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EVALUATION OF SOME MICROBIAL INOCULANTS AND MICRONUTRIENT FERTILIZERS ON THE PERFORMANCE OF SOYBEAN AND MAIZE IN THE NORTHERN SAVANNAH ZONES OF GHANA

BY

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BSc. Agriculture (Hons.)

JULY, 2014

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(BSc. AGRICULTURE)

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

MASTER OF SCIENCE

IN

SOIL SCIENCE (MICROBIOLOGY OPTION)

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DECLARATION

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

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ABSTRACT

Three on - station trials were conducted at the experimental fields of CSIR - Savannah Agricultural Research Institute (SARI), to ascertain and confirm the effectiveness of some commercial microbial inoculants and micronutrient fertilizers for improvement of soybean and maize productivity in the Guinea and Sudan savannah agro - ecological zones of Ghana. Teprosyn Mo and Legumefix products were used as treatments for the soybean trial while Teprosyn Zn/P and Eco - T were used for the maize trial. All the treatments were laid out in a Randomised Complete Block Design (RCBD) with three replications each. A significant ($P < 0.05$) response to Legumefix on soybean nodule dry weight was observed in Kpongu and Manga but not Nyankpala. None of the treatments for soybean and maize significantly ($P < 0.05$) increased shoot dry matter. Teprosyn Mo + Legumefix, Legumefix and Teprosyn Mo increased soybean grain yield by 205.62%, 135.54% and 110.24% respectively over the control in Manga. In Nyankpala, the application of Legumefix and Teprosyn Mo + Legumefix increased soybean grain yield significantly by 22.43% and 42.10% respectively relative to the control while no significant response was observed among treatments on soybean grain yield in Kpongu. The combined application of Teprosyn Mo + Legumefix was the most profitable (VCR = 2.65) among the treatments in Manga.

Teprosyn Zn/P, Eco - T and Teprosyn Zn/P + Eco - T treatments had no significant ($P < 0.05$) effect on plant height, yield and N and P nutrient uptake of maize in all the study locations. However, the return on investments from the products used for maize showed that Eco - T and Teprosyn Zn/P + Eco - T were the most profitable (VCR > 2) in both Manga and Nyankpala.

DEDICATION

This dissertation is dedicated with love and gratitude to Dr. Joseph Sarkodie-Addo.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDICES

CHAPTER ONE

1.0 INTRODUCTION

Increased global agricultural production over the years has been as a result of a bundle of "Green Revolution" technologies, namely improved varieties, increased fertilizer and pesticide use, and improvements in water supply through irrigation (Wissuwa *et al*., 2009). Although there is a growing need for chemical fertilizers to enhance crop yields (Morris *et al*., 2007; World Bank, 2008) due to the inherently poor nature of most soils in sub - Saharan regions, about 60% of African smallholder farmers are unable to afford the high prices of chemical fertilizers (Yanggen *et al*., 1998). As a result, the continuous cultivation without replenishment has led to further decline of nutrients especially nitrogen (N) and phosphorus (P) from the soil pool (Manyong *et al*., 2001) resulting in low crop yields which pose a serious threat to food security in the continent. Moreover, the production and intensive application of chemical fertilizers in agriculture has led to a series of environmental problems (Richter and Roelcke, 2000; Zhu *et al*., 2000) and damage to the ecological state of agricultural systems (Villarreal *et al*., 2003).

In order to increase food production in Africa, efforts are now geared towards approaches of internal and renewable resources and the use of effective management practices (Wani and Lee 1996; Sturz *et al*., 2000) to increase food production without compromising on sustainable agriculture. This has led to the promotion of commercial biological and chemical products intended to restore or enhance the fertility and organic matter content of soils in an eco - friendly manner (Laditi *et al*., 2012). Some of these products include, (among others), seed coatings, leaf sprays,

N inhibitors and conditioners with humic acids which aim at improved nutrient use efficiency through specialized formulations (Zirhahwakuhingwa, 2012).

In Ghana, soybean and maize are widely cultivated in the Northern savannah zones, where it is well adapted. However, production of these crops are faced with a number of constraints including low soil fertility, irregular rainfall pattern, drought, inadequate access to certified seed and poor agronomic practices which result in poor yields. Evidently, commercial products hold enormous prospects in improved and sustainable plant production (Berg *et al*., 2010; Adeseyome and Egamberdieva, 2013). However, knowledge of BNF technologies, research adoption and utilisation of microbial inoculant is relatively new and low in Ghana and its potential benefits remain largely untapped by smallholder farmers. Furthermore, some commercial biological products exist in the market, but their effects on crop yield fluctuate from crop to crop, site to site, and from season to season, depending on the survival of the introduced microorganism on seed or roots or in the soil (Nowak 1998; Khalid *et al*., 2004). Although, most of these products claim increase in crop productivity, their true benefits cannot be vouched for (Simiyu *et al*., 2013). Furthermore, information regarding the use of microbial and micronutrient products on improving soybean and maize production in Ghana is scanty. Preliminary trials conducted in Kenya, Nigeria and Ethiopia to test some commercial microbial and micronutrient products showed varied responses on growth and yield parameters measured.

The general objective of this study was therefore to assess the effectiveness of some commercial microbial inoculants and micronutrient fertilizers for improved soybean and maize productivity in smallholder farms in the Northern savannah zones of Ghana.

The specific objectives were therefore to:

a. evaluate the effect of microbial inoculants and micronutrient fertilizers on the growth and yield of soybean and maize in two different agro - ecologies.
b. determine the effects of microbial inoculants and micronutrient fertilizers in enhancing plant nutrient uptake (N, P).
c. to evaluate the economic benefits of the selected commercial microbial and chemical products.

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

a. the use of commercial microbial inoculants and micronutrient fertilizers have no effect on the growth and yield of soybean and maize.
b. commercial microbial inoculants and micronutrient fertilizers do not increase plant nutrient uptake (N, P).
c. the use of commercial microbial and chemical products by smallholder farmers in the Northern Savannah zones of Ghana is not economically profitable.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Beneficial soil microorganisms

Sound and ecologically compatible environmentally friendly techniques in agriculture, capable of providing adequate nourishment/sustenance for the increasing human population and of improving the quality and quantity of certain agricultural products have become a growing need worldwide (Anna *et al*., 2012). For these reasons, the application of beneficial microorganisms or reduction in the use of chemicals has so far attracted much interest (Dobbelaere *et al*., 2003; Burdman *et al*., 2000). Beneficial microorganisms such as diazotrophs bacteria, biological control agents (BCAs), plant growth promoting rhizobacteria (PGPRs) and fungi (PGPFs) play a key role in this major challenge, as they fulfil important ecosystem functions for plants and soil (Raaijmakers *et al*., 2009; Hermosa *et al*., 2011). Conversely, over the past 150 years, bacteria and fungi have been repeatedly demonstrated to promote plant growth and suppress plant pathogens, this knowledge is yet to be extensively exploited in agricultural biotechnology (Berg, 2009).

2.1.1 The nature and symbiotic relationship of rhizobia with legumes

Rhizobium is a general name given to phylogenetically diverse group of soil bacteria that form nitrogen fixing symbioses with leguminous plants with the help of the nitrogenase enzyme to convert atmospheric N to ammonia (FAO, 2006).

Biological nitrogen fixation (BNF) is seen as a cheap way to get renewable nitrogen in agriculture as it uses photosynthetically produced energy and is environmentally cleaner (Albareda *et al*., 2009).

The BNF process involves a series of events that are necessary for symbiotic nitrogen fixation which includes pre - infection, root colonization, root adhesion, hair branching, hair curling, infection, nodule initiation, bacterial release, bacteroid development, nodule function, nitrogen fixation, complementary functions and nodule persistence (Gage, 2004). In agriculture, the best known and most exploited symbiotic N_2 - fixing bacteria are those belonging to the family *Rhizobiaceae* (Rhizobia) and include the following genera: *Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium, Mesorhizobium, and Allorhizobium* (Graham and Vance, 2000; Farrand *et al*., 2003)

Soybean (*Glycine max.* L.) is mainly nodulated by *Bradyrhizobium japonicum* and *B. elkanii* (Barros de Carvalho, 2013) but most soils usually lack these specific strains. It is therefore important to inoculate seeds with relevant strains of bacteria before sowing, especially if the crop is to be grown for the first time on the land. Inoculation of soybean seeds with the appropriate rhizobia strains provides high numbers of viable effective rhizobia around the root region which allows rapid colonization and nodulation (Deaker *et al*., 2012; Lemus, 2012).

Many research experiments have clearly justified the positive effect of inoculation on soybean nodulation and consequently on growth (Kadiata *et al*., 2012; Solomon *et al*., 2012; Yamakawa and Saeki, 2013). A study by Mburu *et al*. (2011), on evaluation of biological commercial inoculants on soybean production in Bungoma County, Kenya concluded that the use of rhizobia inoculants on soybean production resulted in 40% increase in grain yield when compared with the control. According to Tahir *et al*. (2009), BNF capacity of legumes is a vital process for sustaining crop land management and is an effective and efficient source of nitrogen supply to plants under favourable atmospheric and environmental conditions. According to

Chen (2008), Legumefix which is a rhizobia inoculant helps to boost the natural population of beneficial nitrogen - fixing bacteria to form effective nodules that are responsible for effective BNF. However, a study by Ulzen (2013) on assessing the need for inoculation of soybean and cowpea at Tono in the Kassena Nankana District of the Upper East region of Ghana concluded that Legumefix inoculation had no significant effect on shoot biomass, pod number and grain yield of soybean and cowpea.

2.1.2 Factors limiting BNF and rhizobia inoculation

Under tropical conditions, soybean has to be inoculated with effective rhizobia strains in order to successfully fix nitrogen as it's not an indigenous crop to Africa (Okogun and Sanginga, 2003). However, the introduction of superior strains of rhizobia into the soil does not always guarantee a higher BNF and consequently increased yield (Lupwayi *et al*., 2000). The process is generally influenced by biotic and abiotic factors that affect the survival of the introduced strain, infectivity, and BNF efficiency.

2.1.2.1 Biotic factors influencing BNF and rhizobia inoculation

The genetic potential of the rhizobia and how it interacts with components of the environment ultimately determines the success of a BNF practice with legume inoculation. According to Adamovich and Klasens (2001), the process depends on the occurrence and survival of rhizobia in soils and also on their effectiveness. Furthermore, soybean response to inoculation is regulated and influenced by the number and quality of indigenous rhizobia (Abaidoo and Woomer, 2008).

2.1.2.1.1 The presence and quality of indigenous rhizobia population

Resident soil rhizobia, including indigenous rhizobia and those naturalized through past inoculation, have been proven to compete for nodule occupancy with introduced rhizobia strains, impacting negatively on inoculation success (Denton *et al*., 2002) especially when large populations of infective rhizobia are present in the soil (Thies *et al*., 1991). Many soils contain large number of indigenous rhizobia that are not powerful for nitrogen fixation, but have good compatibility with environment, and have highly competitive ability (Khavazi *et al*., 2005).

Several researches have shown that tropical rhizobia are diverse with sub - groups of varied symbiotic specificity and effectiveness (Thies *et al*., 1991; Mpepereki *et al*., 1996) which poses a barrier to the benefits of inoculation (Shamseldin and Werner, 2004). However, in practice, the presence of a large indigenous population of compatible rhizobia does not necessarily preclude response to inoculation, provided the introduced inoculant rhizobia strains are competitive and highly effective (Giller, 2001; Osunde *et al*., 2003). This suggests that, by increasing the numbers of inoculant strains, it is possible to increase the number of nodules they occupy (Danso, 1990) and increase the BNF potential. Hence, the population size of effective indigenous soil rhizobia is a reliable index for the capacity of a legume crop to derive N through BNF and to determine whether or not the legume will respond to added rhizobia or fertilizer N (Thies *et al*., 1991; Turk *et al*., 1993).

2.1.2.1.2 Biological agents

A study by Mulongoy (1992), reported that, biological constraints that affect rhizobia inoculation and BNF directly or indirectly include defoliation (e.g., pruning and lopping) which decreases the photosynthetic ability of legumes, which impairs N_2 fixation and can lead to nodule decay. Intercropping legumes with non - leguminous

crops can result in competition for water and nutrients and negatively affect N_2 fixation and also interference of insects and nematodes with nodule formation, development, and function and thereby affect the amount of N_2 fixed.

2.1.3 Abiotic factors influencing BNF and rhizobia inoculation

The amount of N fixed by the legume - rhizobia association varies considerably with environmental and edaphic factors which affect the host - plant (macrosymbiont) and the rhizobium (microsymbiont). The major factors include soil temperature, moisture, soil pH (Rayar, 2000), available soil N, phosphorus deficiency and various microelements like Cu, Mo, Co, B which are necessary for atmospheric nitrogen (N_2) fixation (Harold *et al*., 1992).

2.1.3.1 Temperature

Soil temperature generally inhibits legume BNF through its effect on nodulation, nodule establishment and nitrogenase activity when it is either too high or too low (Whitehead, 1995) but differs between species and varieties. According to Michiels *et al*. (1994), high soil temperature reduces the survival of rhizobia in soil and inhibits nodulation and N_2 - fixation. The optimal soil temperature for fixation should range between 25 ℃ to 30 ℃ (Bohner, 2009). However, a continually cool root zone temperature can significantly delay the onset of nitrogen fixation compared to an optimum soil temperature (Abendroth *et al*., 2006).

2.1.3.2 Moisture and drought

The agronomic definition of drought is a temporary or durable change in the plant water status, affecting its functioning, and it is related to a decrease in soil water content (Katerji *et al*., 2011). It is the most important environmental factor contributing to crop yield loss, including soybean where symbiotic fixation of

atmospheric nitrogen (N_2) is sensitive to even modest soil water deficits (Sinclair *et al*., 2007), although they have been suggested as drought tolerant and adapted in terms of plant survival (Serraj *et al*., 1999). This is because legumes are more sensitive to osmotic stress than their microsymbiont rhizobia (Zahran, 1999). Ladrera *et al*. (2007) proposed oxygen limitation, carbon shortage, and regulation by nitrogen metabolism to be the three major factors involved in drought effects on BNF. Limiting N_2 fixation with soil dryness causes yield reductions due to inadequate N for protein production, which is a critical seed product (Sinclair *et al*., 2007).

A study by Pimratch *et al*. (2008) on soybean showed that, lines with high nitrogen fixation at pod filling stage had higher yield under water stress than those having low nitrogen fixation. Also, soil moisture stress affects the soil microbial community in two ways: first, reducing the number of water - filled pores and the thickness of water films around soil particles; and second, increasing salt concentration in the soil solution (Kantar *et al*., 2010). Water film characteristics influence the movement of motile bacteria, like rhizobia, and in general, the distribution of soil biota (Tate, 2000). These factors are altered by drought, affecting not only rhizobia activity and distribution in the soil, but also diversity of rhizobia populations (Mnasri *et al*., 2007).

Several researches have reported on the reduced viability of almost all rhizobia species ability to establish symbiosis with crop legumes under drought or much lower under extreme desiccated conditions (Tate, 1995). A study by Kantar *et al*. (2010) on efficient biological nitrogen fixation under warming climates concluded that, rhizobia are in competition for nutrients with other soil microorganisms and, at the same time, exposed to biotic and abiotic factors. Among the latter drought

represents one of the most harmful constraints due to its effect on soil physical and biological characteristics. Drought reduces not only the growth and diversity of free - living rhizobacteria, but also negatively influences plant development. Both factors determine the establishment and activity of an effective N_2 - fixing symbiotic interaction.

2.1.3.3 Soil pH

Soil pH is one of the most important factors influencing legume and rhizobium symbiosis as it reduces the survival of rhizobia in soil, inhibits nodulation and N_2 - fixation. Moyin-Jesu (2008) reported that at lower pH, Fe and Al oxides and their hydroxides react with the available phosphorus and form complexes that are insoluble in soil solution. Lapinskas (2004), noted that rhizobia is highly sensitive to acid pH and soluble Al when the critical soil pH is 4.8 - 5.0, a pH less than 4.6 inhibits their activity but optimum soil pH for nodulation and yield for soybean is between 6.2 and 6.8 (Lapinskas, 1998). Legumes and rhizobium form efficient symbiosis and fix high amounts of biological nitrogen when soil pH is no less than 5.6 – 6.1 (Lapinskas, 1998). On the contrary, under very acidic soil (pH < 4), nitrogen fixation can be reduced up to 30 percent (Abendroth *et al*., 2006). A study by Ambrazaitienė (2003), Hartwig and Soussana, (2001) showed that soil acidification inhibited the root - hair infection process and nodulation because it disrupts the communication process.

