RESPONSE OF COMMON BEAN AND GROUNDNUT TO CO-INOCULATION OF TRICHODERMA AND RHIZOBIA IN AN ACID SOIL

By

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Integrated Soil Fertility Management

The University of Zambia

February, 2019

DECLARATION

I, Gwen Chilombo, hereby declare that all work presented in this dissertation is my own and has never been submitted for a degree at this or any other university.

Signature…….... Date……...

CERTIFICATION OF APPROVAL

The University of Zambia approves this dissertation of Ms. Gwen Chilombo as fulfilling part of the requirements for the award of the degree of Master of Science in Integrated Soil Fertility Management.

Examiner	Signature	Date
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DEDICATION

This dissertation is dedicated to my family who encouraged and supported me throughout this period.

ACKNOWLEDGEMENTS

I would like to acknowledge my God for having given me this opportunity and for having given me strength and seen me through even in the very hard times. I would also like to acknowledge my supervisors Dr. A. M Mweetwa and Dr. B. H. Chishala for their contribution and hard work for which I will be eternally grateful. Special thanks go to the lecturers and supporting staff in the Departments of Soil Science and Plant Science for their support and availability during the course of my research. I am grateful to AGRA for providing funding that made this research possible.

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ABSTRACT

Common bean and groundnut are an important source of protein, fat, carbohydrates, vitamins and minerals for both humans and livestock. These legumes are produced mostly by smallholder farmers whose current yields are far below the potential yield of 2 t/ha for both legumes due to biological constraints, poor soil fertility and high soil acidity. Soil acidity poses the greatest challenge as it also affects availability of important nutrients such as phosphorus. This study investigated the sole and dual inoculation of Rhizobia and Trichoderma to common bean and groundnut in an acid soil. The soil in which these two crops were grown was characterized for: soil reaction, available phosphorus, total nitrogen, cations organic matter and soil respiration before planting and at harvest using standard laboratory procedures. Soils were amended with nitrogen (N) and phosphorus (P) fertilization at the rates of 100 kg N and 80 kg P_2O_5 per ha and seeds were dressed with; 1 g/kg of seed of *Trichoderma harzianum*; 100 g/kg of seed of *Rhizobium tropici*; or a combination of both inoculants at the recommended rates at planting. The experiment was set up in the greenhouse using the Completely Randomized Design (CRD) with the above amendments' and a control without any amendment. The experiment consisted of two sets, one was nodulation and nodule effectiveness which were determined at 51 days after planting and the other was nitrogen and phosphorus accumulation in the above ground biomass, biomass and grain yield which were determined at maturity. To determine differences among soil amendments, data was analysed using Analysis of Variance and Least Significant Difference at 95 % confidence limit. Relationships among parameters were determined using correlation analysis. The results showed that amending soils with inorganic N at a rate of 100 kg N per hectare can depress nodulation even in the presences of high levels of inorganic P. Inoculating common bean and groundnut with the both Rhizobia and Trichoderma increased nodule activity. However, this increase did not translate to an increase in biomass or grain yield. The study also showed a low phosphorus accumulation in Trichoderma inoculated plants in both common bean and groundnut. Therefore, further investigations to study the extent of colonization and accompanying changes in root volume in common bean and groundnut inoculated with Trichoderma are recommended in order to understand the full effect of the Trichoderma.

CHAPTER 1: INTRODUCTION

1.1 Background

Legume production in Zambia is second only to cereal production. Two important legumes commonly grown in Zambia are common bean (*Phaseolus vulgaris*) and groundnut (*Arachis hypogaea*). These are a nutritious source of fat, proteins, carbohydrates, vitamins and minerals for both human beings and livestock (Katundu *et al.,* 2014). Groundnut are a good source of vegetable oil (Egbenni *et al.,* 2010), while the bean provides an alternative source of protein to animal protein. These legumes can sustain soil fertility through their ability to fix atmospheric nitrogen in the soil, especially when included in crop rotations and intercrop (Zulu, 2014).

