T, Enrich, Eco-T + Enrich, 100% NPK (RR).

Twenty kilogrammes of P per hectare and 20 kg K/ha were applied to all plots in the form of triple superphosphate (TSP) and muriate of potash (MOP), respectively for the soybean experiment as basal treatment. Treatments other than the 100% NPK (RR) in the maize experiment also received 45 kg N/ha, 30 kg P/ha and 30 kg K/ha as a basal treatments. The TSP and MOP were applied once two weeks after planting whiles the urea was split applied; 1/3 two weeks after planting and the remaining 2/3 six weeks after planting. The fertilizers were applied by the band placement method to ensure fertilizer use efficiency and also to reduce weed growth. The treatments were arranged in a Randomized Complete Block Design (RCBD) with three replications each.

3.3 Soil sampling and sample preparation

Eight core samples were taken from each plot using an auger at a depth of 0 - 20 cm. The soil samples were then bulked and thoroughly mixed to obtain homogenous samples from which subsamples were taken for physico-chemical analysis and enumeration of rhizobia. The samples were sieved with a 2 mm mesh sieve to remove broken sticks and other debris before the analyses were carried out

3.4 Laboratory analysis

3.4.1 Determination of soil physical properties

3.4.1.1 Particle size distribution

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

Calculation;

% Sand = $[H_1 + 0.2 (T_1 – 20) – 2] \times 2$

% Clay = $[H_2 + 0.2 (T_2 – 20) – 2] \times 2$

% Silt = 100 - (% Sand + % Clay)

where:

H_1 = 1st hydrometer reading at 40 seconds

T_1 = 1st temperature reading at 40 seconds

T_2 = Temperature reading at 3 hours

H_2 = 2nd hydrometer reading at 3 hours

-2 = Salt correction to be added to hydrometer reading

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

3.4.2 Determination of soil chemical properties

3.4.2.1 Soil pH

This was determined using a glass electrode (H19017 Microprocessor) pH in a 1:2.5 soil to distilled water ratio. A 20 g air-dried soil was weighed into a 100 ml beaker. To this, 50 ml distilled water was added and stirred thoroughly for 20 minutes. The soil – water suspension was allowed to stand for 15 minutes. After calibrating the pH meter with buffer solution of pH 4.0 and 7.0, the pH was read by immersing the electrode into the upper part of the suspension.

3.4.2.2 Soil organic carbon

The modified Walkley and Black procedure as described by Nelson and Somers (1982) was used to determine organic carbon. The procedure involves a wet combustion of the organic matter with a mixture of potassium dichromate and sulphuric acid after which the excess dichromate was titrated against ferrous sulphate. One gram soil was weighed into a conical flask. A reference sample and a blank were included in separate conical flasks. Ten millilitres of 0.166 M (1.0 N) potassium dichromate solution was added to the soil and the blank flask. To this, 20 ml of concentrated sulphuric acid was carefully added from a measuring cylinder, contents were then swirled and allowed to stand for 30 minutes on an asbestos mat. Distilled water (250 ml) and 10 ml concentrated orthophosphoric acid were added

and the mixture allowed to cool. One milliliter of diphenylamine indicator was added and titrated with 1.0 M ferrous sulphate solution.

Calculation:

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

where:

M = molarity of the ferrous sulphate solution

V_1 = ml ferrous sulphate solution required for blank titration

V_2 = ml ferrous sulphate solution required for sample titration

g = weight of air – dry sample in grams

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

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

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

3.4.2.3 Total nitrogen

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

digest was made to 100 ml with distilled water and mixed well. A 10 ml aliquot of the digest was transferred to the reaction chamber and 20 ml of 40% NaOH solution was added followed by distillation. The distillate was collected over 4% boric acid. Using bromocresol green as an indicator, the distillate was titrated with 0.02 *N* HCl solution. A blank distillation and titration was also carried out to take care of N traces in the reagents as well as the water used.

Calculation:

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

where:

A = volume of standard HCl used in the sample titration

B = volume of standard HCl used in the blank titration

N = Normality of standard HCl

3.4.2.4 Available phosphorus

The readily acid – soluble forms of phosphorus were extracted with Bray No. 1 solution as outlined by Olsen and Sommers (1982). Phosphorus in the sample was determined on a spectrophotometer (210 VGP Buck scientific) by the blue ammonium molybdate with ascorbic acid as a reducing agent. A 5 g soil was weighed into 100 ml extraction bottle and 35 ml of Bray 1 solution (0.03 M NH_4F and 0.025 M HCl) was added. The bottle was placed on a reciprocal shaker and shaken for 10 minutes and filtered through Whatman No. 42 filter paper. An aliquot of 5 ml of the filterate was pipetted into 25 ml flask and 10 ml colouring reagent (ammonium paramolybdate) was added followed by a pinch of ascorbic acid. After

mixing well, the mixture was allowed to stand for 15 minutes to develop a blue colour. The colour was measured using the spectrophotometer at 660 nm wavelengths. A standard series of 0, 1.2, 2.4, 3.6, 4.8, and 6.0 mg P/L was prepared by pipetting respectively 0, 10, 20, 30, 40 and 50 ml of 12.0 mg P/L in 100 ml volumetric flask and made to volume with distilled water. The available phosphorus was then extrapolated from the standard curve.