Additionally, a research by Lapinskas (2008) on biological nitrogen fixation in acid soils of Lithuania showed that soil acidity is the decisive factor in the distribution and symbiotic efficiency of *Rhizobium leguminosarum* bv. *trifolii*, *Rhizobium leguminosarum* bv. *viciae*, *Sinorhizobium meliloti* and *Rhizobium galegae*. *Rhizobium leguminosarum* bv. *trifolii* was mostly distributed and tolerant on acid

soils. In a slightly acid soil, the average amount of rhizobia was 540.0 × 10^3 cfu g^{-1} soil. In highly acid and medium acid soil (pH KCl 4.1 - 5.0) *Rhizobium galegae* and *Synorhizobium meliloti* were not found (Lapinskas, 2008).

2.1.3.4 Soil available nitrogen

Several researches have shown that high soil N level in the root zone inhibits legume nodulation, nodule establishment and nitrogenase activity (Abdel-Wahab *et al*., 1996; Arreseigor *et al*., 1997) as it costs less energy for legumes to take up N from soil than fix N biologically from the atmosphere (Cannell and Thornley, 2000). However, the severity of N fixation inhibition by soil mineral N increases with soil mineral N content (Macduff *et al*., 1996). It has been reported that a certain concentration of mineral N in the root zone, defined as "starter N", stimulates nodule establishment and N fixation compared to non - mineral N in some circumstances and the concentrations of "starter N" that stimulate legume BNF vary widely with cultivar and growth conditions but are normally less than 4 m*M* for ammonium (NH_4^+) and less than 2 m*M* for nitrate (NO_3^-) (Gan *et al*., 2004). This is because according to Keyser and Li (1992), 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.

2.1.3.5 Phosphorus deficiency

Phosphorus (P) is an essential element that significantly affects plant growth and metabolism (Abel *et al*., 2002). It enhances the photosynthetic rate, enzymatic activity, energy transfer, root development, uptake and transfer of other nutrients, nodulation and N_2 - fixation by symbiotic bacteria, water use efficiency, reproductive growth and maturation, seed number, seed size, and seed germination

(Synder, 2000). It has been observed that, P is a limiting factor for N fixers, particularly for grain legumes, which may lead to reductions in fixation greater than in plant growth as compared to other nutrients (Ballachanda and Kashchandra, 2007). Its deficiency in soybean results in poor nodulation, reduced seed viability, and decreased percentage of fully developed seeds (Bishnoi *et al*., 2007). However, P is the most limiting nutrient in most soils after nitrogen (Cao *et al*., 2002; Han *et al*., 2005; Wang *et al*., 2005; Jiang *et al*., 2006) as it is easily fixed in mineral or organic forms that are unavailable to plants (Marschner, 1995). Under P shortage conditions, legumes may lose the distinct advantage of an unlimited source of symbiotic N (Sulieman *et al*., 2008) while increased supply of phosphorus results in abundant nodules (Gowariker *et al*., 2009). Thus, a decrease in phosphorus requirement mainly reflects on reducing the leaf area, shoot dry matter and phosphorus content in shoot and root (Waswa, 2013). However, root growth has inverse relations with P because under deficiency, root growth is stimulated as a strategy to improve the phosphorus nutrition (Ahmed, 2007).

Symbiotic N_2 fixation has a higher P requirement for maximum activity than growth supported by nitrate assimilation because of high energy requirements in the reduction of atmospheric N_2 by the nitrogenase system and P deficiency conditions also result in reduced nodule number and mass (Rotaru and Sinclair, 2009). On the other hand, the effect of low phosphorus on nodule formation and functions may be due to low exchange between shoot and nodules, which is the proper way to decrease leaf photosynthesis with the decline in the available phosphorus (Tsvetkova and Georgiev, 2003).

Hoque and Haq (1994) also observed similar results when they treated several legumes with Rhizobium and phosphorus and they found an increase in the number of nodules and maximum growth features with inoculation and phosphorus.

2.2 Maize response to fungal inoculation

Fungi of the genus *Trichoderma* are free - living opportunistic root colonizing fungal plant symbionts (Harman, 2004) that induce numerous changes in plant gene expression and physiology (Shoresh and Harman 2010). Among the phenotypic changes are increased systemic resistance to plant diseases (Alfano *et al*., 2007; Djonovic *et al*., 2006) increased growth of plants and roots, including an increase in fertilizer use efficiency and uptake (Shoresh and Harman 2008; Yedida *et al*., 2001) and a generalized increase in resistance to abiotic stresses (Yildirim *et al*., 2006). The interaction of *Trichoderma* strains with plants are well documented (Harman *et al*., 2004; Benítez *et al*., 2004). According to Harman *et al*. (2004), *Trichoderma spp*. increased nutrient uptake through enhanced root growth or promoted availability of necessary nutrients leading to growth of the plants. Also, *Trichoderma* reduced the concentrations of substances in soil that are inhibitory to plant growth (Wang *et al*., 2000).

Altomare *et al*. (1999), also reported that *T. harzianum* 1295 - 22 could improve nitrogen use efficiency and could solubilize a number of poorly soluble nutrients, such as Mn^{4+}, Fe^{3+} and Cu^{2+} leading to plant growth and development by reducing oxidized metallic ions to increase their solubility and also produces siderophores that chelate iron (Altomare *et al*., 1999). Root colonization by *Trichoderma spp.* also enhances root and shoot development, crop productivity, resistance to abiotic stresses, and nutrients uptake (Azarmi *et al*., 2011). Other direct effects on plants include increased leaf greenness that is probably related to increased photosynthetic

rate (Harman, 2000; Harman and Shoresh, 2007) and increased percentages of germination and rates of germination of seeds (Björkman *et al*., 1998). The mechanisms of *Trichoderma* - plant interaction involve colonization and infection of the outer layer roots by the *Trichoderma* strain (Yedidia *et al*., 1999; Yedidia *et al*., 2000). Once infection occurs, a zone of chemical interaction develops at these sites within which the *Trichoderma* hyphae are walled off by the plant but are not killed (Harman *et al*., 2004a; Harman and Shoresh, 2007). Chemical elicitors from *Trichoderma* produced by the walled off hyphae interact with putative plant receptors (Harman *et al*., 2004a; Harman and Shoresh, 2007).

Several recent reports indicate that the fungi enhances tolerance to abiotic stresses during plant growth (Yildirim *et al*., 2006; Bae *et al*., 2009), in part due to improved root growth, improvement in water - holding capacity of plants (Harman, 2000), or enhancement in nutrient uptake (i.e., potassium) (Yildirim *et al*., 2006); whereas, in the absence of stress, plant growth may (Bae *et al*., 2009) or may not (Yildirim *et al*., 2006) be enhanced. However, in the interaction between T - 22 and maize, root and shoot growths were increased in both sterilized and nonsterilized soils and in the presence of soil fungicides (Harman *et al*., 2004). Eco - T is a wettable powder formulation of the fungus *Trichoderma harzianum* strain produced by Plant Health Products of USA (www.planthealth.co.za).

2.3 The role of micronutrients in plant growth and development

Plants require at least 16 essential elements for optimal growth and development. Nutrients requires in largest amounts are termed macronutrients of which nitrogen, phosphorus, and potassium are needed in relatively large amounts. The micronutrients consists of boron, copper, chlorine, iron, manganese, molybdenum, and zinc (Brady and Weil, 2002) which occur in very small amounts in both soils and

plants. However, they are of equal importance as the macronutrients because if any element is lacking in the soil or not adequately balanced with other nutrients, growth suppression or even complete inhibition may result (Mengel *et al*., 2001). Through their involvement in various enzymes and other physiologically active molecules, these micronutrients are important for gene expression, biosynthesis of proteins, nucleic acids, growth substances, chlorophyll and secondary metabolites and metabolism of carbohydrates and lipids (Gao *et al*., 2008). Physical and chemical characteristics such as soil texture, soil organic matter and soil pH affect the availability and uptake of micronutrients (Mckenzie, 2001).

2.3.1 Effect of molybdenum in legume production

Molybdenum (Mo) is a trace element required for growth of most biological organisms including plants and animals and a transition element, which can exist in several oxidation states (Brent *et al*., 2005). The functions of Mo in leguminous plants include nitrate reduction, nodulation, nitrogen fixation and general metabolism (Togay *et al*., 2008). A study by Hristozkova *et al*. (2006) showed that Mo was required for normal plant growth and reduction supply to the growth medium decreased activities of nitrate reductase and glutamine synthetase involved at initial steps of nitrate assimilation. Hale *et al*. (2001) stated that Mo is a component of some bacterial nitrogenase and, therefore, is especially important for plants that live in symbiosis with nitrogen - fixing bacteria and also essential for nitrate reductase and nitrogenase enzyme activity (Westermann, 2005). A multi - locational trial by Johansen *et al*. (2006b) at farmers' fields in Bangladesh showed that, the efficiency of seed treatment with Mo may be further enhanced by adding rhizobium as yield increases were 37% - 90% over the untreated control.

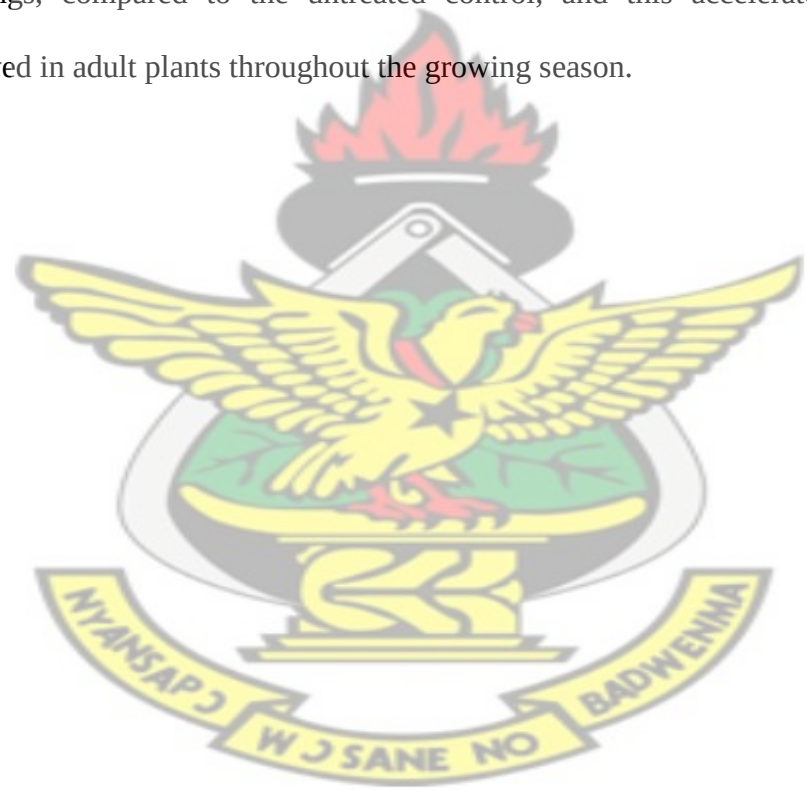
Experiments with soybean and common bean have shown that Mo fertilization can enhance the nitrogen fixing symbiosis through increased nitrogenase activity rates and larger nodules (Vieira *et al*., 1998). Teprosyn Mo a product of Yara Ltd., UK. It is a micronutrient fertilizer which provides vital concentrated nutrition for vigorous seedling emergence, improves seedling resistance to drought and contributes to overall plant health (www.yara.co.uk).

2.3.2 Effect of zinc in cereal production

Soil Zn deficiency is a worldwide problem in the crop production, affecting the growth of the crops in over 50% of agricultural lands (Alloway, 2001). Its deficiency is closely associated with 80% grain yield reduction, beside reduction in grain Zn content and other nutrients in cereal crops (Cakmak *et al*., 1998). Factors affecting soil zinc availability are high soil pH values (Shuman, 1980), carbonate content (Karimian, 1995), and organic matter, further soil texture and sorption capacity as well as the mainly studied zinc interaction with other elements such as iron, copper and manganese (Marschner, 1986), and especially phosphorus (Sankhyan and Sharma 1997, Deusoza *et al*., 1998). Orabi and Abdel-Aziz (1982), reported a positive relationship between Zn and P as the application of phosphorus to soil increased the uptake of Zn as well as the Zn - content in maize plants by exerting many and varied functions in plant metabolism and hence inadequate phosphate supply to the plant seriously affects numerous metabolic processes.

Seed treatment with Teprosyn Zn/P compound has been recommended by the manufacturer as an option for manipulating seedling growth via increased nutrient use efficiency as well as physiological processes during later growth stages with the main attribute of establishing a strong root system during seedling growth and early crop development (Richardson, 2007).

A study by Richardson (2007) concluded that Teprosyn Zn/P seed dressing of two maize cultivars, Dekalb 63-69 and Garst 8581, resulted in enhanced root development and top growth of seedlings leading to more efficient water and nutrient use, as well as drought tolerance. This is believed to be due to enhanced efficiency of nutrient use by plants following seed treatments with Teprosyn Zn/P as it prepared the crop for the coming season thus enabling optimization of their genetic yield potential at harvest (Singh, 2003). In support of these findings, Hunter (2001) reported that pea seed treated with Teprosyn Zn/P resulted in vigorous top growth of seedlings, compared to the untreated control, and this accelerated growth was observed in adult plants throughout the growing season.



2.4 Summary of literature review

Food crop production needs to be increased substantially to reduce hunger and food insecurity in sub-Saharan Africa (SSA). Due to the inherently poor nature of most soils in the region, external inputs such as chemical fertilizers and pesticides are necessary to boost crop production. The problems and concerns of economic constraints and environmental pollution associated with the use of chemical fertilizers, herbicides and pesticides have led to the search for alternative strategies and methods to combat limiting soil nutrients for sustainable agriculture.

The exploitation of biological nitrogen fixation (BNF) by legume - rhizobium symbiosis is seen by researchers as a low and sustainable technical solution compatible with the socio - economic conditions of small - scale farmers in SSA and a very promising approach to enhance the fertility of soil. There are several different physiological and environmental factors, such as temperature, water logging, water stress, salinity, combined nitrogen levels, pH and available nutrients that affect the effectiveness of microbial inoculants. Since this is an emerging technology in SSA, commercial microbial and chemical products need to be evaluated under different agro - ecological conditions to confirm whether these products have the capability to improve soil fertility and crop production as well as assessing under which conditions for realizing potential benefits.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location and description of soil in the study areas

Field trials were conducted in three (3) study locations in the Northern savannah agro - ecological zones of Ghana during the 2013 major cropping season. The trials were conducted at the experimental fields of the CSIR - Savannah Agricultural Research Institute at Kpongu (Latitude 09 ^{o} 59' 34.0'' N and Longitude 002 ^{o} 31'' 30.3' with an elevation of 315 m above sea level) in the Upper West region; Nyankpala (Latitudes 09 ^{o} 36' 31.3''N and Longitude 001^{o} 02' 14.1'' W with an elevation of 195 m above sea level) in the Northern region and Manga (Latitude 11^{o} - 01' N and Longitude 00 ^{o} - 16 ^{o} W with and elevation of 249 m above sea level) in the Upper East region.

The Upper West and Northern regions both fall within the Guinea savannah zone, whereas the Upper East region is located in the Sudan savannah zone. Rainfall distribution in the Northern savannah zone is unimodal, with an average annual rainfall of about 1000 - 1200 mm annually (SARI, 1996). In the Guinea savannah zone, the rainy season extends from May to October and the rest of the year is dry. The Sudan savannah has similar conditions but rainfall amounts are lower (900 - 1000 mm) and the dry period is longer (November - May). The Northern savannah zone records mean temperatures between 26 °C and 30 °C, with little variation throughout the year.