Smaling *et al*. (1990) estimated that soils in Sub Sahara Africa are being depleted annually at rates of 22 kg/ha for nitrogen (N), 2.5 kg/ha for phosphorus (P) and 15 kg/ha for potassium (K). The decline in soil fertility is most evident in medium and high rainfall areas which are high in bean and groundnut production in Zambia. Due to high rainfall of bases are leached to the subsoil leading to an accumulation of hydrogen and aluminum ions in the soils which causes soil acidity. Soil acidity has direct and indirect effects on plant growth. The direct effect is ascribed to the presence of toxic levels of exchangeable aluminum (Al) and the indirect effect is with regard to strains of Rhizobia that may not have tolerance to soil acidity resulting in poor nodulation in some legumes (Basu *et al.,* 2008). Despite the fact that legumes enhance soil fertility through biological nitrogen fixation (BNF) (Giller *et al.,* 2011), poor legume productivity (common bean; 0.3 to 0.5 ton/ ha and groundnut; 0.5

ton/ha) on most smallholder farms in Zambia is attributed to declining soil fertility (Mweetwa *et al*., 2014).

In order to address problems associated with low soil fertility, recommended inorganic fertilizer application rates are far beyond the reach of smallholder farmers due to high cost and limited accessibility (Sikombe *et al.,* 2008). Enhancing the interaction between legumes and microbes in the rhizosphere is another way of improving soil fertility and a way of reducing the use of chemical fertilizers. The use of microorganisms in crop production has demonstrated beneficial effects on plant growth. Microorganisms such as Trichoderma fungi have been reported to increase production in some legume crops (Serna *et al*., 2011). Trichoderma help the host plant overcome different stress conditions such as low water stress and soil acidity (Parkash and Aggarwal., 2009). Trichoderma are free-living imperfect fungi that is highly interactive with plant roots (Saber *et al.,* 2009). When legumes are in a symbiotic relationship with Trichoderma, the microorganism extend the roots of the legume through their mycelia, increasing the nutrient absorbing surface area. The mycorrhizal fungus also releases enzymes and organic acids which can solubilise P in the soil, thereby increasing its availability and plant uptake. Mycorrhizal infection has a value for legumes because nodulation and symbiotic nitrogen fixation by Rhizobia requires an adequate supply of P (Saber *et al.,* 2009). Some of the stress conditions that the plant will tolerate in this symbiotic relationship are soil acidity, aluminum toxicity and water stress. This microorganism can inhibit the growth of a variety of potential pathogens such as *Botrylis cinerea, Mucorpiri formis and Fusarium undum*. All these attributes of the fungus can result in an increase crop production.

Another group of microorganisms that are beneficial in legume production are Rhizobia. These are bacteria that are very important in biological nitrogen fixation. In legumes, they form symbiotic relationships that form nitrogen fixing nodules (Antoun *et al.,* 1998). The nodules are colonized by the bacteria which then differentiate into a distinct cell type known as bacteriod capable of fixing N.

Microorganism interactions can be parasitic, symbiotic, competitive or predacious. The type of interaction among the microorganisms will determine the success of the inoculation. For microorganisms to be co-inoculated their interaction should be positive. This is because inoculants will only be successful if the microorganisms flourish when they are introduced into the soil or on the seed (Sylvia *et al.*, 2005). Co-inoculating legumes with these Trichoderma and Rhizobia has been reported to improve soil fertility and plant growth (Saber *et al.,* 2009). Their combined inoculation to plants has been shown to result in a synergistic increase in legume yield compared to individual inoculations (Morel *et al.*, 2012). This considerable increase in growth and dry weight could be attributed to the increase in uptake of nutrients such as N, P, K and micronutrients, and water by the fungus (Guru *et al.,* 2011). The increased uptake in nutrients encourages the effective functioning of Rhizobia in legumes. This enhances soil fertility as more nitrogen is fixed in the soil by the bacteria and less mineral fertilizer is required for legume production.