Calculation:

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

where:

a	=	mg P/l in the sample extract
b	=	mg P/l in the blank
g	=	sample weight in grams
mcf	=	moisture correction factor
35	=	volume of extraction solution
15	=	final volume of the sample solution

3.4.2.5 Extraction of exchangeable cations

Calcium, magnesium, potassium and sodium in the soil were determined in 1.0 *M* ammonium acetate (NH_4OAc) extract (Black, 1965). A 10 g sample was transferred into a leaching tube and leached with a 250 ml of buffered 1.0 *M* ammonium acetate (NH_4OAc) solution at pH 7. Hydrogen plus aluminum were determined in 1.0 *M* KCl extract as described by Page *et al*. (1982).

3.4.2.5.1 Determination of exchangeable calcium and magnesium

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

Calculation:

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

where:

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

W = weight in grams of air – dry soil extraction

0.01 = concentration of EDTA used

3.4.2.5.2 Determination of exchangeable potassium and sodium

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

Calculations:

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

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

where:

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

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

g = air – dried sample weight of soil in grams

mcf = moisture correction factor

3.5 Enumeration of 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)

3.6 Plant tissue analysis of soybean and maize

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

3.7 Quality assessment of inoculants used in the study

The quality of the inoculants used for the study was assessed and the parameters evaluated were:

- Viable cell count
- pH
- Moisture content

3.7.1 Viable cell count

The spread plated technique employed by Zuberer (1994) was used to estimate the number of viable cells in the inoculants. Physiological saline solution (0.85% NaCl) was used as diluent for all tenfold dilution series. A 10-fold serial dilution from 10^{-1} to 10^{-10} was prepared with each inoculant for which 100 ul of aliquots of each dilution were spread onto Yeast Extract Mannitol Agar and Potato Dextrose Agar for the Eco-Rhiz-Soya and Eco-T respectively starting from the highest dilution. Each dilution plate culture was replicated three times. The plates were then kept for 8 days at 28 ˚C in an incubator after which the dilution level having colonies between 30 - 300 were used for the estimation. The number of colony forming units (Cfu) was determined using the formula below:

$$\text{Cfu} = \text{Number of colonies counted x } \frac{\text{Dilution factor}}{\text{Aliquot plated}}$$

3.7.2 pH

This was determined using glass electrode (H19017 Microprocessor) pH meter in a 1:2.5 inoculant to distilled water ratio. Ten grams of the inoculant was weighed into a 100 ml beaker. To this, 25 ml distilled water was added from a measuring cylinder, stirred thoroughly for 20 minutes. The inoculant – water suspension was allowed to stand for 15 minutes. After calibrating the pH meter with buffer solution at pH 4.0 and 7.0, the pH was read by immersing the electrode into the upper part of the suspension.

3.7.3 Moisture content

The moisture content of the inoculants were determined according to the procedure described in America Association of Cereal Chemists (AACC, 2000). Five grams of the sample was weighed into a moisture dish which had been previously dried in an oven and weighed. The uncovered dish was then dried in the oven for 3 hours at a temperature of 105±5°C. The dish was covered and was transferred to desiccators and weighed quickly as soon as the dish was cooled. The heating and weighing procedure was repeated until successive weights did not differ by more than one milligram. The moisture content was determined using the relation below;

Calculation

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

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

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

3.8 Data collection

3.8.1. Nodule count and nodule dry weight

Ten soybean plants at 50% flowering 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 ˚C for 48 hours. The dry weights of the nodules were then recorded.

3.8.2 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.

3.8.3 Number of pods per plant

Ten soybean plants were harvested from the four middle rows of each plot at physiological maturity and the pods carefully plucked and counted. The mean number of pods per plant was then determined for each treatment.

3.8.4 Soybean grain yield

Harvesting of the soybean was done in an area of 3.75 m^2 from each plot at physiological 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).

3.8.5 Maize stover and grain yield

At physiological maturity, maize was harvested from the three middle rows from a net plot area of 1.6 m x 3.6 m. The cobs were then oven dried at 70 ^{o}C for 72 hours after which the grains were shelled and the grains weighed.

The stover yield was determined by weighing the total biomass from each plot in the net area on the field after which fresh and dry weights of five representative plants were taken to the laboratory following drying in the oven at 70 ^{o}C for 72 hours. The grain and stover yield were determined on per hectare basis as follows:

$$\text{Stover yield (kg/ha)} = \frac{\text{FWSPP} \times \text{DWSSx } 10000}{\text{FWSSSx Net area}}$$

$$\text{Grain yield (kg/ha)} = \frac{\text{weight of grains} \times 10000}{\text{Net area}}$$

Where;

FWSPP = Fresh weight of stover per net plot

FWSSS = Fresh weight of sub sample stover

DWSS = Dry weight of sub sample stover

3.9 Statistical analysis

The data collected were subjected to Analysis of Variance (ANOVA) using Genstat statistical software version 12. Significant differences were assessed at 5% (p = 0.05) level of significance and the means separated using least significance difference

(LSD) procedure. All count data were transformed logarithmically (Kihara *et al*., 2011) before being subjected to ANOVA.

CHAPTER FOUR

4.0 RESULTS

4.1 Soil physico-chemical analysis and MPN count of the study sites

The physico - chemical properties and the MPN of indigenous rhizobia population of the top soil (0 - 20 cm) before treatments application are as shown in Table 4.1.