Soils in the Northern savannah zones generally have a sandy texture, inherently low in organic matter which limits their moisture - holding capacity and potential for

growing annual crops. The dominant soil type in the Guinea savannah is Savannah Ochrosol (FAO, 1998). Soil in the UER is underlain by granites interspersed with some pyroclastic rocks while the NR soils are essentially Voltaian sandstones, giving easily worked light soils but prone to concretions and hardpan (Runge-Metzger, 1993).

The dominant soil types in the UWR are laterite, sandy and sandy loam (Savannah Ochrosols) which are poor in organic matter and nutrients as a result of the absence of serious vegetative cover due to bush burning, overgrazing and protracted erosion and heavy leaching.

3.2 Field preparation and planting of soybean and maize

The experimental fields were weeded, ploughed and harrowed after which the field layout was done. The trial was split in two main plots. One main plot was used to plant soybean and the second was allocated to the maize plant with plot sizes measuring 4.5 × 4.5 m each and an alley of 1 m between plots, (were demarcated in all the experimental sites). Soybean variety "Jenguma" (medium maturing) and maize variety "Obaatanpa" (medium maturing) were obtained from the Savannah Agricultural Research Institute and planted at the rate of three seeds per hole of about 5 - 7 cm deep at a spacing of 50 cm × 5 cm for soybean and a spacing of 80 × 40 cm, for maize. At about 2 - 3 weeks after planting (WAP), plants were thinned to two seedlings per hill. A germination test was undertaken before sowing.

3.3 Treatments and Experimental Design

Field experiments were conducted in all the experimental sites using four (4) treatments for each crop designated as T1- Control, T2 - Teprosyn Mo, T3 - Legumefix and T4 - Teprosyn Mo + Legumefix for soybean and T1 - Control,

T2 - Teprosyn Zn/P, T3 - Eco - T and T4 - Teprosyn Zn/P + Eco - T for maize. The source and composition of the microbial inoculants and micronutrient fertilizers used are presented in Appendix 1.

Nitrogen fertilization as urea (46% N) at a rate of 90 kg N ha^{-1} was divided into three equal doses to all the maize plots. One third dose was applied two weeks after planting (WAP) and the remaining two third dose was applied six weeks later. The banding method of fertilizer application was used to ensure fertilizer use efficiency. The experiments were laid out in a Randomised Complete Block Design (RCBD) with three replications each.

3.3.1 Inoculation of seeds

Two hundred and fifty gram of soybean and maize seeds were pre - treated with the different treatments at the concentration specified by the manufacturers for each treatment (Appendix 1). Teprosyn Mo, a micronutrient fertilizer was mixed with the soybean seeds at a rate of 20 mL (kg seed)$^{-1}$ a day prior to planting. Legumefix, a rhizobia inoculant was coated with the soybean seed at a rate of 4.0 g kg^{-1} seed after wetting the seeds. Tepeosyn Zn/P, a micronutrient fertilizer was mixed with maize seeds at a rate of 20 mL (kg seed)$^{-1}$ a day prior to planting. Eco - T, a fungal inoculant was applied to the maize seeds at a rate of 1.5 g kg^{-1} seed.

The seeds were placed in paper bags and kept under a shade for 30 - 45 minutes with intermittent mixing before planting. The growth response of soybean to the various seed treatments was quantified against an untreated control.

3.3.2 Treatment imposition

The trials consisted of 4 plots of 4.5 m × 4.5 m with 1 m alleys between plots in all the experimental sites as shown in Figure 3.1. For soybean, the Control plots were

planted first followed by Teprosyn Mo, Legumefix and Teprosyn Mo + Legumefix plots. With maize, the control plots were planted first followed by Teprosyn Zn/P, Eco - T and Teprosyn Zn/P + Eco - T plots. These precautions for imposing the treatments were carefully adhered to in order to avoid cross contamination between the plots.

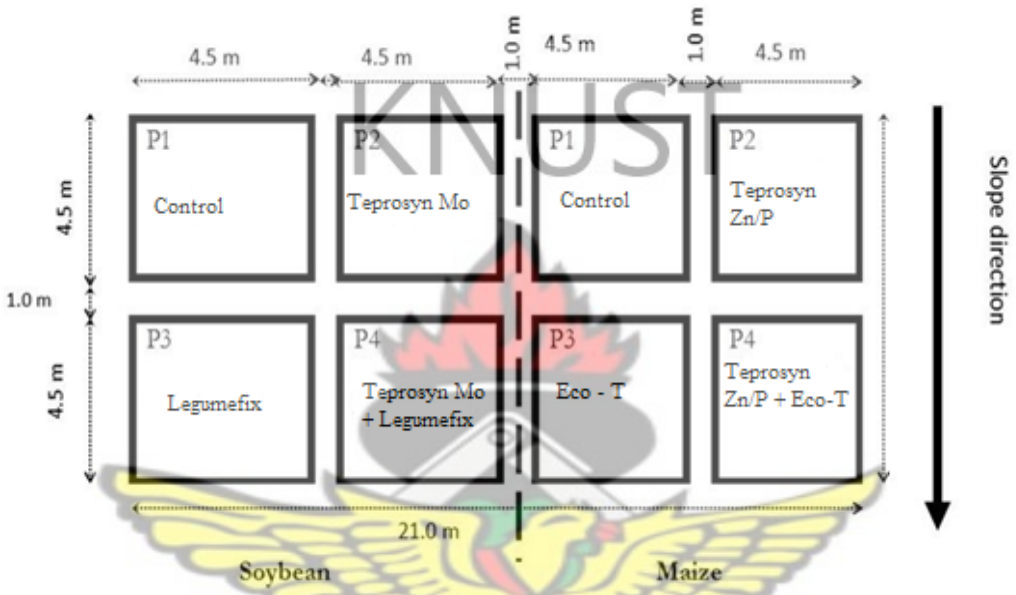


Figure 3.1. Experimental layout and treatments

3.4 Soil sampling and sample preparation

Eight (8) core soil samples from a depth of 0 - 15 cm were collected from the various experimental sites following a 'W' design per replicate block for the initial soil analysis. A composite sample was taken after bulking which was air - dried, grounded and sieved through a 2 mm mesh sieve. After sieving, samples were put into polythene bags awaiting laboratory analysis.

3.4.1 Laboratory chemical analysis

Laboratory chemical analyses were conducted on both soil and plant samples which were obtained from the various experimental sites.

3.4.1.1 Determination of soil chemical properties

3.4.1.1.1 Soil pH

This was determined by using a Suntex mv / Temp pH meter (701) in a 1: 2.5 soil to distilled water (soil : water) ratio according to the electrometric method described by Page (1982). 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.

3.4.1.1.2 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 (10 mL) of 0.166 *M* (1 *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 *M* ferrous sulphate solution.

Calculation:

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

where:

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)

3.4.1.1.3 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:

$$\% \text{ N} = \frac{M \times (\text{a} - \text{b}) \times 1.4 \times \text{mcf} \times \text{v}}{\text{s} \times \text{t}}$$

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

3.4.1.1.4 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 a Whatman No. 42 filter paper. An aliquot of 5 mL of the filterate was pipetted into 25 mL flask and 10 mL colouring reagent (ammonium paramolybdate) was added followed by a pinch of ascorbic acid. After mixing well, the mixture was allowed to stand for 15 minutes to develop a blue colour. The colour was measured using a 21D spectrophotometer at 660 nm wavelength. The available phosphorus was extrapolated from a standard curve.

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

3.4.1.1.5 Extraction of exchangeable cations

Calcium, magnesium, potassium and sodium in the soil were determined in 1 *M* ammonium acetate (NH_4OAc) extract (Black, 1986). A 10 g sample was transferred into a leaching tube and leached with 250 mL of buffered 1 *M* ammonium acetate

(NH_4OAc) solution at pH 7. Hydrogen plus aluminium were determined in 1 *M* KCl extract as described by Page *et al*. (1982).

3.4.1.1.5.1 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 *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:

$$Ca + Mg\,(cmol\ (+)/kg) = \frac{0.01 \times (V_a - V_b) \times 1000}{0.1 \times W}$$

where:

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

V_a = mL of 0.01 *M* EDTA used in the sample titration.

V_b = mL of 0.01 *M* EDTA used in the blank titration.

0.01 = concentration of EDTA used

3.4.1.1.5.2 Determination of calcium

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 millilitres of 0.01 *M* calcium chloride solution was titrated with 0.01 *M* EDTA in the presence of 25 mL 1 *M* ammonium acetate solution to provide a standard pure blue colour. The titre value of calcium was recorded.

3.4.1.1.5.3 Exchangeable potassium and sodium

Exchangeable 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 a 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. One hundred millilitres of 1 *M* NH4OAc 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:

$$\text{Exchangeable K (cmol kg}^{-1}\text{ soil)} = \frac{(a-b) \times 250 \times mcf}{10 \times 39.1 \times s}$$

Exchangeable Na (cmol kg^{-1} soil) = $\frac{(a-b) \times 250 \times mcf}{10 \times 23 \times s}$

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

3.4.1.1.6 Exchangeable acidity

Exchangeable acidity (defined as the sum of Al and H) was determined by titration method after extraction with 1 *M* potassium chloride (Page *et al*., 1982). A 50 g soil sample was put in 200 mL plastic bottle and 100 mL of 1 *M* KCl solution added. The bottle was capped and shaken for 1 hour on a mechanical - electric shaker and then filtered. A 50 mL portion of the filtrate was taken with a pipette into a 250 mL 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:

Exchangeable acidity (cmol kg^{-1} soil) $= \frac{(a-b) \times M \times 2 \times 100 \times mcf}{s}$

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

3.4.1.1.7 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 ($Al^{+} + H^{+}$).

3.4.1.2 Determination of soil physical properties

Particle size distribution was determined by the hydrometer method (Day, 1953). A 50 g air dry soil was weighed into a 250 mL beaker and 100 mL of dispersing agent, sodium hexa - metaphosphate, was added to the soil.

The content of the beaker was weighed into a shaking cap and fitted to a reciprocal shaker at 400 rpm overnight (18 hours) which was then transferred to 1 L sedimentation cylinders and made up to the mark with distilled water. It was then placed on the bench and hydrometer readings taken at a 40 seconds interval followed by 3 hours interval. A hydrometer was used to measure the density of the suspension of soil and water at various times and the temperature was also taken at each hydrometer reading. The percent sand, silt and clay were calculated as follows:

$$\%Sand = 100 - [H1 + 0.2 (T1 - 20) - 2] \times 2$$

$$\%Clay = [H2 + 0.2 (T2 - 20) - 2] \times 2$$

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

where:

H1 = 1st hydrometer reading at 40 seconds

T1 = 1st temperature reading at 40 seconds

T2 = Temperature reading at 3 hours

H2 = 2nd hydrometer reading at 3 hours

-2 = Salt correction to be added to hydrometer reading

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

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

3.5 Plant tissue analysis

At 50% podding, 10 plant samples were randomly collected from the two border rows of each plot. The shoots as well as the seeds of the plants were separated, weighed, oven - dried at 60 ℃ for 72 hours and milled in a stainless steel mill, after which the nitrogen and phosphorus contents were determined.

3.5.1 Determination of total nitrogen in plant samples

Kjeldahl's method described under section 3.4.1.1.3 was used in the determination of total nitrogen in the plant biomass.

3.5.2 Determination of phosphorus in plant samples

Total phosphorus in plant samples was determined using the spectrophotometric vanadium phosphomolybdate method. One gram of plant sample was weighed into a digestion tube. One millilitre of digestion mixture ($HCIO_4HNO_3$) was added. It was digested and made up to 500 mL in a volumetric flask. Ten millilitres of the digest

was measured into a 50 mL volumetric flask. Ten millilitres of vanadomolybdate was then added. Distilled water was added to make the required volume. It was then shaken vigorously and kept for at least 30 minutes and colour development was read on a 430 nm spectrophotometer. The percentage transmittance was recorded; and absorbance and the P content were then determined from a standard curve.

3.6 Determination of rhizobia population in the experimental sites

Estimation of the native rhizobia population was determined using the most probable number (MPN) plant infection technique (Somasegaran and Hoben, 1994). Soil samples from a depth of 0 - 15 cm was taken, bulked and a subsample taken, sealed and stored at 4 ℃ and a composite sample was used for the evaluation of rhizobia population of the experimental sites.

3.6.1 Pre - germination of seeds

Soybean seeds of uniform colour, size and undamaged were selected (Maingi *et al*., 1999), placed in a 250 mL Erlenmeyer flask and surface sterilized with 95% ethanol to remove waxy materials and trapped air and drained away after 10 seconds. Hydrogen peroxide (H_2O_2) (3%) solution was added in sufficient volume to immerse the seeds completely. Contents were swirled gently to bring the seeds and sterilant into contact. The sterilant was drained off after 5 minutes. Rinsing was done in six changes of sterile distilled water. Aseptic procedures were observed throughout the rinsing (Somasegaran and Hoben, 1994).

The seeds were then incubated at a temperature of 30 °C for germination. After 2 - 4 days, there was emergence and seedlings whose radicles attained a length of 1 - 2 cm after the incubation period were considered ready for transferring to growth pouches.

3.6.2 Preparation of nutrient solution

Plastic growth pouches containing a formulation of 50 mL nitrogen - free mineral nutrient solution (composition in Appendix 2) were arranged in wooden racks. Soybean seeds with straight radicles were inserted into holes created on top of the paper wick growth pouches using forceps and kept under greenhouse conditions to avoid contamination. Addition of nutrient solution was made as and when necessary.

3.6.3 Inoculation and estimation of rhizobia population

The seedlings were maintained for eight days in the growth pouches in wooden racks sorted for uniformity and arranged in quadruplicates before inoculation with serial dilutions of the soil samples. For the preparation of the serial dilutions, 100 g of the composite soil sample were diluted in 400 mL of distilled water. A five - fold dilution series was made from 5^{-1} to 5^{-6}.

Growth pouches with the seedlings were set in quadruplicates in the greenhouse and inoculated with 1 mL of the dilutions following the procedure of Somasegaran and Hoben (1994). After inoculation with the soil samples the plants were maintained in the greenhouse for 4 weeks after which they were harvested. The number of rhizobia per gram was calculated by using the formula below:

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

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

d = lowest dilution (first unit)

v = volume of aliquot applied to plant

3.7 Quality assessment of the microbial inoculants used in the study

The quality of the bacterial and fungal inoculants used for the experiment was determined.

3.7.1 Viable cell count

The number of viable cells in the rhizobia and fungal inoculant were estimated by using the spread plate technique described by Zuberer (1994) and pour plate technique respectively. A 10 - fold serial dilution from 10^{-1} to 10^{-10} was prepared with the inoculant for which 100 µL of aliquots of each dilution were spread onto Yeast Extract Mannitol Agar (YEMA) (Appendix 3) with the rhizobia inoculant starting from the highest dilution using three replicate for each dilution.

With the fungal inoculant, 10 - fold serial dilution from 10^{-1} to 10^{-10} was prepared with the inoculant for which 100 µL of aliquots of each dilution were added onto to sterile petri plates and mixed with liquid potato dextrose agar (PDA) that has been cooled to about 45 ℃. The plates were then incubated for 8 days at a temperature of 28 ℃ in the incubator. The number of colony forming unit (cfu) after the incubation period constitutes the population density. Plates having between 30 - 300 colonies were counted and viable cell counts were calculated using the equation below:

$$\text{CFU / mL} = \text{No. of colonies counted} \times \frac{1000}{\text{aliquot plated}} \times \frac{1}{\text{dilution factor}}$$

3.7.2 Determination of pH

This was determined by using a Suntex mv / Temp pH meter (701) in a 1: 2.5 inoculant to distilled water (soil : water) ratio according to the electrometric method described by Page (1982). Ten grams of the inoculant was weighed into a 100 mL beaker. To this 25 mL distilled water was then added and stirred thoroughly for 20

minutes and allowed to stand for 15 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.

3.7.3 Determination of moisture content

The moisture content of the microbial inoculants was determined by measuring the weight loss of sample that was placed onto aluminium foil cup of known weight. The cup and its contents was oven dried at a temperature of 105 ± 5 ℃ (AACC, 2000). The moisture content was determined based on the weight loss of sample before and after drying.