1.2 Statement of the Problem

Groundnut and common bean are grown mostly in areas with acid and poor fertility soils in Zambia resulting in low yields. This has necessitated the use of mineral fertilizers and increased cost of production. Despite the potential benefits of

microbial inoculants to enhance plant tolerance to low fertility and high acidity, there is limited research done in Zambia to quantify this benefit.

1.3 Objectives

The objective of this study was to determine the response of common bean and groundnut to co-inoculation with Trichoderma and Rhizobia.

1.3.1 Specific Objectives:

1. To determine the effect of co-inoculating bean and groundnut with Rhizobia and Trichordema on biomass yield
2. To determine the effect of co-inoculating bean and groundnut with Rhizobia and Trichordema on biological nitrogen fixation
3. To evaluate the uptake of N and P by bean and groundnut co-inoculated with Rhizobia and Trichordema

1.4 Justification

Bean and groundnut are widely grown by smallholder farmers in Zambia and are a major source of food and nutrition (protein) security for rural households. However, yields are low at 0.56 and 0.58 ton/ha for groundnut and common bean respectively, without adequate fertilization compared to potential yields of 2 t / ha. Co-inoculation with Trichoderma and Rhizobia could address N and P deficiencies in soil and contribute to productivity of the two crops.

1.5 Hypotheses

1) Co-inoculation with Trichoderma and Rhizobia in acid soils results in a significant increase in biomass yield of common bean and groundnut

2) Co-inoculation with Trichoderma and Rhizobium increases the biological nitrogen fixation in common bean and groundnut in an acid soil.

3) Co-inoculation of Trichoderma and Rhizobia in acid soils results in a significant increase in P and N uptake by common bean and groundnut.

CHAPTER 2: LITERATURE REVIEW

2.1 Groundnut production in Zambia

Groundnut is a self-pollinating, indeterminate, annual, herbaceous legume. Groundnut is an important oil food and forage crop that is generally distributed in tropical, subtropical and warm temperate zones (Smartt, 1994). In Zambia, it is widely grown by small scale farmers particularly women who are generally resource poor farmers (Zulu, 2014; Ross and De Klerk, 2012). Over 700,000 households grow, consume and trade groundnut in Zambia (Ross and De Klerk, 2012). Mukuka and Shipekesa (2013) reported that as of 2011/2012 farming season groundnut production was second only to maize by small scale farmers in terms of hectares planted (Table 1). A total of 248, 106 hectares nationwide were used in groundnut production in the 2013/2014 farming season in Zambia as given in Table 1. Although groundnut are grown in the whole of Zambia, half the country's groundnut are produced in the Eastern and Northern provinces during the 2009/10 farming season as shown in Table 2 (Ross and De Klerk, 2012).

Table 1: Crop production in terms of hectares planted in Zambia

Crop	Hectares planted	Crop	Hectares planted
Maize	1,375,787	**Virginia Tobacco**	9,398
Sorghum	16,829	**Burley Tobacco**	7,133
Rice	40,974	**Common bean**	110,171
Millet	39,323	**Bambara nuts**	6,765
Sunflower	65,513	**Cowpeas**	7,792
Groundnut	248,106	**Velvet bean**	132
Soya bean	55,434	**Coffee**	11
Seed cotton	133,462	**Sweet Potatoes**	47,381
Irish potatoes	988		

Source: CSO Crop Forecast – 2013-2014

Table 2: Groundnut produced per province in 2009/10 (metric tons)

Province	Groundnut production (metric tonnes)
Lusaka	1,719
Central	19,687
Southern	23,024
Western	6,120
Northern	34,859
North Western	5,229
Eastern	49,854
Copperbelt	9,466
Luapula	13,775

Source: CSO (CFS 2009/2010)