Table 4.1. Physico - chemical analysis and MPN count of the experimental sites

Soil parameter	Kpongu		Akukayili	
	Soybean	Maize	Soybean	Maize
pH (1:1) (H_2O)	7.91	7.82	7.15	6.93
Organic carbon (%)	0.69	0.41	1.04	0.41
Total nitrogen (%)	0.06	0.05	0.11	0.09
Available P (mg/kg)	5.99	4.72	17.47	15.42
Exchangeable K ($cmol_{(+)}$/kg)	0.15	0.16	0.08	0.06
Exchangeable Mg ($cmol_{(+)}$/kg)	0.08	0.80	1.07	0.92
Exchangeable Ca ($cmol_{(+)}$/kg)	1.34	1.36	1.87	1.62
Exchangeable Na ($cmol_{(+)}$/kg)	0.07	0.10	0.04	0.03
Sand (%)	72.20	76.06	66.05	63.14
Silt (%)	16.80	17.50	29.95	30.09
Clay (%)	6.00	6.40	4.00	6.00
Textural class	Loamy sand	Loamy sand	Sandy loam	Sandy loam
MPN (cells/g of soil)	31.90	-	43.60	-

4.2 Quality assessment of inoculants

The results of quality parameters of the inoculants used for the study are presented in Table 4.2. The pH of the products was 7.2 and 6.5 for the Eco-Rhiz-Soya and Eco-T respectively. The moisture content of the Eco-Rhiz-Soya was less than 40% while

that of the Eco-T was less than 1%. Additionally, viable cell count of 1.68 x 10^8 and 5.7 x 10^7 were obtained in the Eco-Rhiz-Soya and Eco-T respectively.

Table 4.2. Quality assessment of the products used for the study

Quality indicator	Commercial products	
	Eco-Rhiz-Soya	**Eco-T**
pH	7.2	6.5
Moisture content (%)	34	0.8
Viable cell count (cfu/g)	1.68 x 10^8	5.7 x 10^7

4.3 Soybean experiment

4.3.1 Nodule number of soybean

The effect of the treatments on nodule number at both study sites is as shown in Table 4.3 At Kpongu, the highest number of nodules was produced by combined Eco-T and Eco-Rhiz-Soya treatment (16.2 nodules/plant) which was 25% higher than that of the control whiles the least (9.6 nodules/plant) was produced by the urea treated plots. The number of nodules produced by Eco-T + Eco-Rhiz-Soya was not statistically different from that of Eco-Rhiz-Soya but differed significantly ($P<0.05$) from that of the control, Eco-T and the urea treatments. At Akukayili, Eco-T + Eco-Rhiz-Soya produced the highest number (55.6 nodules/plant) of nodules which was about 14% higher than the control. However, this was not significantly different from counts obtained for control, Eco-T, and Eco-Rhiz-Soya treatments but differed significantly from counts obtained for plots treated with urea. It can thus be said that

the combined application of the inoculants produced a relatively higher number of nodules than sole use of the products.

4.3.2 Nodule dry weight of soybean

Nodule dry weights of soybean at both sites are as shown in Table 4.4 At Kpongu, Eco-T + Eco-Rhiz-Soya treated plots produced the highest nodule dry weight (74.6 kg ha^{-1}) which represented about 44% higher biomass than that of the control while the urea treated plots produced the least (31.3 kg ha^{-1}) nodule dry weight. The nodule dry weight recorded for Eco-T + Eco-Rhiz-Soya treatment plot was not significantly different from that recorded for Eco-T, Eco-Rhiz-Soya and the control but was significantly different from that of the urea at Kpongu. Nodule dry weight was increased by 32.8% and 21.6% respectively for Eco-T + Eco-Rhiz-Soya and Eco-Rhiz-Soya, relative to the control. At Akukayili, Eco-T + Eco-Rhiz-Soya produced the highest nodule dry weight of 107.3 kg ha^{-1} while the urea treated plots had the least (40.3 kg ha^{-1}). The nodule dry weight produced by Eco-T + Eco-Rhiz-Soya was not significantly different from that of Eco-Rhiz-Soya, Eco-T and the control but differed from that of the urea treated plots.

Table 4.3. Effect of the treatments on nodule number at Kpongu and Akukayili

Treatment	Kpongu	Akukayili
Control	9.6^{b}	33.9^{ab}
Eco-T	9.4^{b}	28.9^{ab}
Eco-Rhiz-Soya	14.3^{bc}	42.2^{b}
Eco-T + Eco-Rhiz-Soya	16.2^{c}	56.6^{b}
Urea	5.5^{a}	17.1^{a}
CV (%)	11.6	10.80

*Means with the same superscript are not significantly different at P<0.05

Table 4.4. Effect of the treatments on nodule dry weight at Kpongu and Akukayili

Treatment	Kpongu (kg ha^{-1})	Akukayili (kg ha^{-1})
Control	56.1^{b}	74.7^{ab}
Eco-T	55.7^{b}	79.9^{ab}
Eco-Rhiz-Soya	68.2^{b}	90.60^{b}
Eco-T + Eco-Rhiz-Soya	74.6^{b}	107.3^{b}
Urea	31.3^{a}	40.29^{a}
CV (%)	18.1	28.4

*Means with the same superscript are not significantly different at P < 0.05

4.3.3 Shoot biomass yield of soybean

Table 4.5 shows the shoot biomass yield of the treatments at both locations of the study. At Kpongu, the highest shoot biomass yield of 5486 kg ha^{-1} was produced by urea treated plots, although this was not significantly different ($P>0.05$) from biomass produced by Eco-T + Eco-Rhiz-Soya and Eco-Rhiz-Soya but differed significantly ($P<0.05$) from those of the Eco-T and control. Urea application increased the shoot biomass yield by 64.6% relative to the control while Eco-T + Eco-Rhiz-Soya, Eco-Rhiz-Soya and Eco-T inoculation increased the shoot biomass by 45.2%, 37.3% and 8.8% respectively. At Akukayili, Eco-T + Eco-Rhiz-Soya produced the highest shoot biomass yield of 5707 kg ha^{-1} while the least biomass yield of 3620 kg ha^{-1} was produced by the control. The shoot biomass yield produced by Eco-T + Eco-Rhiz-Soya did not differ significantly from that of Eco-Rhiz-Soya and the urea but differed significantly from biomass yield obtained from the Eco-T and the control. A shoot biomass yield increase of 57.6% relative to the control was produced by Eco-T + Eco-Rhiz-Soya whiles increases of 35.4%, 33.7% and 1.2% were recorded for Eco-Rhiz-Soya, urea and Eco-T, respectively.