3.8 Soybean agronomic data collected

3.8.1 Nodule number

At 50% podding, ten (10) soybean plants were randomly collected per plot (avoiding central rows) for evaluation of nodulation (nodule number and dry weight per plant). The plants were carefully uprooted by digging 15 cm around the plant using a spade and washed gently with clean water to remove all attached soil from the roots and the nodules. Nodules were carefully counted and nodule numbers determined and oven - dried at 60 ℃ for 48 hours to determine dry matter weights.

3.8.2 Shoot and root dry weight

At 50% podding, ten (10) soybean plants were randomly harvested from the two second rows of each plot for evaluation of shoot and root dry weights. The plants were separated into shoots and roots and oven - dried at 60 ℃ for 72 hours and dry weights recorded for each sample. The shoots were later milled for laboratory analysis.

3.8.3 Number of pods per plant

Ten (10) soybean plants were harvested at 50% podding from the net plot of each experimental plot, excluding the outer rows and the outer guard plants in each row and pods were detached. The number of pods obtained for each plot was divided by the 10 plants and recorded as the number of pods per plant.

3.8.4 Grain yield

Seed yield of soybean plants were harvested at the physiological maturity stage, air - dried, threshed and winnowed. The grains were oven - dried at 60 ℃ for 72 hours. The dry weights for each plot were then determined and used to estimate the grain yield per hectare (Okogun *et al*., 2005).

3.9 Maize agronomic data collected

3.9.1 Plant height

At physiological maturity, ten (10) maize plants were randomly selected from the net plot excluding the border rows in each plot and their heights measured from the base of the plant to the tassel using a tape measure.

3.9.2 Stover yield

At harvest, five (5) maize plants were randomly selected from the net plot in each plot and their stovers collected and oven - dried at 60 ℃ for 72 hours to determine the mean dry weights for each plot.

3.9.3 Grain yield

All plants on the plots were harvested at full maturity excluding the border rows by cutting at the ground level and weighed. The grains were oven - dried at 60 ℃ for

72 hours. The dry weights for each plot were then determined and used to estimate the grain yield per hectare (Okogun *et al*., 2005).

3.10 Economic analysis

Economic analyses of the commercial products used during the field trials were undertaken using partial budgeting. Additional cost and benefit of the soyean treatments (Teprosyn Mo, Legumefix and Teprosyn Mo + Legumefix) were calculated relative to the control. Additional cost was inccured through the purchasing price of the products per hectare (USD 4.25, USD 0.22, USD 4.47 for Teprosyn Mo, Legumefix and Teprosyn Mo + Legumefix respectively). Labour per hectare and seed treatment per hectare did not account for any additional cost in all the study sites. Additional benefit comprised revenue from additional soybean grain yield over the control obtained from the application of the various treatments. The farm - gate price of soybean grain was obtained as USD 40 per 100 kg bag in all the study locations.

Additional cost and benefit of the maize treatments (Teprosyn Zn/P, Eco - T and Teprosyn Zn/P + Eco - T) were calculated in relation to the control. The additional cost incurred through the purchasing price of the products per hectare were USD 4.25, USD 0.04, USD 4.29 for Teprosyn Zn/P, Eco - T and Teprosyn Zn/P + Eco - T respectively. Additional benefit comprised revenue from additional maize grain yield over the control. The farm - gate price of the maize grain was USD 25 per 100 kg bag in all the study locations.

Prices were collected in local currencies and converted to US dollars at the prevailing exchange rates (GH¢ 1 to USD 2). Gross margin (GM) was calculated as the difference between the total revenue and the total variable cost. The profitability of

product application was estimated by the value to cost ratio as explained by Nziguheba *et al*., (2010) and it is expressed as:

$$VCR = \frac{(Y - Yc)}{X}$$

where;

Y = the monetary value of the crop harvested in intervention (treated) plots,

Yc = the monetary value of the crop harvested in control plots, and

X = the monetary cost of inputs (seeds and fertilizers).

3.11 Statistical analysis

All data collected were subjected to Analysis of Variance (ANOVA) using Genstat Statistics (GENSTAT) package (Genstat, 2010). The relationship between parameters measured was established using simple correlation. Means of treatments were separated by the least significant difference (LSD) test at 5% probability level (Steel and Torrie, 1987). All count data were transformed logarithmically (Kihara *et al*., 2011) before being subjected to ANOVA.

CHAPTER FOUR

4.0 RESULTS

4.1 Selected physico - chemical properties and MPN of the experimental sites

The results of the physical and chemical properties of the soil at the three study locations are as presented in Table 4.1. The textural class at the three study sites were loamy sand in Kpongu and Manga and sandy loam in Nyankpala. The pH values of the study sites ranged from acidic to moderately acidic (4.12 - 5.53). The organic carbon (OC) levels at all the study sites were very low; 0.66% in Kpongu, 0.40% in Manga and 0.44% in Nyankpala. Total N at all the study sites were generally low ranging from 0.02 - 0.06%. Results recorded on available phosphorus (P) were 1.96 mg kg^{-1}, 1.24 mg kg^{-1} and 2.70 mg kg^{-1} for Kpongu, Manga and Nyankpala respectively which is below the critical range (10.0 - 14.0 mg kg^{-1}). Generally, the fertility status of the study sites was very low. The indigenous rhizobia population (IRP) estimation of each soil sample from the study sites were 3.19×10^1 colony forming units (cfu) per gram of soil, 2.79×10^1 cfu g^{-1} of soil and 4.36×10^1 cfu g^{-1} of soil and for Kpongu, Manga and Nyankpala respectively.

Table 4.1. Physico - chemical and biological properties of the study sites

Parameter	Kpongu	Manga	Nyankpala
pH (1:2.5 H_2O)	5.53	4.12	5.37
Organic Carbon (%)	0.66	0.44	0.40
Total Nitrogen (%)	0.06	0.02	0.04
Available Phosphorus (mg kg^{-1})	1.96	1.24	2.70
Exchangeable K ($cmol_{(+)}$ kg^{-1})	1.13	0.07	0.19
Exchangeable Ca ($cmol_{(+)}$ kg^{-1})	0.46	0.16	0.37
Exchangeable Mg ($cmol_{(+)}$ kg^{-1})	0.35	0.16	0.29
Exchangeable acidity (Al + H) ($cmol_{(+)}$ kg^{-1})	0.84	1.00	1.00
Particle size distribution			
Sand (%)	76.45	80.38	66.59
Clay (%)	6.40	4.99	6.35
Silt (%)	17.15	14.63	27.06
Texture	Loamy sand	Loamy sand	Sandy loam
IRP (cells g^{-1} of soil)	3.19×10^1	2.79×10^1	4.36×10^1

*Means represent duplicate samples

*IRP - Indigenous Rhizobia Population

4.2 Quality assessment of microbial inoculants used in the study

The results of viable cell counts, moisture content and pH of the microbial inoculants used in the study are as shown in Table 4.2. The viable cell counts of Legumefix and Eco - T were 5.8×10^7 and of 5.7×10^7 respectively. pH values of 7.1 and 6.2 were

recorded for Legumefix and Eco - T respectively while moisture contents of 39% (Legumefix) and 0.45% (Eco - T) were recorded.

Table 4.2. Quality of the microbial products used in the study

Inoculant	Viable cell count (cfu g^{-1} carrier)	pH (1:2.5 H_2O)	Moisture content (%)
Legumefix	5.80×10^7	7.10	39.00
Eco - T	5.70×10^7	6.21	0.45

4.3 Growth, yield and nutrient uptake of soybean

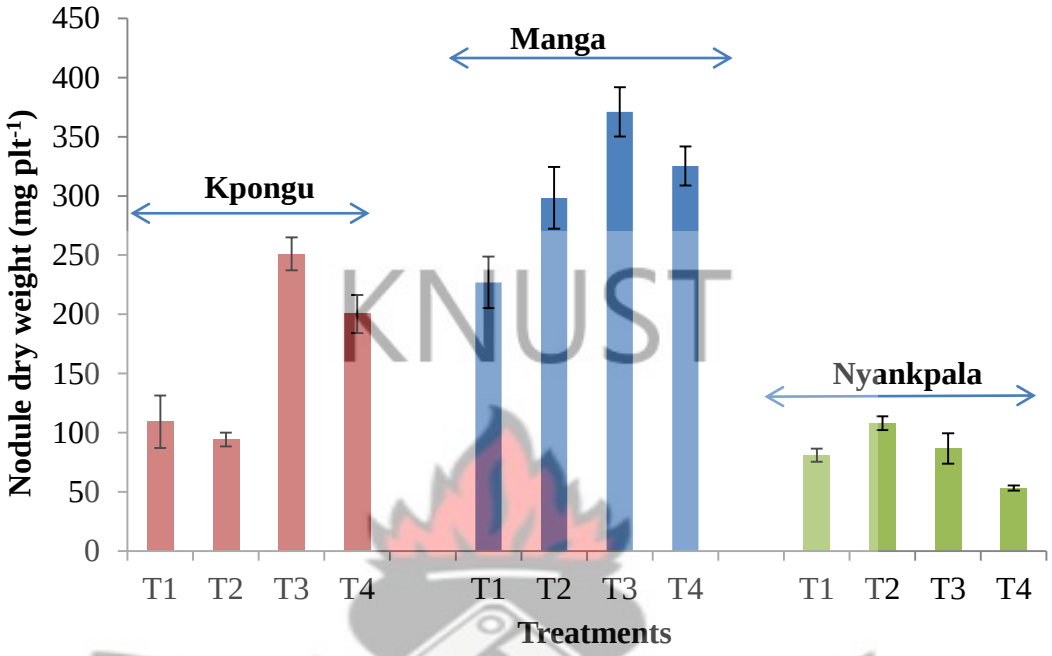
4.3.1 Dry weight of soybean nodules as influenced by Legumefix and Teprosyn Mo

Results of the effects of rhizobia inoculation and micronutrient fertilizer on soybean nodulation at the three (3) study sites are as illustrated in Figure 4.1. At 50% podding, the highest nodule dry weight in Kpongu was observed in Legumefix (251 mg plt^{-1}) which was significantly ($P < 0.05$) different from the control and Teprosyn Mo which produced the least nodule dry weight (94 mg plt^{-1}). The application of Legumefix and Legumefix + Teprosyn Mo to soybean increased nodule dry weight significantly by 130% and 83.49% respectively over the control.

In Manga, Legumefix was significantly ($P < 0.05$) effective in eliciting increased nodulation response (371 mg plt^{-1}) over the control. Furthermore, nodule dry weight increased by 63.44%, 43.17% and 31.28% for Legumefix, Teprosyn Mo + Legumefix and Teprosyn Mo respectively over the control.

In Nyankpala, no significant ($P > 0.05$) differences were observed among treatments. However, Teprosyn Mo and Legumefix increased nodule dry weight by 33.33% and

7.04% over the control. The least nodule dry weight was recorded in Teprosyn Mo + Legumefix (53.3 mg plt^{-1}).



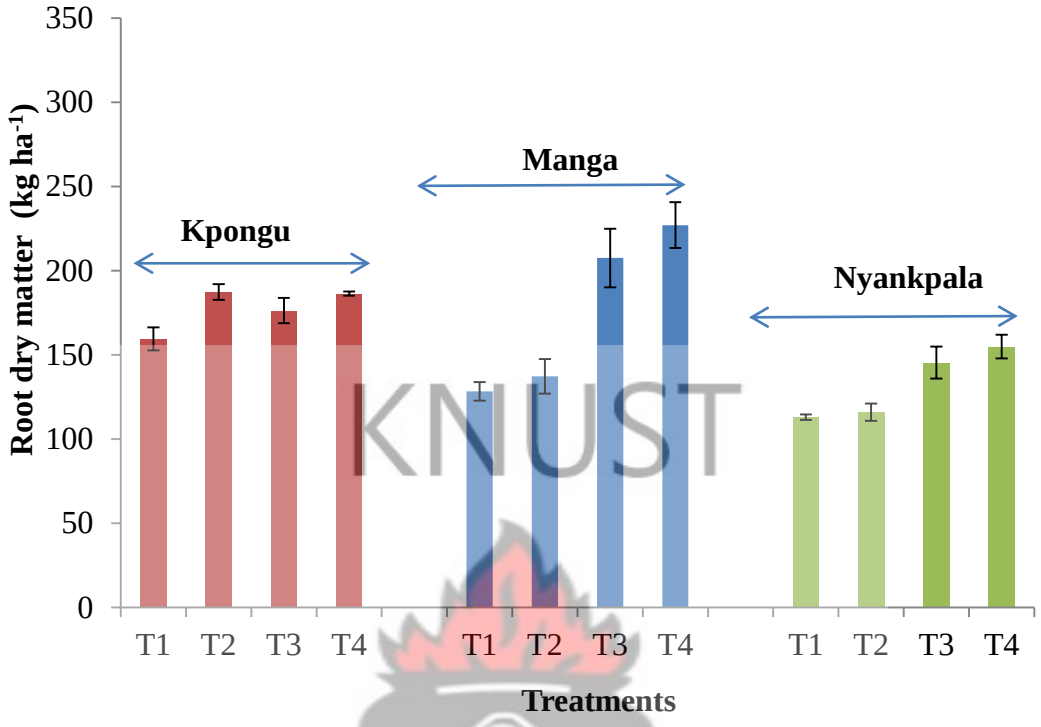
Key: T1 = Control, T2 = Teprosyn Mo, T3 = Legumefix, T4 = Teprosyn Mo + Legumefix. Error bars represents the mean ± SED between treatments within the study areas.

Figure 4.1. Soybean nodule dry matter in the three study sites as influenced by the application of rhizobia inoculant and Teprosyn Mo

4.3.2 Soybean root dry matter as influenced by Legumefix and Teprosyn Mo

The effects of rhizobia inoculation and micronutrient fertilization on soybean root dry matter are as shown in Figure 4.2.

Teprosyn Mo recorded the highest root dry weight (187.30 kg ha^{-1}) in Kpongu while the control recorded the least (159.60 kg ha^{-1}). The application of Legumefix and Teprosyn Mo + Legumefix significantly ($P < 0.05$) increased root dry weight by 63% and 77% respectively over the control in Manga. At Nyankpala, the highest root dry weight was produced by Teprosyn Mo + Legumefix (155.00 kg ha^{-1}) while the control produced the least (113.10 kg ha^{-1}).

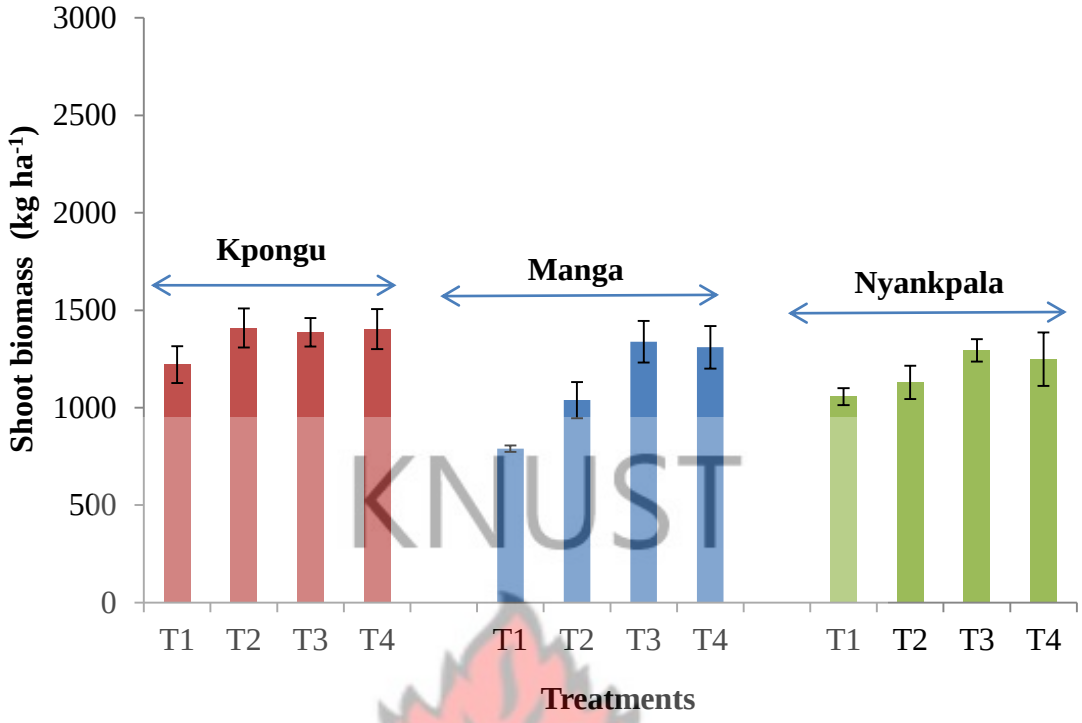


Key: T1 = Control, T2 = Teprosyn Mo, T3 = Legumefix, T4 = Teprosyn Mo + Legumefix. Error bars represents the mean ± SED between treatments within the study areas.