The production of groundnut relies on a nutrient supply of potassium, calcium, phosphorus, zinc, iron and magnesium (Smartt, 1994). Phosphorus is particularly critical for root growth and energy storage and transfer (Ncho *et al.,* 2013). Its importance in energy transfer and storage is due to the high Adenosine Triphosphate (ATP) requirements for nitrogenase function (Qian *et al.,* 2007). Nitrogenase enzyme complex converts dinitrogen gas to ammonia which is subsequently protonated in the presence of water to yield ammonium ion. The complex consists of dinitrogenase reductase (Fe – protein) which gathers electrons for the reaction and dinitrogenase

(Mo Fe – protein) which uses these electrons to produce ammonium, which then protonates to ammonium ion. In theory 16 ATPs are required for binding and dissociation of the iron protein and molybdenum iron protein in the nitrogenase complex (Whalen and Sampedro, 2010). Phosphorus directly affects BNF by modulating nodule growth, nodule formation and functioning.

The groundnut crop requires not less than 600mm of rainfall in a season for it to grow well; 500 -700 mm per annum will be satisfactory for good yields (Woomer, 2010). Worldwide, most of the crop is produced in areas that receive 500 to 1000 mm of rainfall (Purseglove, 1988). Areas in Zambia famous for groundnut production receive rainfall of about 1000mm. In these areas soils are highly leached with low soil pH of about 4.6 to 5 (DFID, 2005). This soil pH is below the optimal range of 5-7. Due to heavily leached soils and high acidity, production in these areas is 0.56 tonnes per hectare (CSO, 2014), lower than the potential groundnut production of 2 tonnes per hectare (Purseglove, 1988). Groundnut plants are tolerant to 5.5 - 6.5 acidity levels but require readily available Ca to prevent pops (Woomer, 2010).

Low pH levels in most groundnut production areas result in P fixation thereby reducing plant available P (Basu *et al*., 2008). Because groundnut have a very high demand for P, this deficiency results in poor groundnut productivity (Dakora *et al*., 1987).

Groundnut have been shown to be a major crop used in rotation and intercrops in conservation farming because of its ability to fix atmospheric N at 150 kg per annum (Zulu, 2014, 2010). This is more than the recommended rate for mineral fertilizer application of some crops. For nodule formation to take place, there has to be atleast

an amount of 10 – 30 kg of N /ha in the soil. Due to heavy leaching of soils in Northern, North Western and Eastern provinces of Zambia, this amount of N in the soils is not available. This limits the amount of N fixed by the crop, and reduces the potential benefits derivable from its relationship with the Rhizobia.

2.2 Bean production in Zambia

Common bean is the most important grain legume for human consumption in the world (Stajkovic *et al*., 2011). It provides a cheap source of protein, energy, minerals and vitamins (Ross and De Klerk, 2012), whose production and marketing has potential for improving rural livelihoods (Birachi *et al*., 2011). Common bean is commonly used as an intercrop with cereals in some farming systems (Birachi, 2012). An increase in the production of a cereal crop in a rotation with a legume was attributed to an increase in the total nitrogen pool in the soil that was as a result of biological Nitrogen fixed by the common bean (Hayat *et al*., 2008).

Currently common bean is cultivated on 110,739 hectares of land in Zambia Table 1. The average production yield of common bean in Zambia is 0.58 ton/ha (CSO, 2014). This is lower than the potential yield of 2 ton/ha (Gronemeyer *et al*., 2014). In Zambia, common bean is grown in agro-ecological regions II and III which receive more than 800 mm and 1000 mm of rain per annum, respectively. The crop is grown mainly in Northern, North Western and Eastern provinces, with Northern Province ranking the highest in terms of production (Birachi, 2012). Common bean grows well at pH (water) of not less than 5.8 and not more than 7.0 (Burt, 2005).