Table 4.5. Effect of treatments on shoot biomass yield at Kpongu and Akukayili

Treatment	Kpongu (kg ha^{-1})	Akukayili (kg ha^{-1})
Control	3332^{a}	3620^{a}
Eco-T	3624^{ab}	3665^{a}
Eco-Rhiz-Soya	4575^{bc}	4900^{ab}
Eco-T + Eco-Rhiz-Soya	4840^{c}	5707^{b}
Urea	5486^{c}	4840^{ab}
CV (%)	12.7	18.2

*Means with the same superscript are not significantly different at P<0.05 (LSD)

4.3.4 Pod count of soybean

Pod number for the treatments is as shown in Table 4.6. At Kpongu, Eco-T + Eco-Rhiz-Soya produced the highest number of pods (52.0) while the least (33.5) was produced by the control. There was an increase of 13.2% pods produced by the Eco-T + Eco-Rhiz-Soya relative to the control. At Akukayili, Eco-Rhiz-Soya produced the highest (76.8) number of pods while the least (43.5) was produced by the control. The pod number of the Eco-Rhiz-Soya treated plots was not significantly different (P>0.05) from pod numbers produced by Eco-T + Eco-Rhiz-Soya, and the urea but differed significantly (P<0.05) from the control and Eco-T.

4.3.5 Grain yield of soybean

Table 4.7 shows the effect of the treatments on grain yield. Grain yield recorded at Kpongu did not show significant differences among the treatments but significant

differences were observed between the treatments at Akukayili. At Kpongu, the highest grain yield (1800 kg ha^{-1}) was produced by the combined Eco-T + Eco-Rhiz-Soya while a least of 1507 kg ha^{-1} was produced by the control. Eco-T + Eco-Rhiz-Soya inoculation increased soybean seed yield relative to the control by 19.4%. At Akukayili, the highest grain yield of 2471 kg ha^{-1} was produced by Eco-T + Eco-Rhiz-Soya while the least (1724 kg ha^{-1}) was recorded for the control. Eco-T + Eco-Rhiz-Soya inoculation increased grain yield relative to the uninoculated control by 43%.

Table 4.6. Effect of the treatments on pod number at Kpongu and Akukayili

Treatment	Kpongu	Akukayili
Control	33.5^{a}	43.5^{a}
Eco-T	37.8^{ab}	54.7^{ab}
Eco-Rhiz-Soya	49.0^{ab}	76.8^{c}
Eco-T + Eco-Rhiz-Soya	52.0^{b}	71.8^{bc}
Urea	46.83^{ab}	63.3^{bc}
CV (%)	20.0	16.0

*Means with the same superscript are not significantly different at $P < 0.05$

Table 4.7. Effect of the treatments on grain yield at Kpongu and Akukayili

Treatment	Kpongu (kg ha^{-1})	Akukayili (kg ha^{-1})
Control	1507^{a}	1724^{a}
Eco-T	1520^{a}	2085^{ab}
Eco-Rhiz-Soya	1589^{a}	2269^{b}
Eco-T + Eco-Rhiz-Soya	1800^{a}	2471^{b}
Urea	1638^{a}	1743^{a}
CV (%)	13.9	11.8

*Means with the same superscript are not significantly different at P<0.05

4.3.6 Shoot nitrogen and phosphorus uptake of soybean

The effects of the treatments on shoot nitrogen and phosphorus uptake are as shown in Table 4.8. At Kpongu, the highest shoot N (154.7 kg ha^{-1}) was produced by urea while the least (97.4 kg ha^{-1}) was produced by the control. Similarly, the highest (11.69 kg ha^{-1}) shoot P uptake was produced by the urea treated plots with the control recording the least (6.94 kg ha^{-1}). At Akukayili, soybean inoculation with Eco-T + Eco-Rhiz-Soya produced the highest shoot N uptake (177.6 kg ha^{-1}) while the least (98.6 kg ha^{-1}) was produced by the control. The urea produced the highest shoot P uptake (15.08 kg ha^{-1}) whiles the least (7.77 kg ha^{-1}) was by the control. Both shoot N and P were significantly increased by the Eco-T + Eco-Rhiz-Soya inoculation relative to the control.

4.3.7 Total nitrogen and phosphorus uptake in soybean grain

Table 4.9 shows the effect of the treatments on grain nitrogen and phosphorus uptake at both locations of study. At Kpongu, both grain N and P contents did not vary significantly ($P > 0.05$) among the treatments. The Eco-T + Eco-Rhiz-Soya produced the highest grain N (145.9 kg ha^{-1}) and seed P (4.79 kg ha^{-1}) while the least of 111.7 kg ha^{-1} grain N and 4.27 kg ha^{-1} grain P was produced by the control. At Akukayili, grain N content was significantly ($P < 0.05$) influenced by inoculation with Eco-T + Eco-Rhiz-Soya recording the highest (188.6 kg ha^{-1}) while the least (112.3 kg ha^{-1}) was produced by the control. The highest grain P (5.88 kg ha^{-1}) was produced by the Eco-Rhiz-Soya treated plots while the least (4.50 kg ha^{-1}) was obtained by the urea treated plots although, the differences were however not significant ($P > 0.05$).