Figure 4.2. Soybean root dry matter in the three study sites as influenced by the application of rhizobia inoculant and Teprosyn Mo

4.3.3 Soybean shoot biomass as influenced by Legumefix and Teprosyn Mo

Biomass yield of soybean shoot under the various treatments in the study sites are as presented in Figure 4.3. In Kpongu, increased shoot biomass yields of 15.48%, 14.91% and 13.60% were obtained for Teprosyn Mo, Teprosyn Mo + Legumefix and Legumefix respectively over the control. In Manga, the highest shoot biomass was recorded in Legumefix (1338 kg ha^{-1}) while the control recorded the least (790 kg ha^{-1}) which was however not statistically significant ($P > 0.05$). The shoot biomass in Nyankpala ranged from 1057.00 - 1294.00 kg ha^{-1}. Percentage increases over the control were 22.42%, 18.17% and 6.91% for Legumefix, Teprosyn Mo + Legumefix and Teprosyn Mo respectively.



Key: T1 = Control, T2 = Teprosyn Mo, T3 = Legumefix, T4 = Teprosyn Mo + Legumefix. Error bars represents the mean ± SED between treatments within the study area

Figure 4.3. Soybean shoot biomass in the three study sites as influenced by the application of rhizobia inoculant and Teprosyn Mo

4.3.4 Soybean grain yield as influenced by Legumefix and Teprosyn Mo

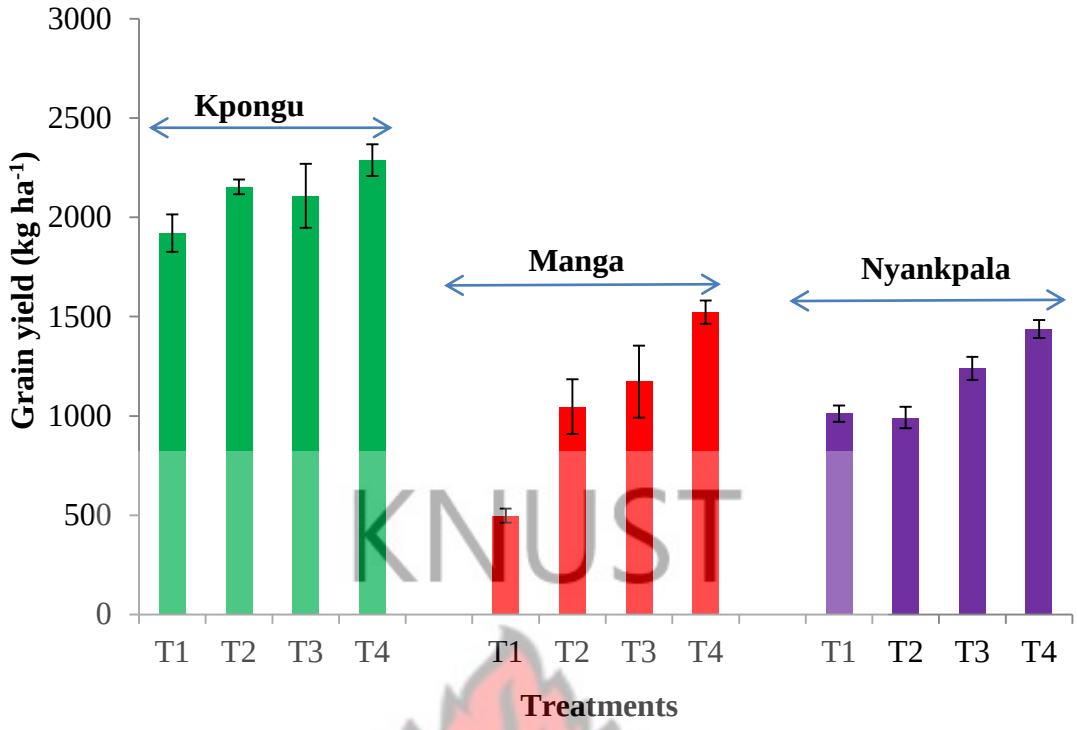
Figure 4.4 shows the effect of the Legumefix, Teprosyn Mo and its combination on soybean grain yield in Kpongu, Manga and Nyankpala.

There was no significant ($P > 0.05$) effect of Legumefix and Teprosyn Mo on soybean grain yield in Kpongu. However, an increase of 9.73%, 12.13% and 19.10% were observed in grain yield by the application of Legumefix, Teprosyn Mo and Teprosyn Mo + Legumefix respectively over the control.

Significant ($P < 0.05$) differences existed among some treatments for soybean grain yield in Manga where the highest grain yield of 1522 kg ha^{-1} was obtained by Teprosyn Mo + Legumefix while the control recorded the lowest grain yield

(498 kg ha^{-1}). Furthermore, Teprosyn Mo + Legumefix, Legumefix and Teprosyn Mo increased grain yield by 205.62%, 135.54% and 110.24% respectively over the control.

In Nyankpala, the application of Teprosyn Mo + Legumefix increased grain yield significantly ($P < 0.05$) by 42.10% while Legumefix recorded an increase of 22.43% but was not statistically significant ($P > 0.05$) relative to the control. Teprosyn Mo however, resulted in a 2.08% decline in soybean grain yield.



Key: T1 = Control, T2 = Teprosyn Mo, T3 = Legumefix, T4 = Teprosyn Mo + Legumefix. Error bars represents the mean ± SED between treatments within the study areas.

Figure 4.4. Soybean grain yield in the three study sites as influenced by the application of rhizobia inoculant and Teprosyn Mo

4.3.5 Nitrogen and phosphorus partitioning into soybean shoot biomass and grain

Data on the effects of Legumefix and Teprosyn Mo and its combination on soybean shoot and grain N and P uptake in the three study sites are as presented in Tables 4.3 - 4.6. The amount of N in the shoot ranged from 26.20 - 40.70 kg ha^{-1}, 20.40 - 38.10 kg ha^{-1} and 19.50 - 39.50 kg ha^{-1} in Kpongu, Manga and Nyankpala respectively. Shoot N uptake was not significantly ($P > 0.05$) affected by any of the treatments in all the study sites (Table 4.3).

The amount of nitrogen (N) in the soybean grain was not significantly ($P > 0.05$) affected by the treatments in Kpongu (Table 4.4). In Manga, the amount of nitrogen in soybean grain was not significantly ($P > 0.05$) different among the treatments except the control (Table 4.4). Teprosyn Mo + Legumefix (94.60 kg ha^{-1}), Legumefix (68.80 kg ha^{-1}) and Teprosyn Mo (67.00 kg ha^{-1}) resulted in significantly ($P < 0.05$) higher grain N uptake than the control which recorded the least grain N uptake (29.30 kg ha^{-1}). In Nyankpala, the application of Teprosyn Mo + Legumefix produced significantly ($P < 0.05$) higher grain N (86.40 kg ha^{-1}) than Teprosyn Mo (62.30 kg ha^{-1}). Teprosyn Mo + Legumefix and Legumefix increased grain N uptake over the control by 33.33% and 18.67% respectively (Table 4.4).

Application of Legumefix, Teprosyn Mo and Teprosyn Mo + Legumefix did not significantly ($P > 0.05$) increase shoot P uptake in Kpongu (Table 4.5). However, increases of 39.02%, 40.24% and 42.68% over the control were observed for Legumefix, Teprosyn Mo + Legumefix and Teprosyn Mo applications respectively. Application of Legumefix significantly ($P < 0.05$) increased shoot P uptake relative to the control in Manga (Table 4.5). Teprosyn Mo + Legumefix and Teprosyn Mo resulted in increased shoot P uptake of 15.24% and 4.76% respectively over the control but the increases were not significant ($P > 0.05$). In Nyankpala, Teprosyn Mo increased shoot P uptake (3.46 kg ha^{-1}) significantly ($P < 0.05$) compared to the control (2.14 kg ha^{-1}) (Table 4.5). The application of Legumefix did not significantly ($P > 0.05$) increase shoot P uptake over the control. The lowest shoot P uptake was observed in Teprosyn Mo + Legumefix.

The P uptake in soybean grain was not significantly ($P > 0.05$) affected by the treatments that were applied in Kpongu (Table 4.6). However, phosphorus uptake in the grain varied from 5.39 to 6.17 kg ha^{-1}. The application of Teprosyn Mo +

Legumefix and Legumefix and Teprosyn Mo resulted in grain phosphorus uptake of 2.99 kg ha^{-1}, 1.82 kg ha^{-1}, and 1.30 kg ha^{-1} respectively at Manga (Table 4.6). In Nyankpala, soybean grain P uptake ranged from 1.61 kg ha^{-1} - 1.96 kg ha^{-1} while the lowest was recorded by Teprosyn Mo (1.61 kg ha^{-1}) (Table 4.6).

Table 4.3. Effect of rhizobia inoculant and Teprosyn Mo on soybean shoot N uptake

Treatments	Kpongu	Manga	Nyankpala
	← kg ha^{-1} →		
Control	26.20	20.40	19.50
Teprosyn Mo	40.60	29.00	25.30
Legumefix	40.70	35.30	39.50
Teprosyn Mo + Legumefix	37.30	38.10	35.10
Lsd (5%)	ns	ns	ns
CV (%)	19.40	25.10	24.20

Table 4.4. Effect of rhizobia inoculant and Teprosyn Mo on soybean grain N upta

Treatments	Kpongu	Manga	Nyankpala
	← kg ha^{-1} →		
Control	123.70	29.30	64.80
Teprosyn Mo	139.20	67.00	62.30
Legumefix	138.70	68.80	76.90
Teprosyn Mo + Legumefix	142.90	94.60	86.40
Lsd (5%)	ns	33.92	22.43
CV (%)	16.50	26.20	15.50

Table 4.5. Effect of rhizobia inoculant and Teprosyn Mo on soybean shoot P uptake

Treatments	Kpongu	Manga	Nyankpala
	←	kg ha^{-1}	→
Control	1.64	1.05	2.14
Teprosyn Mo	2.34	1.10	3.46
Legumefix	2.28	1.77	1.07
Teprosyn Mo + Legumefix	2.30	1.21	0.98
Lsd (5%)	ns	0.69	1.11
CV (%)	18.40	27.00	29.10

Table 4.6. Effect of rhizobia inoculant and Teprosyn Mo on soybean grain P uptake

Treatments	Kpongu	Manga	Nyankpala
	←	kg ha^{-1}	→
Control	5.39	1.07	1.62
Teprosyn Mo	6.17	1.30b	1.61
Legumefix	5.97	1.82	1.71
Teprosyn Mo + Legumefix	6.11	2.99	1.96
Lsd (5%)	ns	0.74	ns
CV (%)	18.60	20.60	18.30

4.3.6 Relationships between growth and yield parameters of soybean

A simple correlation coefficient was worked out to ascertain the degree of relationship between the various growth and yield parameters of soybean in the study locations (Tables 4.7 - Table 4.9). Positive significant ($P < 0.05$) correlations were observed between biomass dry weight and grain yield and root dry weight and nodule dry weight in Kpongu (Table 4.7). In Manga, no significant ($P < 0.05$) correlations were observed between the growth and yield parameters measured (Table 4.8). The correlation (r) between nodule dry weight and root dry weight was 0.03 which were the only variables to give a positive significant ($P < 0.05$) correlation in Nyankpala (Table 4.9).

Table 4.7. Pearson correlation matrix of some measured plant parameters in Kpongu

	Biomass Dry Weight	Root Dry Weight	Nodule Dry Weight	100 Seed Weight	Grain Yield
Biomass Dry Weight		0.42*	0.09*	0.15*	0.03**
Root Dry Weight	0.42*		0.03**	0.19*	0.15*
Nodule Dry Weight	0.09*	0.03**		0.67*	0.27*
100 Seed Weight	0.15*	0.19*	0.67*		0.30*
Grain Yield	0.03**	0.15*	0.27*	0.30*	

*-No significance P > 0.05, **-Significance P < 0.05.

Table 4.8. Pearson correlation matrix of some measured plant parameters in Manga

	Biomass Dry Weight	Root Dry Weight	Nodule Dry Weight	100 Seed Weight	Grain Yield
Biomass Dry Weight		0.76*	0.43*	0.21*	0.62**
Root Dry Weight	0.76*		0.63*	0.19*	0.33*
Nodule Dry Weight	0.43*	0.63*		0.28*	0.23*
100 Seed Weight	0.21*	0.19*	0.28*		0.57*
Grain Yield	0.62**	0.33*	0.23*	0.57*	

*-No significance P > 0.05

Table 4.9. Pearson correlation matrix of some measured plant parameters in Nyankpala

	Biomass Dry Weight	Root Dry Weight	Nodule Dry Weight	100 Seed Weight	Grain Yield
Biomass Dry Weight		0.17*	0.15*	0.33*	0.48*
Root Dry Weight	0.17*		0.03**	0.46*	0.49*
Nodule Dry Weight	0.15*	0.03**		0.08*	0.49*
100 Seed Weight	0.34*	0.46*	0.08*		0.25*
Grain Yield	0.48*	0.49*	0.49*	0.25*	

*-No significance P > 0.05, **-Significance P < 0.05.

4.4 Economic assessment of Legumefix and Teprosyn Mo

A partial budget and value to cost ratio analyses were undertaken to assess the profitability of sole application of Teprosyn Mo, Legumefix and their combined effect (Teprosyn Mo + Legumefix) under field conditions (Table 4.10).

The economic analysis showed that additional benefits were achieved when Teprosyn Mo, Legumefix and Teprosyn Mo + Legumefix were applied except for Teprosyn Mo in Nyankpala (Table 4.10). The value cost ratio analysis also showed that all the treatments had a VCR below 2 except the combined application of Teprosyn Mo + Legumefix in Manga (VCR = 2.65) (Table 4.10).

Table 4.10. Economic analysis of commercial products for soybean

Study sites	Treatments	Product price *(USD ha^{-1})	Grain price *(USD)	Gross margin *(USD)	Value cost ratio
Kpongu	Control	0.00	768.40	-	-
	Teprosyn Mo	4.25	861.60	532.38	0.59
	Legumefix	0.22	843.20	777.36	0.48
	Teprosyn Mo + Legumefix	4.47	915.20	520.14	0.92
Manga	Control	0.00	199.20	-	-
	Teprosyn Mo	4.25	418.80	89.58	1.42
	Legumefix	0.22	469.20	403.36	1.80
	Teprosyn Mo + Legumefix	4.47	608.80	213.74	2.65
Nyankpala	Control	0.00	404.80	-	-
	Teprosyn Mo	4.25	396.4	67.18	-0.05
	Legumefix	0.22	495.6	429.76	0.60
	Teprosyn Mo + Legumefix	4.47	575.2	180.14	1.10

*USD 1 = GH¢ 2

4.5 Growth, yield and nutrient uptake of maize

4.5.1 Maize plant height as influenced by Eco - T and Teprosyn Zn/P

Results of the effects of the fungal inoculant and micronutrient fertilizer on plant height of maize for Kpongu, Manga and Nyankpala are as presented in Table 4.11. No significant ($P > 0.05$) differences were observed among the treatments applied in Kpongu. In Manga, percentage increases over the control were 6.94% and 2.08% for Teprosyn Zn/P + Eco - T and Eco - T respectively while Teprosyn Zn/P recorded the lowest plant height (1.42 m). Similar results were obtained in Nyankpala, where Teprosyn Zn/P + Eco - T and Eco - T had average plant height of 1.86 m and 1.81 m respectively with the lowest observed in Teprosyn Zn/P (1.63 m).