Although common bean is associated with several species of Rhizobia in a symbiotic relationship (Gronemeyer, 2014), nitrogen fixation is considered to be low (63-120

kg/ha/year) in comparison to other legumes (Stajkovic *et al*., 2011). This is because it has a short growing season (Ogut *et al.,* 2005). This makes common bean production more sensitive to low N and P soil content than other legumes. Nodulated legumes can use mineral nitrogen fertilizer application in the soil, but this reduces biologically fixed nitrogen (Woomer, 2010). Nonetheless legumes grow well if there is some mineral N available as nodules form. An amount of 10 – 13 kg of N/ha is required at planting to increase total BNF over the crops life span (Woomer, 2010).

Bean originated in the mid altitude neo-tropics and by its nature is not well adapted to soils with poor fertility (Beeds *et al*., 2014). Tropical soils are mostly poor in fertility due to rapid mineralization of organic matter caused by the warm temperatures and loss of nutrient by leaching. Poor fertility affects root and shoot growth due to deficiencies in N and P in these soils. In sub-Sahara Africa, soil nutrient reserves are being depleted because of continued nutrient mining without adequate replenishment (Mafongoya *et al*., 2006). Problems with P deficiency are particularly severe in Africa where there is the tradition of slash and burn method of cultivation (Sylvia *et al.,* 2005). Phosphorus has a considerable influence on legume – Rhizobia symbiosis, positive effects of nodulation and nitrogenase activity in common bean were observed by Stajkovic *et al* (2011). Under P – deficient conditions P fertilization will usually result in enhanced nodule number and mass and greater N_2 fixation per plant (Sylvia *et al*., 2005). Therefore, P deficiencies in soils can lead to reduced nitrogen fixation and productivity in common bean. In Zambia, beans are grown in region II and III which are high to medium rainfall areas. The soils in these areas tend to be highly leached and acid (Birachi *et al*., 2011). As a result, farming systems in Zambia that include legumes require P and lime to support

better legume growth and BNF. This increases the cost of common bean production to the small holder farmers who have to deal with other constraints such as; water stress, weed competition and damaged plants caused by disease and pests (Wagara and Kimani, 2007) which are also costly to crop management.

2.3 Microbial inoculation practices in Zambia

One of Zambia's Sustainment Developmental Goals is to reduce poverty and improve household and national food security (Mwila *et al.*, 2006 and Weitz et al., 2015). Poor soil fertility and low availability of mineral fertilizers in most African countries limits food production which results in a major development problem (UNDP, 1994). This has resulted in the introduction of programmes such as the Fertilizer Input Support Programme (FISP) in Zambia which mostly supports maize production.

Little emphasis has been put to support the use of Biofertilizers for crop production. Biofertilizers are products containing active or latent strains of soil microorganisms, either bacteria alone or in combination with algae or fungi that increase the nutrient availability and uptake of mineral nutrients (Mirparsa *et al.*, 2016). In general, they contain free-living organisms associated with root surfaces but may also include endophytes that are able to colonize the intercellular or even intracellular spaces of plant tissues without causing apparent damage to the host plant (Banayo *et al.*, 2012). The benefits of using microbial inoculants as a biofertilizer is that they do not just increase crop yield, but also their environment friendliness and can be utilized as an alternative or a complement to inorganic nitrogen fertilizers. Biofertilizers do not lead to plant and environmental pollution as maybe the case with some mineral fertilizers (Salem and Midan, 2012). The use of some inoculants as biofertilizers has

proven to be economically beneficial and necessary in all soils deficient in N through improved BNF (Chianu *et al.*, 2011). Inoculation is necessary in areas with low plant beneficial microorganisms such as Rhizobia and Mycorrhiza. In Zambia, limited work has been done on the promotion of inoculation of common bean and groundnut, while a great deal has been done on soy bean (Sikombe *et al*., 2003). Currently, an inoculant for soybean (*Glycine max*) is produced at Mt Makulu Research Station in Zambia. However, the use of this inoculant is mostly in the Central province of Zambia (UNDP, 1994). Inoculants for other crop species found in Zambia are imported; examples of these include CIAT 899, Eco-T, and TAL 1383. CIAT 899 and TAL 1383 are inoculants used in P. vulgaris production while Eco-T is an inoculant used in wheat, soy bean, potatoes, pasture, fruit trees, vegetable, tunnels, nursery crops and turf (Thies *et al.,* 1991).