Table 4.8. Effect of the treatments on soybean shoot nitrogen and phosphorus uptake

Treatment	Kpongu		Akukayili	
	N (kg ha^{-1})	P (kg ha^{-1})	N (kg ha^{-1})	P (kg ha^{-1})
Control	97.4^{a}	6.94^{a}	98.6^{a}	7.77^{a}
Eco-T	110.6^{ab}	7.15^{a}	124.5^{ab}	10.00^{ab}
Eco-Rhiz-Soya	122.7^{ab}	8.52^{ab}	163.4^{b}	10.19^{ab}
Eco-T + Eco-Rhiz-Soya	133.8^{bc}	10.33^{ab}	177.6^{b}	13.13^{bc}
Urea (100 kg N/ha)	154.7^{c}	11.69^{b}	159.4^{b}	15.08^{c}
CV (%)	12.8	22.3	18.7	16.90

*Means with the same superscript are not significantly different at $P < 0.05$

Table 4.9. Effect of the treatments on soybean grain nitrogen and phosphorus uptake

Treatment	Kpongu		Akukayili	
	N (kg ha^{-1})	P (kg ha^{-1})	N (kg ha^{-1})	P (kg ha^{-1})
Control	111.7^{a}	4.29^{a}	112.3^{a}	4.98^{a}
Eco-T	124.0^{a}	4.20^{a}	140.3^{ab}	4.92^{a}
Eco-Rhiz-Soya	137.6^{a}	4.34^{a}	176.4^{b}	5.88^{a}
Eco-T + Eco-Rhiz-Soya	145.9^{a}	4.79^{a}	188.6^{b}	5.81^{a}
Urea (100 kg N/ha)	127.0^{a}	3.91^{a}	151.1^{ab}	4.50^{a}
CV (%)	24.6	16.4	17.3	15.3

*Means with the same superscript are not significantly different at $P < 0.05$.

4.3.8 Relationship between some growth and yield components of soybean

The results for correlation analysis for pod number, biomass yield, grain yield, grain N and P contents and shoot N and P contents are shown in Table 4.10 Grain yield significantly ($P < 0.05$) correlated with pod number, grain P content, grain N and shoot N uptake. Also, with exception of seed P and grain yield, biomass yield correlated significantly with all the tested variables.

Table 4.10. Pearson correlation coefficient of some measured growth and yield parameters

Variables	Biomass yield	Grain yield	Shoot N	Shoot P	Grain N	Grain P	Pod number
Biomass yield	1						
Grain yield	0.30472	1					
Shoot N	0.85037*	0.51168*	1				
Shoot P	0.71673*	-0.0871	0.55055*	1			
Grain N	0.40134*	0.79974*	0.53868*	0.10899	1		
Grain P	0.04432	0.82309*	0.32817	-0.30261	0.67314*	1	
Pod number	0.53452*	0.71334*	0.61104*	0.11836	0.6701*	0.49529*	1

(*) significant at $P < 0.05$

4.4. Maize experiment

4.4.1 Maize biomass yield

Maize biomass yield did not show significant differences among the treatments at both locations of the study (Table 4.11). However, at Akukayili, the 100% NPK (RR) produced the highest stover yield (5681 kg ha^{-1}) which was 19.7 % higher than that of the control. Similarly, at Kpongu the 100% NPK (RR) produced the highest biomass yield of 5406 kg ha^{-1} which was 24% higher than the 4375 kg ha^{-1} produced by the control treatment.

4.4.2 Maize grain yield

Table 4.12 shows the effect of the treatments on maize grain yield at both locations of study. Maize grain yield did not show significant differences among the treatments

at both locations of the study. However, the highest grain yield was produced by the 100% NPK (RR) while the least was by the control at both study sites. Yield increase of 32 and 26% over the control were produced by the 100% NPK (RR) plots at Kpongu and Akukayili, respectively.

Table 4.11. Effect of the treatments on maize biomass yield

Treatment	Kpongu (kg ha^{-1})	Akukayili (kg ha^{-1})
Control	4375^{a}	4740^{a}
Eco-T	4442^{a}	5074^{a}
Enrich	4619^{a}	5286^{a}
Eco-T + Enrich	4900-^{a}	5153^{a}
100% NPK (RR)	5406^{a}	5681^{a}
CV(%)	11.2	12.7

*Means with the same superscript are not significantly different at P < 0.05

Table 4.12. Effect of the treatments on maize grain yield

Treatment	Kpongu (kg ha^{-1})	Akukayili (kg ha^{-1})
Control	3039^{a}	3002^{a}
Eco-T	3449^{a}	3277^{a}
Enrich	3548^{a}	3365^{a}
Eco-T + Enrich	3669^{a}	3393^{a}
100% NPK (RR)	4028^{a}	3793^{a}
CV(%)	12.2	13.2

*Means with the same superscript are not significantly different at P < 0.05

4.4.3 Maize shoot nitrogen and phosphorus

The effect of the treatments on shoot N and P uptake are shown in Table 4.13. At both study locations, significant differences were not observed between the treatments with regard to both shoot N and P uptake. However, at Kpongu, the 100% NPK (RR) produced the highest shoot N (49.5 kg ha^{-1}) while the Eco-T treated plots produced the least shoot N (33.2 kg ha^{-1}). Also, the highest shoot P (12.69 kg ha^{-1}) was produced by the 100% NPK (RR) while the least (7.17 $kgha^{-1}$) was produced by Eco-T treated plots. At Akukayili, 100% NPK (RR) produced the highest shoot N uptake (46.2 kg ha^{-1}) while the control produced the least (32.5 kg ha^{-1}). The 100% NPK (RR) produced the highest shoot P uptake (9.45 kg ha^{-1}) and the least of 6.8 kg P ha^{-1} was produced by the control.