Table 4.11. Effect of Eco - T and Teprosyn Zn/P on maize plant height

Treatments	Kpongu	Manga	Nyankpala
	←	m	→
Control	1.87	1.44	1.71
Teprosyn Zn/P	1.82	1.42	1.63
Eco - T	1.87	1.47	1.81
Teprosyn Zn/P + Eco - T	1.86	1.54	1.86
Lsd (5%)	ns	ns	ns
CV (%)	6.40	4.20	7.80

4.5.2 Maize stover yield as influenced by Eco - T and Teprosyn Zn/P

The effects of the fungal inoculant and micronutrient fertilizer on maize stover yield from the experimental sites are as presented in Table 4.12. In Kpongu, none of the products applied had significant ($P < 0.05$) effect on maize stover yield at harvest. Teprosyn Zn/P however, resulted in 22.44% increase in maize stover yield over the control.

There was no significant ($P > 0.05$) difference between the treatments for maize stover yield in Manga. However, Teprosyn Zn/P + Eco - T, Eco - T and Teprosyn Zn/P produced stover yields of 24.26%, 10.65% and 10.65% respectively over the control.

In Nyankpala, the highest stover yield was recorded in the combined effect of Teprosyn Zn + Eco - T (223.00 kg ha^{-1}) but was not significantly ($P > 0.05$) different from the control (183.00 kg ha^{-1}).

Table 4.12. Effect of Eco - T and Teprosyn Zn/P on maize stover yield

Treatments	Kpongu	Manga	Nyankpala
	← kg ha^{-1} →		
Control	205.00	169.00	183.00
Teprosyn Zn/P	251.00	187.00	170.00
Eco - T	192.00	187.00	184.00
Teprosyn Zn/P + Eco - T	199.00	210.00	223.00
Lsd (5%)	ns	ns	ns
CV (%)	27.80	27.90	26.70

4.5.3 Nitrogen and phosphorus partitioning into maize stover and grain

The results of the effect of the fungal inoculant and micronutrient fertilizer on maize stover N is as shown in Tables 4.13 - 4.16.

In Kpongu, stover N uptake was not significantly affected by all the treatments applied. However, Teprosyn Zn/P recorded the highest stover N uptake (2.11 kg ha^{-1}) while the control recorded the least (Table 4.13). The accumulation of N in the stover biomass was not enhanced significantly ($P > 0.05$) by the application of the fungal inoculant and micronutrient fertilizer in Manga where stover N uptake ranged from 1.50 kg ha^{-1} - 2.51 kg ha^{-1} (Table 4.13). Similarly, the effect of the fungal inoculant and micronutrient fertilizer on maize stover N was not significantly ($P > 0.05$) different compared to the control in Nyankpala (Table 4.13). Conversely, Eco - T, Teprosyn Zn/P + Eco - T and Teprosyn Zn/P increased stover N uptake by 25.49%, 18.95% and 2.61% respectively over the control.

Table 4.14 shows the effects of the fungal inoculant and micronutrient fertilizer on N uptake of maize grain in the experimental sites. The N uptake in the maize grain was not significantly ($P > 0.05$) affected by the treatments that were imposed in Kpongu. Nitrogen uptake in the grain of maize in Manga ranged between 19.20 kg ha^{-1} - 27.90 kg ha^{-1}. No significant ($P > 0.05$) differences were observed among the treatments. The effect of the fungal inoculant and micronutrient fertilizer on N content of maize grain was not significant ($P > 0.05$) compared to the control in Nyankpala. The application of Teprosyn Zn/P + Eco - T and Eco T increased grain N uptake of maize by 31.97% and 2.19% respectively over the control. Teprosyn Zn/P recorded the lowest grain N content (30.70 kg ha^{-1}).

The effect of application of the fungal inoculant and micronutrient fertilizer in Kpongu did not augment maize stover P content significantly ($P > 0.05$) compared to the control (Table 4.15). However, Teprosyn Zn/P + Eco - T and Teprosyn Zn/P increased maize stover P by 13% while a lower P uptake was observed with Eco - T application relative to the control (0.15 kg ha^{-1}). The addition of fungal inoculant and micronutrient fertilizer did not improve maize stover P uptake significantly in Manga. Teprosyn Zn/P + Eco - T gave the highest stover P (0.08 kg ha^{-1}) while Teprosyn Zn/P recorded the same value as the control (0.06 kg ha^{-1}) which produced the least. A comparison of the control and the application of fungal inoculant and micronutrient fertilizer on maize stover P uptake in Nyankpala showed no significant differences among the treatments. On the contrary, the application of Eco – T and Teprosyn Zn/P + Eco - T resulted in a stover P uptake increase of 16.67% and 8.33% respectively over the control. The lowest stover P content was however recorded in Teprosyn Zn/P (0.11 kg ha^{-1}).

Data in Table 4.16 shows the response of the fungal inoculant and micronutrient fertilizer application on maize grain P content in the study sites. The amount of P in the grain ranged from 1.72 - 2.42 kg ha^{-1} in Kpongu. The application of Teprosyn Zn/P and Eco - T resulted in increased grain P uptake of 3.41% and 37.5% respectively over the control, while Teprosyn Zn/P + Eco - T recorded the lowest (1.72 kg ha^{-1}). Grain P uptake was however not significantly ($P > 0.05$) affected by all the treatments imposed. In Manga, fungal inoculation and micronutrient fertilization affected grain P uptake, but was not statistically significant ($P > 0.05$). Teprosyn Zn/P, Eco - T and Teprosyn Zn/P + Eco - T application gave a rise to 25.22%, 46.96% and 63.48% increase in grain P uptake over the control which recorded the least (1.15 kg ha^{-1}). A similar trend was observed in Nyankpala with no

significant (P > 0.05) variations among the treatments. Nutrient P grain uptake ranged from 2.00 kg ha^{-1} to 3.73 kg ha^{-1}.

Table 4.13. Effect of Eco - T and Teprosyn Zn/P on maize stover N uptake

Treatments	Kpongu	Manga	Nyankpala
	←	kg ha^{-1}	→
Control	1.32	1.50	1.53
Teprosyn Zn/P	2.11	2.08	1.57
Eco - T	1.42	2.49	1.92
Teprosyn Zn/P + Eco - T	2.06	2.51	1.82
Lsd (5%)	ns	ns	ns
CV (%)	23.90	21.40	23.30

Table 4.14. Effect of Eco - T and Teprosyn Zn/P on maize grain N uptake

Treatments	Kpongu	Manga	Nyankpala
	←	kg ha^{-1}	→
Control	33.00	19.30	31.90
Teprosyn Zn/P	21.90	19.20	30.70
Eco - T	32.40	24.10	32.60
Teprosyn Zn/P + Eco - T	30.90	27.90	42.10
Lsd (5%)	ns	ns	ns
CV (%)	15.30	20.40	20.20

Table 4.15. Effect of Eco - T and Teprosyn Zn/P on maize stover P uptake

Treatments	Kpongu	Manga	Nyankpala
	←	kg ha^{-1}	→
Control	0.15	0.06	0.12
Teprosyn Zn/P	0.17	0.06	0.11
Eco - T	0.14	0.07	0.14
Teprosyn Zn/P + Eco - T	0.17	0.08	0.13
Lsd (5%)	ns	ns	ns
CV (%)	27.10	21.20	26.80

Table 4.16. Effect of Eco - T and Teprosyn Zn/P on maize grain P uptake

Treatments	Kpongu	Manga	Nyankpala
	←	kg ha^{-1}	→
Control	1.76	1.15	2.00
Teprosyn Zn/P	1.82	1.44	2.69
Eco - T	2.42	1.69	3.27
Teprosyn Zn/P + Eco - T	1.72	1.88	3.73
Lsd (5%)	ns	ns	ns
CV (%)	21.30	26.60	27.10

4.5.4 Maize grain yield as influenced by Eco - T and Teprosyn Zn/P

The effects of Teprosyn Zn/P, Eco - T and Teprosyn Zn/P + Eco - T on maize grain yield in Kpongu, Manga and Nyankpala are as shown in Table 4.17. In Kpongu, the highest grain yield was obtained by the application of Eco - T (2093 kg ha^{-1}) followed by the application of Teprosyn Zn/P + Eco - T (2006 kg ha^{-1}) which resulted in 8.28% and 3.78% increase in grain yield respectively over the control. Application of Teprosyn Zn/P resulted in 11.38% reduction in maize grain yield relative to the control.

The application of Teprosyn Zn/P + Eco - T, Eco - T and Teprosyn Zn/P produced maize grain yields of 1861 kg ha^{-1}, 1514 kg ha^{-1} and 1185 kg ha^{-1} respectively in Manga while control produced the lowest grain yield (1155 kg ha^{-1}).

No significant ($P > 0.05$) variation was observed for grain yield among all the treatments tested in Nyankpala. Grain yields ranged from 2740 kg ha^{-1} to 3673 kg ha^{-1}. The application of Teprosyn Zn/P + Eco - T and Eco - T produced were 24.93% and 19.63% respectively over the control.

Table 4.17. Effect of Eco - T and Teprosyn Zn/P on maize grain yield

Treatments	Kpongu	Manga	Nyankpala
	←	kg ha^{-1}	→
Control	1933.00	1155.00	2940.00
Teprosyn Zn/P	1713.00	1185.00	2740.00
Eco - T	2093.00	1514.00	3517.00
Teprosyn Zn/P + Eco - T	2006.00	1861.00	3673.00
Lsd (5%)	ns	ns	ns
CV (%)	21.60	25.40	26.50

4.6 Economic analysis of Eco - T and Teprosyn Zn/P

A partial budget and value to cost ratio analyses was undertaken to assess the profitability of sole application of Teprosyn Zn/P, Eco - T and Teprosyn Zn/P + Eco - T under field conditions (Table 4.18).

The economic analysis showed that additional benefits were achieved when Eco - T, and Teprosyn Zn/P + Eco - T were applied in all the study locations except Teprosyn Zn/P (Table 4.18). In Kpongu, the value cost ratio analysis of all the treatments had a VCR below 2. However, Eco - T and Teprosyn Zn/P + Eco - T recorded a VCR above 2 except Teprosyn Zn/P in Manga and Nyankpala.

Table 4.18. Economic analysis of commercial products for maize

Study sites	Treatments	Product price *(USD ha^{-1})	Grain price *(USD)	Gross margin *(USD)	Value cost ratio
Kpongu	Control	0.00	483.25	-	-
	Teprosyn Zn/P	4.25	428.25	99.03	-1.40
	Eco - T	0.04	523.25	498.56	1.14
	Teprosyn Zn/P + Eco - T	4.29	501.50	147.59	0.46
Manga	Control	0.00	288.75	-	-
	Teprosyn Zn/P	4.25	296.25	-32.97	0.21
	Eco - T	0.04	378.50	353.81	2.80
	Teprosyn Zn/P + Eco - T	4.29	465.25	111.34	4.86
Nyankpala	Control	0.00	735.00	-	-
	Teprosyn Zn/P	4.25	685.00	355.78	-1.13
	Eco - T	0.04	879.25	854.56	3.60
	Teprosyn Zn/P + Eco - T	4.29	918.25	353.91	4.14

*USD 1 = GH¢ 2

CHAPTER FIVE

5.0 DISCUSSION

5.1 Selected physico - chemical properties and MPN of the experimental sites

The soil fertility status at Kpongu, Manga and Nyankpala was generally low (Table 4.1). This is in line with Buri *et al*. (2009), who reported that soil nutrient levels in the Savannah zones of Ghana are particularly low with high pH values, low organic matter, nitrogen and available P levels. The results of the MPN study showed that the soil of Nyankpala had relatively higher indigenous rhizobia population than that of Kpongu and Manga (Table 4.1). Although the presence of large numbers of native rhizobia is noted to interfere with inoculation response according to Thies *et al*. (1991), soybean growing in a soil with a small number of effective rhizobia (20 - 50 cells g^{-1} soil) will likely respond to inoculation. Also in practice, the presence of a large indigenous population of compatible rhizobia does not necessarily preclude response to inoculation, provided the inoculant rhizobia strains are competitive and highly effective (Giller, 2001; Osunde *et al*., 2003).

5.2 Quality assessment of the microbial inoculants used in the study

The quality of an inoculant is evaluated by the number of viable rhizobia it contains (Stephens and Rask, 2000) and the number available to participate in the infection process at the point of use (Catroux *et al*., 2001). The viable cell count of the Legumefix was within the minimum number of cells g^{-1} that a peat - based rhizobia inoculant must contain (Table 4.2). According to Olsen *et al*. (1994), the acceptable level of rhizobia required in an inoculant varies worldwide (between 10^7 and 10^9 cfu

g^{-1} carrier) and no set of common international standards exists. The pH and moisture content were also within the survival range of rhizobia.

5.3 Soybean growth, yield and nutrient uptake

5.3.1 Effect of Legumefix and Teprosyn Mo on nodule dry weight

In Kpongu, Legumefix increased nodule dry weight significantly over the control and Teprosyn Mo (Fig. 4.1). This is in agreement with Katulanda (2011), who observed that rhizobia inoculation significantly increased nodule dry weight of soybean over control. According to Jonas *et al*. (2011), response to inoculation by soybean is strongly influenced by the number of effective rhizobia in the soil. However, Singleton and Tavares (1986) and Thies *et al*. (1991) had shown that soybean growing in a soil with a small number of effective rhizobia (20 - 50 cells g^{-1} soil) will likely respond to inoculation. The results also indicated that Teprosyn Mo treated plots (Fig. 4.1) produced the lowest nodule dry weight. This is in agreement with the results of Jongruaysup *et al*. (1993) who reported that the total number of nodules per plant was reduced by molybdenum application. It can also be attributed to the acidic nature of the soil (pH 5.53) in the study area. Molybdenum serves an additional function to help root nodule bacteria to fix atmospheric N in legumes (Campo *et al*., 2000). In the soil solution, Mo exists as an anion at soil pH above 4 (Lindsay, 1991) but becomes deficient under pH levels below 6.0 (Reddy *et al*., 1997). Several reports have indicated no improvement or toxicity from Mo seed - coating through suppressive effects of salts used as Mo sources on *Bradyrhizobium* affecting bacterial survivability and nodulation (Albino and Campo, 2001; Campo *et al*., 2000). This observation was however, in contrast with those of Krishnappa *et al.*

(1992) and Noor *et al.* (1997) who indicated that nodule number and dry weight of cowpea were increased by Mo application and enhanced the rate of nitrogen fixation.

In Manga, Legumefix recorded the highest nodule dry weight rhizobia (Fig. 4.1). However, the lowest indigenous rhizobia population (2.79×10^1) was observed in Manga soil and as such responded significantly to rhizobia inoculation. According to Slattery and Pearce (2002), where there are low (< 50 Rhizobium bacteria g m^{-1} soil) naturalised populations of rhizobia specific to a target legume, the introduction of new strains by seed inoculation is normally successful. Therefore the introduced strain provided enough viable and effective rhizobia to participate in the infection process (Catroux, 2001) for higher nodulation. Nodule dry weight in the sole Teprosyn Mo and combined Teprosyn Mo + Legumefix treatments did not differ from the control in Manga (Fig. 4.1). This could be explained by the high acidic level of the soil as stated by Reddy *et al*. (1997) that, in acidic soils (pH < 5.5) Mo availability decreases as anion adsorption to soil oxides increase. According to Albino and Campo (2001), due to the saline or acidic sources of micronutrients, seed treatment with Mo can damage the rhizobia, drastically affecting the survival of inoculated bacteria on the seeds, thus resulting in reduced nodulation and N_2 fixation (EMBRAPA, 2006).