4.4.4 Maize grain nitrogen and phosphorus

Table 4.14 shows the effect of the treatments on maize grain N and P for both study sites. At both locations, no significant differences were observed among the treatments for both grain N and P uptake. At Kpongu, the highest grain N uptake (27.86 kg ha^{-1}) and P uptake (11.13 kg ha^{-1}) was produced by the 100% NPK (RR) while the least of 21.6 kg ha^{-1} and 5.86 kg ha^{-1} was respectively produced by the control. At Akukayili, 100% NPK (RR) produced the highest grain N uptake (27.07 kg ha^{-1}) while the control produced the least (19.90 kg ha^{-1}). Similarly, the 100% NPK (RR) produced the highest shoot P (9.18 $kgha^{-1}$) and the control produced the least of 5.13 kg ha^{-1}.

Table 4.13. Effect of the treatments on maize shoots nitrogen and phosphorus content

Treatment	Kpongu		Akukayili	
	N (kg ha^{-1})	P (kg ha^{-1})	N (kg ha^{-1})	P (kg ha^{-1})
Control	33.6^{a}	7.66^{a}	32.5^{a}	6.80^{a}
Eco-T	33.2^{a}	7.17^{a}	38.9^{a}	8.72^{a}
Enrich	42.1^{a}	9.11^{a}	39.6^{a}	8.83^{a}
Eco-T + Enrich	45.7^{a}	8.24^{a}	39.9^{a}	7.39^{a}
100% NPK (RR)	49.5^{a}	12.69^{a}	46.2^{a}	9.45^{a}
CV (%)	22.0	23.6	23.9	23.8

*Means with the same superscript are not significantly different at P < 0.05

Table 4.14. Effect of the treatments on maize grain nitrogen and phosphorus

Treatment	Kpongu		Akukayili	
	N (kg ha^{-1})	P (kg ha^{-1})	N(kg ha^{-1})	P (kg ha^{-1})
Control	21.60^{a}	5.86^{a}	19.90^{a}	5.13^{a}
Eco-T	23.45^{a}	6.64^{a}	21.33^{a}	7.08^{a}
Enrich	24.34^{a}	9.63^{a}	23.89^{a}	7.10^{a}
Eco-T + Enrich	25.21^{a}	7.77^{a}	23.56^{a}	8.19^{a}
100% NPK (RR)	27.86^{a}	11.13^{a}	27.07^{a}	9.18^{a}
CV (%)	9.8	24.0	13.3	22.6

*Means with the same superscript are not significantly different at P < 0.05

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physico-chemical characteristics and rhizobia population of the experimental sites

The properties of the top (0 - 20 cm) soil obtained before treatments application showed that the pH was moderately alkaline at Kpongu and neutral at Akukayili in accordance with the description given by Bruce and Rayment (1982). Organic carbon was low and moderate at Kpongu and Akukayili respectively. The soils at both study sites were low in total nitrogen. Bruce and Rayment (1982) reported that soils with total nitrogen in the range of 0.05 - 0.15% are described as low in N content. Similarly, based on the classification by Holford and Cullis (1985), available P was low and moderate at Kpongu and Akukayili respectively. According to interpretations by Metson (1961), the exchangeable K, Ca and Na were very low at both study locations while exchangeable Mg was moderate at Akukayili but very low at Kpongu. The textures of the soils were sandy loam for Kpongu and loamy sand for Akukayili.

The MPN counts of rhizobia (31.9 and 43.6 cells/g of soil) in the soils used for the study indicated that the indigenous rhizobia were low at both sites (Slattery and Pearce, 2002).

5.2 Quality assessment of the products

The aim of inoculation is to provide high numbers of viable effective strains in the rhizosphere to allow rapid colonization in order to maximize yield potential (Deaker *et al*., 2006)**.** Survival is affected by the initial condition of the cells in the inoculant, particularly the moisture status, age, purity, the initial number, the strain and the type of inoculant. Changes in the physiological and morphological characteristics of cells during the maturation of the inoculant have been shown to affect survival (Lucy et al., 2004). Since Ghana does not have quality standards for inoculants, the quality parameters assessed on the products were compared with the Kenyan standards for biofertilizer specifications. The standards permits a minimum viable cells of 10^8 cells/g of a carrier, pH in the range of 6.5-7.5 and moisture content of 30-40% for *Rhizobia* inoculants whereas the minimum viable cell counts for antagonistic fungi is 2 x 10^6. With reference to the standards, it can thus be said that the quality parameters assessed on the Eco-T and the Eco-Rhiz-Soya met quality standards for inoculants specification.

5.3 Effect of the treatments on nodulation

Soybean inoculation with Eco-T + Eco-Rhiz-Soya did not significantly increase nodulation at Akukayili over the uninoculated control (Table 4.3). However, at Kpongu, Eco-T + Eco-Rhiz-Soya inoculation significantly increased the nodule number over the control. The results further showed that the urea treated plots significantly recorded the least nodule dry weight while the inoculated treatments failed to significantly produce more nodule biomass than the control (Table 4.3).

Katulande (2011) and Albareda *et al*. (2009) reported a significant increase in nodule number as a result of *rhizobia* inoculation but these findings go contrary to the results obtained from this study as Eco – Rhiz - Soya failed to significantly increase the nodule number at both study sites. However, the results of this study were similar to those reported by Okogun *et al*. (2005) and Chemining'wa *et al*. (2007) who reported no significant increase in nodulation following *rhizobia* inoculation. Lack of significant increase in the number of nodules could be attributed to the numbers of indigenous *rhizobia* in the soils of the study sites. Thies *et al*. (1991) reported that great response to inoculation could be achieved if the number of indigenous *rhizobia* population is less than 10 cells/gram of soil; the indigenous *rhizobia* population of 31.9 and 43.6 cells/g number of rhizobia at Kpongu and Akukayili, were higher than the threshold population reported by Thies *et al*. (1991) (Table 4.1). The addition of *Trichoderma* to the *Rhizobia* inoculant enhanced nodulation of the soybean at Kpongu (Table 4.2). Badawi *et al* (2011) reported significant increases in nodulation resulting from co - inoculation of *Bradyrhizobium* and *Trichoderma* in peanut. Improvement in nodulation resulting from co-inoculation of the soybean may be due to the benefits of the soybean – *bradyrhizobia* interaction from growth promoting substances such as auxins, flavonoid - like compounds and siderophores, which enhance root proliferation and provide more infection sites for the *rhizobia* and in synchronism enhancing the survival and activity of microsymbiont in the soybean rhizosphere. This could be the reason why 25% and 13% increase in nodule number was realized at Kpongu and Akukayili respectively. Contrary to reports by Shaban and El-Bramawy (2011) that combined inoculation of *Rhizobium* and *Trichoderma* was effective in increasing growth parameters in chickpea under greenhouse

conditions, soybean nodule dry weight was not significantly increased by the co - inoculation of the Eco-T and the Eco-Rhiz-Soya. The low number of nodules and nodule dry weight resulting from the Urea treatment (Tables 4.3 and 4.4) may be due to the inorganic N released from the urea, as high concentrations suppress nodulation in legumes (Herridge *et al*., 1984).

5.4. Effect of the treatments on shoot biomass yield of soybean

Soybean biomass yield was significantly increased following Eco-Rhiz-Soya inoculation at both study sites (Table 4.5). Additionally, the Eco-Rhiz-Soya treatment as well as its combination with Eco-T produced biomass that did not significantly differ from the urea treated plots but differed significantly from the control and the sole Eco-T treatments (Table 4.5). This could be due to the essential role of *Bradyrhizobium* in enhancing plant growth and N_2-fixation as reported by Mekhemar *et al*. (2005). In a study by Rabia and Shamin (2012) under greenhouse conditions, dual inoculation of *Trichoderma* and host specific *rhizobia* on Vigna mungo produced biomass that was significantly higher than the fertilizer treatments yet the biomass yield of the host specific *rhizobia* treatment was also significantly higher than the dual inoculation. The findings of Rabia and Shamin (2012) go contrary to the results of this study because Eco-Rhiz-Soya, Eco-Rhiz-Soya + Eco-T and the urea produced shoot biomass yield which were not significantly different from each other. The differences in the results might be due to differences in crops, *Rhizobia* and *Trichoderma* strains used as well as the rate of nitrogen applied.

5.5 Effect of treatments on grain yield of soybean

Soybean grain yield was significantly influenced by the treatments at Akukayili (Table 4.7). Seneviratne *et al*. (2000), Albareda *et al*. (2009) and Katulande (2011) reported significant positive responses in grain yield to inoculation confirming the results obtained at Akukayili. Co - inoculation of *Bradyrhizobium* and *Trichoderma* improved the grain yield of the soybean compared to individual use of the products (Table 4.7). This improvement could be attributed to the complementary effects elucidated by the organisms to enhance the N_2 - fixation performance, as well as nutrient availability and uptake from soil and a healthy rhizosphere, which results in the production of substances like hormones, siderophores, phosphate solubilization and improvement of nutrients and water uptake. Similar studies where dual applications of *Bradyrhizobium* and *Trichoderma* significantly increased yield have been reported by Badawi *et al*. (2012) and Verma *et al*. (2010). Variable differences in grain yield were obtained at both locations of the study confirming the findings of Date (2000) who reported that response to inoculation is highly variable and site specific and that rhizobia inoculation does not always elicit desired results (Chemining'wa *et al*., 2007). Lack of response to inoculation has been attributed to the number and effectiveness of the native *rhizobia* (Singleton and Tavares, 1986; Thies *et al*., 1991), nitrogen availability (Keyser and Li, 1992), pH (Blamey *et al*., 1983) and climatic factors such as moisture, temperature and light (Keyser and Li, 1992). Although both study sites were low in total nitrogen, indigenous population of *rhizobia* and the pH were optimal for *rhizobia* - legume symbiosis (Table 4.1) and therefore, the differences in yield trends at the study sites could be attributed to differences in climate of the study sites.

Grain yield produced by the urea fertilized plots did not differ from that of the Eco-Rhiz-Soya and its combination with Eco-T at Kpongu but at Akukayili, the yield of the urea fertilized plots were significantly lower than that of the sole use of the Eco-Rhiz-Soya and its combination with the Eco-T. This confirms a report by Abayomi et al. (2008) that 24% reduction in yield was obtained following N, P and K application. On the contrary, Thies *et al.* (1991) reported a significantly higher yield due to nitrogen fertilizer application than inoculation.