No significant differences in nodule dry weight were observed among the treatments in Nyankpala (Fig. 4.1). This agrees with the findings of Okogun *et al*. (2005) and Chemining'wa *et al*. (2007) who reported no significant increase in nodulation following rhizobia inoculation. Soils in this study site recorded the highest indigenous rhizobia population of 4.36×10^1. High population density of indigenous and naturalized rhizobia population of 1 x 10^2 rhizobia cell g^{-1} soil has been reported to prevent nodulation and displace applied inoculums (Graham, 1981; Singleton and

Tavares 1986; Brockwell *et al*., 1995). This suggests that failure of this soil to respond to rhizobia inoculation was primarily due to the presence of sufficient number of indigenous rhizobia population to adequately compete with the introduced strain for nodule occupancy (Al-Falih, 2002). Since the indigenous rhizobia are present through the soil while the introduced rhizobia are only present on the seed coat, there is the competitive advantage of the native rhizobia over the introduced strain (Denton *et al*., 2002).

Results of this trial also support the findings of Singleton and Tavares (1986) which showed that indigenous rhizobia populations, with a range of effectiveness from ineffective to highly effective, are capable of meeting crop N demand as long as they are present in sufficient numbers to adequately nodulate the host. On the contrary, a study by Saginga *et al*. (2000) in the moist savanna zone of Nigeria noted that, promiscuous soybean is incapable of nodulating effectively with indigenous rhizobia in all study locations. Bala (2008) equally observed that, it is not clear whether promiscuous soybean cultivars are effectively nodulated by indigenous rhizobia populations in all soils and under all conditions. Fening and Danso (2002) concluded that, *Bradyrhizobia* number and effectiveness vary considerably among locations which may have accounted for the different responses of inoculation on nodule dry weight in the study sites. Eaglesham (1989) also reported that promiscuous soybean varieties show inconsistent response to inoculation. Soil pH among other factors could also contribute to lack of response to rhizobia inoculation (Vinuesa *et al*., 2003; Shamseldin and Werner 2004, 2005; Shamseldin, 2007). According to Brady *et al*. (1990), in acidic soils root systems are poorly developed, form little fine branching roots, which may result in a low number of infection sites, and therefore limit the infection process of the *Bradyrhizobium*.

5.3.2 Contribution of Legumefix and Teprosyn Mo on root dry matter of soybean

The results of the study indicated that addition of Teprosyn Mo, Legumefix and Teprosyn Mo + Legumefix did not have significant effect on root dry weight in Kpongu (Fig. 4.2). This could be due to the poor fertility status of the site especially soil P. Under low soil P, photosynthetic activity is reduced due to the strong competition imposed by the symbioses (Marschner and Dell, 1994), as root growth can be influenced negatively (Piccini *et al*., 1988).

Significant differences were however observed among the treatments in Manga with the highest root dry weight observed in Teprosyn Mo + Legumefix (Fig. 4.2). Physiological function studies by Marschner (1995) and Liu (2000), confirms that Teprosyn Mo indirectly affected the growth of plant roots by enhancing nitrogenase activity, nitrate reductase activity in Legumefix and growth of the nodule of the soybean roots. No significant differences in root dry weight were observed among the treatments in Nyankpala (Fig. 4.2). Eswaran *et al*. (1997) reported that acidic soils characterised by low pH and excess of Al and Mg affects plant roots growth by disrupting the metabolically active cells at the apex which would result in inhibition of root enlongation (Ryan *et al*., 2005; Ciamporová, 2002).

5.3.3 Contribution of Legumefix and Teprosyn Mo on shoot dry matter of soybean

No significant differences in shoot biomass were observed among treatments tested in all the study sites (Fig. 4.3). This is in agreement with Okogun *et al*. (2004, 2005) who reported that inoculation of soybean variety (TGx1448-2E) did not significantly increase the shoot yield. The absence of response to product application on shoot

biomass could be attributed to unfavourable rainfall conditions which occurred during the growing period. A temporary drought, low and poorly distributed rainfall during crop growth in all study sites could be contributing factors (Appendices 4 - 6). It has been suggested that reduced shoot growth of plants whose roots have been subjected to stress such as deficient soil water, deficient aeration, high salinity, or low temperature is caused at least in part by a change in the amount and kind of growth regulators supplied from the roots (Davies and Zhang, 1991). In addition, the existence of low soil fertility of the zones especially N and P could also be a factor. According to Jakobson (1985), low P availability in soils results in a decrease in shoot growth and affects the photosynthetic activity. Although Teprosyn Mo contained some amount of P (15% P_2O_5), it was too little to supplement or compensate for the P deficiency.

5.3.4 Soybean grain yield as influenced by Legumefix and Teprosyn Mo

Treatment effects on grain yield in Kpongu were not significantly different from the control (Fig. 4.4). The increase in nodule dry weight by Legumefix and Teprosyn Mo + Legumefix, did not translate into grain yield. A study by Furseth *et al*. (2012) also confirms that soybean yield did not differ between inoculant products and the controls. The lack of grain yield response could also be attributed to the temporary drought during plant growth and mineral nutrient deficiencies (N, P) of the site which are the major constraints limiting legume N_2 fixation and yield (O'Hara *et al*., 1988). Schultz and Thelen (2008) suggested a potential negative yield response from inoculation under extreme drought conditions occurring during pod fill due to an increased vegetative sink. Salvagiotti *et al*. (2009) suggested a pre - plant field application of deep banded slow - release urea to ensure nodulation is not inhibited and a supplemental nitrogen application at a rate of 22 kg N ha^{-1} at the R3 growth

stage is recommended by Wesley *et al*. (1998). This is because, pods and seeds require great amounts of nitrogen respective to the rest of the plant, containing around 75% of the total nitrogen in the plant which suggest the greatest demand for nitrogen in the R3 to R5 soybean growth stages (Zapata *et al*., 1987). Dahmardeh *et al*. (2010) and Morad *et al*. (2013) reported that, seed inoculation with proper rhizobium strain together with minor amounts of phosphorus at early growth stage stimulate root nodulation and increase biological nitrogen fixation eventually improving yield components such as number of branches per plant, number pods per plants, number seeds per pod and seed weight.

In Manga, significant differences were observed among treatments for grain yield with Teprosyn Mo + Legumefix recording the highest (Fig. 4.4). This is in confirmation with a multi-locational trial by Johansen *et al*. (2006b) at farmers' fields in Bangladesh, which showed that, the efficiency of seed treatment with Mo may be further enhanced by adding rhizobium as yield increases were 37% - 90% over the untreated control. According to Campo *et al*. (2000), Mo in legumes serves as an additional function to help root nodule bacteria to fix atmospheric N resulting in increased yield which accounted for over 200% increase in grain yield. Significant effect of rhizobia inoculation on legume yield is extensive (Okereke *et al*., 2001; Egamberdiyeva *et al*., 2004; Hayat *et al*., 2004; Kazemi *et al*., 2005; Fatima *et al*., 2006; Ndakidemi *et al*., 2006; Abbasi *et al*., 2008; Shahid *et al*., 2009; Ibrahim *et al*., 2011; EI-Shaarawi *et al*., 2011; Abdul-Jabbar and Saud, 2012; Shiri-Janagard *et al*., 2012; Solomon *et al*., 2012). Legumefix, a rhizobia inoculant helps to boosts the natural population of beneficial nitrogen - fixing bacteria to form effective nodules that are responsible for effective BNF (Chen, 2008) and explains the over 100% increase in grain yield compared to the control. This is in confirmation with a series

of field experiments in DR - Congo and Nigeria which resulted in significant yield increases of 80 – 300% with inoculation (Bala, 2008; Ranga-Rao *et al*., 1981)

Significant differences were observed among treatments in grain yield in Nyankpala, while no significant differences were observed in nodulation (Fig. 4.4). Although Teprosyn Mo + Legumefix and Legumefix increased grain yield by 42% and 22% over the control, these increases could not meet the current estimated yield (1.6 t ha^{-1}) and potential yield (2.5 t ha^{-1}) of the soybean variety "Jenguma" (Dugje *et al*., 2009). On the other hand, Teprosyn Mo recorded the lowest grain yield compared to the control. The varied correlation between the nodulation and grain yield could partly be attributed to the lower soil pH of the experimental site. A study by Giller (2001), Chen (2006) and Shamseldin (2007), reported the influence of soil pH as it induces deficiency in some essential nutrients such as P and Mo and also affects the survival of inoculum, distribution of rhizobia, root infection and nodulation and leads to the reduction of number and sizes of nodules (Marschner, 1995). The presence of high numbers of indigenous rhizobia population could also have interfered with nodulation. Since the indigenous rhizobia are present through the soil while the introduced rhizobia are only present on the seed coat, there is the competitive advantage of the native rhizobia over the introduced strain (Denton *et al*., 2002).

5.3.5 Nitrogen and phosphorus partitioning into soybean shoot biomass and grain

The experiment showed that Legumefix and Teprosyn Mo had no significance effect on shoot N compared to the control in all the study sites (Table 4.3). This finding is in contrast with Tahir *et al*. (2011) who observed significant increase in shoot N

concentration of mungbean with sole application of Mo, rhizobia inoculation and the combined effect of the two treatments.

Application of Teprosyn Mo, Legumefix and Teprosyn Mo + Legumefix did not cause significant ($P > 0.05$) increase in soybean grain N uptake in Kpongu (Table 4.4). The lack of significance could be attributed to the low fertility status of the soil (Sanginga *et al*. 1996). Teprosyn Mo + Legumefix, Legumefix and Teprosyn Mo recorded increased N uptake in soybean grain compared to the control in Manga (Table 4.4). This finding is in agreement with the results of Okogun *et al*. (2005) and Bhuiyan *et al*. (2008) that rhizobia inoculation and seed treatment with Mo result in significant increase in grain N uptake over control. Ruiz-Diaz *et al*. (2009) noted that plants with successful symbiosis with *B. japonicum* possess greater plant nitrogen concentration and total plant nitrogen compared to those with poor nodulation.

The combined effect of Teprosyn Mo + Legumefix resulted in significantly ($P < 0.05$) higher grain N uptake relative to Teprosyn Mo while Legumefix alone did not differ from the control in Nyankpala (Table 4.4). The result agrees with the findings of Rabbani *et al*. (2005) who found that the application of rhizobia inoculant in combination with Mo gave statistically significant grain N increase. However, Okogun *et al*. (2005) noted significant increase in grain nitrogen in inoculated plants over uninoculated plants.

No significant differences were observed in treatments on soybean shoot P uptake in Kpongu although the application of Teprosyn Mo, Teprosyn Mo + Legumefix and Legumefix treatment resulted in higher shoot P over the control (Table 4.5). This is in contrast with the findings of Ndakidemi *et al*. (2011) who reported that *Bradyrhizobium* inoculation enhance the uptake of P, K, Ca, Mg, S, Mn, Fe, Cu, Zn,

B and Mo in leguminous plants. Therefore, the lack of significant response in this current research is likely due to the pH and low P levels of the study site (Table 4.1). According to Schachtman *et al*. (1998) and Hinsinger (1998) an increase in rhizosphere pH would decrease P uptake, which has an optimum pH between 5 and 6 and at the same time might favour the formation of relatively insoluble P compounds. In addition, low P might reduce photosynthetic activity due to the strong competition imposed by the symbioses (Marschner and Dell, 1994), which might affect root growth negatively resulting in the incapability of roots to take up sufficient amount of nutrients to the plant tissues (Piccini *et al*., 1988).

Significant ($P < 0.05$) differences were observed among the treatments on shoot P uptake in Manga with the highest shoot P uptake obtained in Legumefix application (Table 4.5). The application of Teprosyn Mo + Legumefix and Legumefix increased root development and may have enhanced its ability to take up significant amount of P into the soybean tissues in Manga. This finding is in agreement with a research by Yan *et al*. (1995a) who concluded that common bean genotypes performed best on low P - available soils with the greatest root mass. According Lopez-Bucio *et al*. (2005a), the root system is important for plant fitness because it provides anchorage, contributes to water use efficiency and facilitates the acquisition of mineral nutrients from the soil.

The only significant difference in shoot P uptake in Nyankpala was recorded by Teprosyn Mo and the control (Table 4.5). According to Nyemba (1986), total phosphorus uptake of plants that were inadequately nodulated were not significantly different from the control indicating that, inadequately nodulated plants were still dependent on nitrogen from the soil the availability of which increased with level of phosphorus in the soil. Research have shown that one of the major consequences of

inadequate P is a decrease in the energy metabolism of the plant and a central reason for this response is the decrease of Pi supply to the mitochondria for the phosphorylation of ADP in ATP (Adu-Gyamfi, 2003). Sa and Israel (1991) reported that low levels of P resulted in low levels of ATP and energy charge in the plant - cell fraction of soybean nodules. In their study, the negative influence of low P availability on energy metabolism resulted in greatly decreased nitrogen fixation activity.

The study did not record any significant difference on soybean grain P uptake among the treatments in Kpongu and Nyankpala, however, Teprosyn Mo + Legumefix and Legumefix significantly increased grain P uptake over the control in Manga (Table 4.6). Okogun *et al*. (2005) reported a significant increase in seed P due to rhizobium inoculation. Hammond *et al*. (2009) noted that, P concentrations in plant tissues are determined largely by the ability of the roots to acquire P from the soil and tissue growth rates. From the root dry matter analysis (Fig. 4.2), no significant differences were obtained with treatment application in Kpongu and Nyankpala while significant differences were found in Manga. This explains that Teprosyn Mo + Legumefix and Legumefix application increased root development to take up significant amount of P into the soybean tissues in Manga but not Kpongu and Nyankpala.

5.3.6 Relationships between growth and yield parameters of soybean

Significant relationship were obtained between biomass dry weight and grain yield and root dry weight and nodule dry weight in Kpongu (Table 4.7). Van den Boogaard *et al*. (1996) observed that a higher biomass production will lead indirectly to a higher grain production. This is in line with Mollasadeghi (2010), who found significant correlations between biomass yield and grain yields. A study by Fabián *et*

al. (2008) also showed high correlation between the nodules weight and the root dry weight. In Manga, no significant correlations were evident between measured variables (Table 4.8) and this may be due to prevailing environmental conditions such as moisture stress which according to Simane *et al*. (1993) results in lower rates of dry matter accumulation, irrespective of the time the stress occurred. A significant correlation was found between root dry weight and nodule dry weight in Nyankpala (Table 4.9). Similar results were found by Kahn (1991), who observed that roots were important as loci for nodulation.

5.4 Returns on investment from Legumefix and Teprosyn Mo

Morris *et al*. (2007) defined profitability when the value cost ratio was 2 or higher. None of the products tested in Kpongu and Nyankpala were profitable as the treatments had a value cost ratio of less than 2 (Table 10). This was probably due to the lack of significant differences between treatments on grain yield except Teprosyn Mo + Legumefix in Nyankpala. However, Teprosyn Mo + Legumefix treatment was the most profitable among the treatments in Manga as it resulted in a value cost ratio of 2.65 (Table 10) and the profitability of this treatment is likely related to grain yield increment.

5.5 Maize growth, yield and nutrient uptake

5.5.1 Maize plant height as affected by Eco - T and Teprosyn Zn/P

No significant differences were observed among the treatments in all the study sites on maize plant height (Table 4.11). The lack of response of Eco- T, Teprosyn Zn/P and their combined application observed in this study could be attributed to the inherently low soil fertility level of the study sites. Grant *et al*. (2001) reported

decreases in plant height as one of symptoms of P deficiency. The low organic matter content of the study sites could also have resulted in the decline of plant height in Teprosyn Zn/P application as low organic matter content in soils give rise to Zn deficiency. According to Katyal and Randhawa (1983) an increase in the organic matter content of a soil will increase its Zn availability. Several researchers have reported that water stress imposed during the vegetative growth phase reduces plant height (Yang and Hsiang, 1992; Abrecht and Carberry, 1993). This result confirms the study by Pholo and Pretorious (2011) who reported no significant effect on maize growth after Teprosyn Zn treatment. It was further explained that, Teprosyn Zn/P contributed to a slight inhibition of seedling growth which strongly suggests that Teprosyn Zn/P most probably has a stimulating effect on seed vigour, germination and early seedling growth, but not on sustainable seedling growth following germination and might be connected to an inhibitory effect on natural growth hormones found in plants.