5.6 Effect of treatments on shoot and grain nitrogen and phosphorus of soybean

Soybean inoculation with the combined Eco-T + Eco-Rhiz-Soya significantly increased the shoot N content at both study sites and shoot P content at Akukayili, while the shoot P content at Kpongu was not significantly increased by inoculation (Table 4.8). Additionally, grain N content was significantly influenced by the combined Eco-T + Eco-Rhiz-Soya at Akukayili but not at Kpongu while grain content P did not differ significantly among the treatments at both study sites (Table 4.9). Badawi *et al*. (2011) reported significant increases in shoot N following dual inoculation of *Bradyrhizobium spp* and *T. harzianum* on peanut and the results of shoot N content from this study agree to the findings of these investigators. These results confirm that co-inoculation of soybean with *Bradyrhizobium* and *Trichoderma* could result in pronounced accumulation of nitrogen in shoot. Hence, the results emphasized the key role of co-inoculation in the improvement of biological nitrogen fixation by soybean–*bradyrhizobia* symbiosis and the enhanced absorption of nutrients from the surrounding environment.

On grain P content, Okogun *et al*. (2005) reported significant increases following *rhizobia* inoculation. However, the results obtained from this study is in contrast to their report since grain P content at both study sites were not significantly influenced by the various treatments (Table 4.9).

5.7 Effect of treatments on grain yield of maize

Maize grain yield did not differ significantly among the treatments at both study sites (Table 4.12). This implies that chemical fertilizers could be supplemented with the Eco-T and the Enrich to obviate the harmful effects of using inorganic fertilizers alone. Patil *et al*. (2012) evaluated the effect of liquid formulations of *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* on wheat under field conditions and reported significant improvement in grain yield. This is contrary to the results of this study as the Enrich treated plots did not produce yield that differed significantly from the control. The inability of the Enrich and Eco-T treated plots to significantly increase the grain yield may be due to the basal NPK received by the control which might have been sufficient under the growing conditions. Although grain yield was not significantly increased by the inoculants, yield increases of 13% and 17% were produced by the combined Eco-T + Enrich at Akukayili and Kpongu, respectively compared to increases of 26.3% and 28% produced by the 100% NPK (RR)

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The quality of the microbial products used for the study (Eco-Rhiz-Soya and the Eco – T) met the quality standards for assessing biofertilizers. Viable cell count, pH and moisture content of 1.68 x 10^8, 7.2 and 34% respectively were recorded for Eco-Rhiz-Soya while 5.7 x 10^7, 6.5 and 0.8% were recorded for Eco-T.

The results showed that dual inoculation of soybean with the Eco - Rhiz-Soya and Eco – T significantly increased nodule number by 69% and 67% at Kpongu and Akukayili respectively. Furthermore, shoot biomass yield increased by 45% and 58% at Kpongu and Akukayili respectively. Grain yield of soybean inoculated with Eco-Rhiz-Soya + Eco-T increased by 19% and 43% over the control at Kpongu and Akukayili respectively. Soybean shoot N uptake was significantly increased ($P<0.05$) by Eco-Rhiz-Soya + Eco-T at both sites of study whereas shoot P uptake was only significantly increased at Akukayili. The soybean inoculation treatments had no significant effect on grain P uptake whiles grain N was significantly increased at Akukayili only. It can thus be deduced that exploitation of co – inoculation of soybean with *Bradyrhizobia* and *Trichoderma* through the use of Eco – Rhiz – Soya and Eco – T respectively could be an efficient strategy for enhancing productivity in soybean.

The study further showed that the Enrich and Eco-T used for maize had no significant effect on the maize grain yield, stover yield, shoot and grain N and P at both study sites.

6.2 RECOMMENDATIONS

Owing to the results obtained from this study, further studies need to be conducted using different varieties of crops. Also, further studies need to be conducted in different agro-ecological zones to ascertain the effectiveness of the products.

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APPENDICES

Appendix 1. Constituent of Potato Dextrose Agar

Material (g/l)	Quantity
Potato	200
Dextrose	20
Agar	20

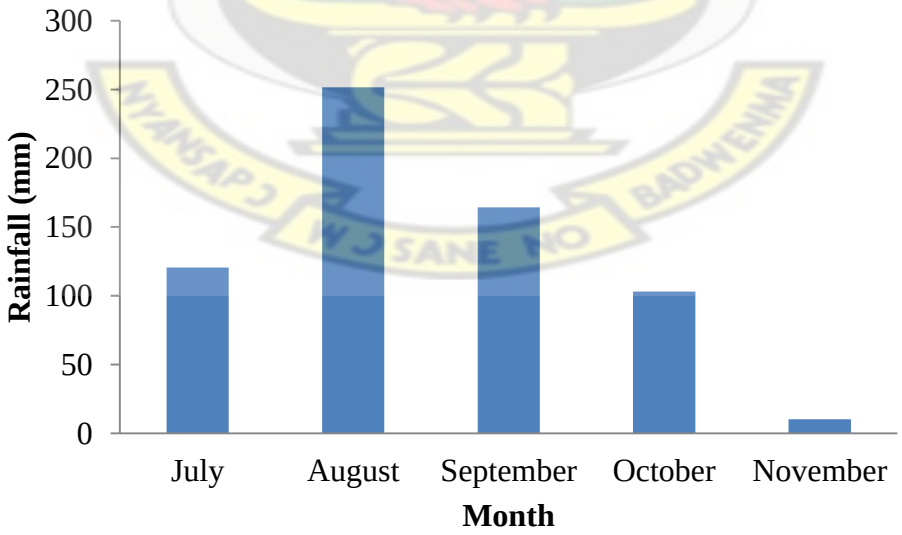
Appendix 2. Constituent of Yeast Extract Mannitol Agar

Chemical (g/l)	Quantity
K_2HPO_4	0.5
NaCl	0.2
$MgSO_4.7H_2O$	0.1
Mannitol	10
Yeast extract	0.5
Agar	15

Appendix 3. Broughton and Dilworth N-free Plant Nutrient Solution

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

Appendix 4. Rainfall distribution at Kpongu



Appendix 5. Rainfall distribution at Akukayili