5.5.2 Maize stover yield as affected by Eco - T and Teprosyn Zn/P

None of the treatments produced significant effects on stover yield in all the study sites (Table 4.12). This is however contrary to studies by Ozbay and Newman (2004), who observed an increase in dry shoot mass due to *Trichoderma* inoculation. Zirhahwakuhingwa (2012) however confirmed the non-significant effect of Teprosyn Zn/P on maize shoot biomass at harvest in a previous study.

The lack of response to *Trichoderma* inoculation is explained by Windham *et al*., (1986) who reported that when soil fertility is increased, the level of increased tomato growth induced by *Trichoderma spp*. was enhanced through the addition of fertilizer which could have provided substrate for proliferation of the fungus thereby increasing its overall performance (Okoth *et al*., 2011). The poor fertility

status of the study sites might have affected the multiplication of the fungus in the soils and Zn availability in terms of organic matter contents of the soils (Katyal and Randhawa, 1983). In addition, yellow coloration of maize leaves from the base to the top at the latter stage of growth was observed in all the study sites which is a major indication of N deficiency. Work done by Ding *et al*. (2005) on effects of nitrogen deficiency on photosynthetic traits of maize hybrids concluded that N deficiency decreased stover weight and grain yield in maize. Total rainfall amounts in all the study sites during the growing season was below 200 mm (Appendices 4 - 6)), which is below the generally required amount (500 - 700 mm) for maize production. Prabhu and Shivaji (2000) reported drought especially in the vegetative period of maize as the main effect of reduction in leaf and stem growth as the crop intercepts less sunlight. Although the enhanced growth responses of several plants following application of *Trichoderma spp*. and Zn under abiotic or physiological stresses have well been documented (Harman, 2000; Harman *et al*., 2004; Yildrim *et al*., 2006; Mastouri and Harman, 2009; Mastouri *et al*., 2010; Shoresh *et al*., 2010; Potarzycki, 2009), this study however, did not show significant effects in stover yields, among the treatments in the different study sites.

5.5.3 Nitrogen and phosphorus partitioning into maize stover and grain

The application of Teprosyn Zn/P, Eco - T and the combined treatment of Teprosyn Zn/P + Eco - T on stover N uptake did not produce significant effect in relation to the control in all the study sites (Table 4.13). This is in contrast with a study by Nzanza *et al*. (2011) who reported positive effects of *Trichoderma* inoculation on shoot N in tomato seedlings. Harman *et al*. (2004) reported that *Trichoderma* spp. increased N uptake in maize shoot through enhanced root growth or availability of necessary nutrients leading to growth of the plants. Altomare *et al*. (1999) attributed increased

nitrogen concentrations in tomato and cucumber shoots inoculated with *T. harzianum* to increased absorptive area observed in the plants as a result of fertilization effect rather than nutrient solubilization of *T. harzianum* inoculation since significant results were obtained with addition of sufficient levels of minerals to the soil. Yedidia *et al*. (2001) also reported the effect of *T. asperelloides* on concentration of minerals and increased plant growth through higher nutrient uptake, but the mechanism was not established. Harman (2000) further explains that *T. harzianum* increases the efficiency of nitrogen use in maize and other crops, resulting in improved yields. The absence of the effects of Teprosyn Zn/P, Eco - T and Teprosyn Zn/P + Eco - T on stover N uptake was expected since the study sites were low in soil nutrients.

The study did not show any significant differences in N uptake in maize among the treatments in all the study sites (Table 4.14). Shoresh *et al*. (2010) however recorded significant increases in nitrogen content with *Trichoderma* treated plants.

The absence of the effects of the Teprosyn Zn/P, Eco - T, and Teprosyn Zn/P + Eco - T on grain N uptake was due to the low N uptake in the stover as a reflection of the low % total N of the study sites.

Application of Teprosyn Zn/P, Eco - T, and Teprosyn Zn/P + Eco - T did not cause any significant increase in stover P uptake of maize in all the study sites (Table 4.15). This trend was expected since the study sites had low available P. According to Waswa (2013), low available soil P can limit the production of plant biomass and phosphorus content in shoot and root. However, P concentrations in all treatments fell within the sufficiency range (0.4 - 0.8%) established for shoot of maize plants (Vandamme, 2008).

The study did not record any significant differences in maize grain P uptake among the treatments tested (Table 4.16). Hammond *et al*. (2009) noted that, P concentration in plant parts is determined largely by the ability of the roots to acquire P from the soil and tissue growth rates. Although *Trichoderma spp*. are known as opportunistic root colonizing fungal plant symbionts (Harman *et al*., 2004) that induce numerous changes in plant gene expression and physiology among which are increased systemic resistance to plant diseases, increased growth of plants and roots, including an increase in fertilizer use efficiency and uptake (Yedida *et al*., 2001; 2003) it did not contribute to increased P concentration in maize grain. The absence of significant response on grain P uptake could be attributed to the the inability of maize root to acquire P due to initial low available P of the study sites.

5.5.4 Maize grain yield as affected by Eco - T and Teprosyn Zn/P

Teprosyn Zn/P, Eco - T and the combined effect of Teprosyn Zn/P + Eco - T applied in all the study sites had no significant ($P > 0.05$) effect on maize grain yield (Table 4.17). The lack of response was similar to the results of Ugur and Sureyya (2008) where *T. harzianum* did not elicit significant response on lettuce yield. Varietal responses of plants to *Trichoderma spp*. have also been reported (Harman 2006; Ruocco *et al*., 2007) and could partly be a factor. In relation to Teprosyn Zn/P treatment, Suresh *et al*. (2013) observed that genotypes vary in their magnitude of response to Zn as a particular genotype may grow well while another genotype may produce much lower yields in the same soil. The absence of response of the treatments to grain yield could also be due to the generally low rainfall pattern (Appendices 4 - 6) together with low soil moisture for plant growth in the course of the planting season. According to Njiru *et al*. (2006), this situation is further worsened by the slow but evident changes in temperature and rainfall in a hotter and

drier environment. Several researches have shown that soil moisture deficiency that causes wilting for 1 - 2 days during tasseling can reduce yield by up to 28% and 6 - 8 days wilting stage can cause a reduction in yield of about 50%, which cannot be made up by later precipitation or irrigation (Tweneboa, 2000). Prabhu and Shivaji (2000) and Bänziger *et al*. (2000) also observed the sensitivity of maize to moisture stress and around flowering. With reference to the fertility status of the study sites, Pandey *et al*. (2000) observed that, nitrogen supply has substantial effects on plant growth, development and grain yield, as it is a fundamental constituent of many leaf cell components, particularly those associated with the photosynthetic apparatus, including carboxylating enzymes and proteins of membranes. A report by Muchow (1994) showed that the response of grain yield to N deficiency was associated with much larger effects on biomass production. Zelonka *et al*. (2005) also attributed an increase in grain yield to the promotion of the formation of photosynthetic pigments and therefore higher rate of photosynthesis.

5.6 Returns on investment from Eco - T inoculant and Teprosyn Zn/P

The profitability of the treatments used in the study sites were defined when the value to cost ratios was 2 or higher (Morris *et al*., 2007). The value cost ratio varied between sites. None of the products applied in Kpongu were profitable (Table 4.18). The application of Eco - T and Teprosyn Zn/P + Eco - T was highly profitable in Manga and Nyankpala as it resulted in a value to cost ratio above 2, but not Teprosyn Zn/P (VCR < 2).

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

This study has shown that, in Kpongu, only Legumefix had significant effect on nodulation. Teprosyn Mo, Legumefix and Teprosyn Mo + Legumefix had no significant effect on root dry weight, shoot biomass and grain yield of soybean.

In Manga, Legumefix produced nodule dry weight (371 mg plt^{-1}) which was significantly higher than the control (227 mg plt^{-1}). Teprosyn Mo + Legumefix (2.99 kg ha^{-1}) and Legumefix (1.82 kg ha^{-1}) however, produced significantly higher grain P uptake in soybean compared to the control (1.07 kg ha^{-1}). Biomass dry weight and shoot N uptake were not affected significantly by the imposition of Teprosyn Mo, Legumefix and Teprosyn Mo + Legumefix.

No significant differences were observed in Nyankpala between Teprosyn Mo, Legumefix, Teprosyn Mo + Legumefix and the control for nodule dry weight, root dry weight, biomass dry weight and shoot N and grain P uptake. Percentage increases of 22.43 and 42.10% were recorded in grain yield for Legumefix and Teprosyn Mo + Legumefix respectively over the control. Furthermore, percentage increases of 18.67 and 33.33% were recorded in grain N uptake for Legumefix and Teprosyn Mo + Legumefix respectively over the control. Teprosyn Mo (3.46 kg ha^{-1}) increased shoot P uptake significantly over the control (2.14 kg ha^{-1}), Legumefix (1.07 kg ha^{-1}) and Teprosyn Mo + Legumefix (0.98 kg ha^{-1}).

The economic analysis showed Teprosyn Mo + Legumefix to be the most profitable treatment (VCR = 2.65) in terms of input costs and benefits in increased grain yield in Manga.

Teprosyn Zn/P, Eco - T and Teprosyn Zn/P + Eco - T had no significant effect on plant height, yield and N and P nutrient uptake of maize in all the study locations. However, the economic analysis of the study showed that the application of Eco - T and Teprosyn Zn/P + Eco - T were highly profitable (VCR > 2) in both Manga and Nyankpala. The profitability of Eco - T was however, likely related to its relatively low application rate. Nevertheless, the use of Teprosyn Zn/P resulted in a value cost ratio less than 1 in Kpongu, Manga and Nyankpala.

It is therefore recommended that to achieve maximum benefits from these products at the study locations, both N and P sources should be applied to the soil as a starter in order to ensure good plant establishment. There is also the need for extensive agro - ecological zone evaluation of the agricultural commercial products because of the varied responses of these products in the various agro - ecological zones.

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APPENDICES

Appendix 1. Source and composition of treatments tested

Product	Producer	Rate of Application (per ha^{-1})	Details
Teprosyn Mo	Yara Ltd., UK	20 mL (kg seed)$^{-1}$	P_2O_5 (15 %), Mo (15.5 %), mix 1 day prior to planting
Legume fix	Legume Technology Ltd., UK	4.0 g kg^{-1} seed, peat - based inoculant	*Rhizobium spp.*, coat the seed at the planting time
Teprosyn Zn/P	Yara Ltd. UK	20 mL (kg seed)$^{-1}$	N (4%), P_2O_5 (12%), Zn (19.4%) mix 1 day prior to planting
Eco - T / T22	Plant Health Care Inc., USA	seed treatment at 1.5 g per kg of seed	*Trichoderma harzianum* strain, Rifai KRL AG2

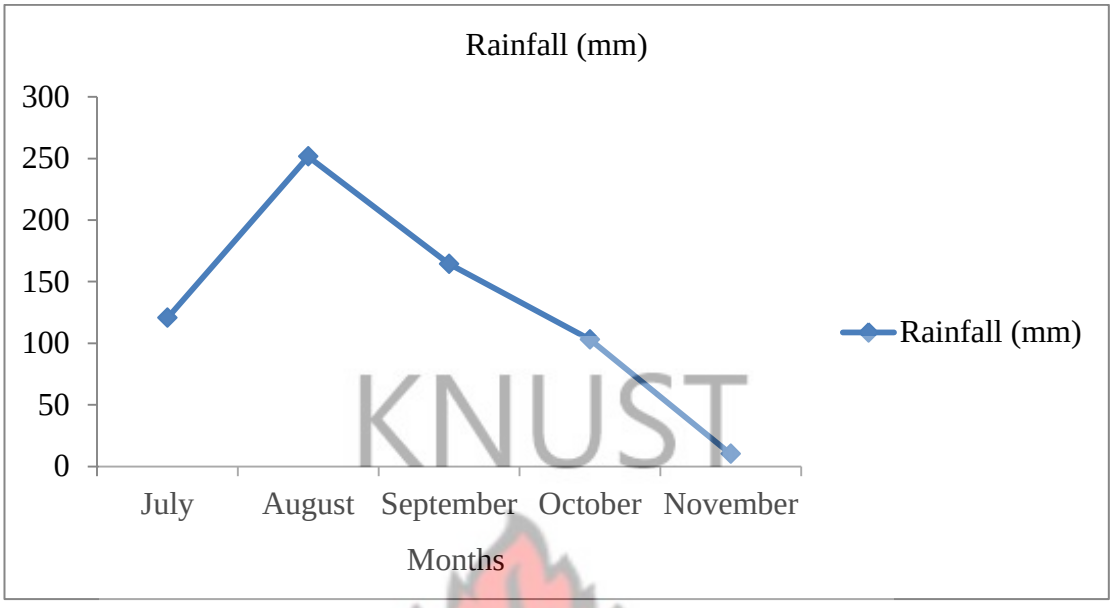
Appendix 2. Nutrient composition of the modified Broughton and Dillworth's nitrogen - free mineral solution

Stock	Compound	Amount g / L	Active Ingredient
1	$CaCl_2.2H_2O$	294.1	Ca
2	KH_2PO_4	136.1	P
3	Fe-citrate	5.4	Fe
	$MgSO_4.7H_2O$	123.3	Mg
	K_2SO_4	87.0	K
	$MnSO_4.H_2O$	0.338	Mn
4	H_3BO_3	0.247	B
	$ZnSO_4.7H_2O$	0.288	Zn
	$CuSO_4.5H_2O$	0.1	Cu
	$NaMoO_2.2H_2O$	0.048	Mo
	$CoSO_4.7H_2O$	0.056	Cu

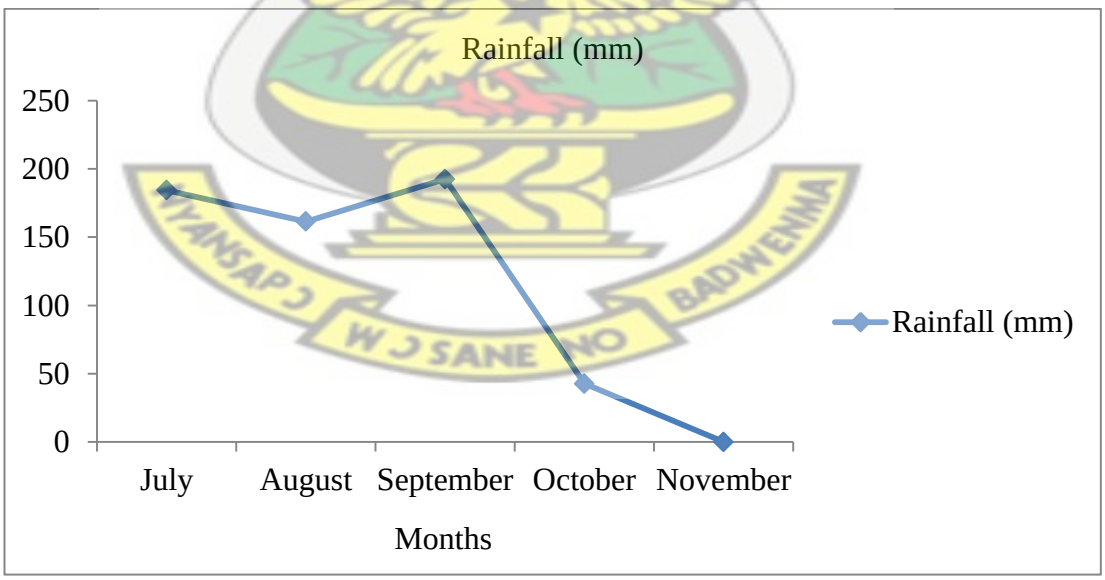
Appendix 3. Nutrient composition of standard YMA

Stock	Compound	Amount
1	K_2HPO_4	0.5 g
2	$MgSO_4.7H_2O$	0.1 g
3	NaCl	0.2 g
4	Mannitol	10.0 g
5	Yeast extract	0.5 g
6	Agar	15 g
7	Distilled water	1 L

Appendix 4. Rainfall distribution during the 2013 major cropping season at Kpongu



Appendix 5. Rainfall distribution during the 2013 major cropping season at Manga



Appendix 6. Rainfall distribution during the 2013 major cropping season at Nyankpala