2.3.1 Rhizobia

Rhizobia are heterotrophic soil bacteria that form symbiotic relationships with leguminous plants. Rhizobia are broadly classified in two categories; the fast growing (Rhizobia) and the slow growing (Bradyrhizobia) (Boss and Hemantaranjan, 2005). The genus is further classified according to the interaction the bacteria has with the plant. Not all Rhizobia nodulate all legumes, rather specific Rhizobia form symbiosis with specific legumes or groups of legumes. This matching system between Rhizobia and legumes categorizes them into cross inoculation groups (Roy *et al.,* 2006).

Rhizobia converts inert dinitrogen gas from the atmosphere to ammonium that can be used by the plant (Sylvia *et al*., 2005). Rhizobia forms associative symbioses with plants, feeding on photosynthates from the plants root system and in turn providing

nitrogen to plant by reducing N_2 to NH_3. The bacteria first invade the plant root and forms nodules in which atmospheric N is converted to plant available N. The enzyme system responsible for the conversion of dinitrogen gas to ammonium (dinitrogenase) is inactivated by free oxygen, therefore a mechanism for controlling the diffusion of oxygen into nodules is constructed (Smarrt,1994). This construction is a cost to the plant in the symbiosis of N fixation. The oxygen is needed for the production of 16 ATPs that are required to drive the reaction. This oxygen is carried by leghemoglobin in the nodules, therefore active nodules are identified by the pink colour of the oxy – leghemoglobin (Whalen and Sampedro, 2010). The quantity of N provided by the bacteria is influenced by plant genotype and age, the occurrence of pests or disease plant damage, changes in physical and chemical soil conditions and agricultural practices that affect root structure (Sylvia *et al*., 2005). Soil acidity is one of the major soil chemical influences of N fixation. This is due to the fact that most bacteria function optimally at pH 6.5 to 8.0 (in 0.01M $CaCl_2$). Soil pH levels outside this range render the bacteria inactive (Whalen and Sampedro, 2010).

In the first 2 to 3 weeks after emergence of the legume plant, an evolved complex signal exchange mechanism takes place; this allows a specific bacterial species to induce its host plant to form invasion structures called Nod factors, which are lipochitooliga saccharides, through which the bacteria can enter the legume plant (Jones *et al*., 2007). In the absence of these specific bacteria, nodulation may not take place. Therefore, an inoculation with Rhizobia culture is usually recommended particularly where the crop has been introduced recently; or has been grown for several years; or where native Rhizobia population is inadequate and/or ineffective (Roy *et al*., 2006). As early as 1857, artificial inoculation with specific bacteria was

shown to result in legume root nodulation and N fixation (Donahue et al., 1983). The optimization of an inoculant lies in the identification and matching of the legume cultivar to the Rhizobium strain (Sikombe *et al*., 2003). The specificity is the extent that the frequency of nodulation and total nitrogen content was maximized when each individual plant species was inoculated with its own isolate of Rhizobium (Elhassan *et al*.,2010*).* Inoculation with a specific strain has been shown to enhance the amount of dry weight of nodules, nitrogen fixed and yield in common bean. However, on the other hand, groundnut is a promiscuous host which often grows well without inoculation because it can be nodulated by and fix dinitrogen with a range of soil Rhizobia (Thompson, 1991). Several strains of Rhizobia have been identified with common bean such as *R. etli, R leguminosanum* bv., *R. phaseoli, R. gallicum* and *R. tropici. Rhizobium tropici* strains are more successful in acid conditions as they were recovered from bean nodules from plants in acid soils (Romero, 2003). Crops that are nodulated by the same Rhizobium strain are in the same cross inoculation group (Sylvia *et al*., 2005). However, little research has been done to determine whether common bean and groundnut are in the same cross inoculation group.

The amount of N fixed by bean and groundnut in a season can be atleast 45 kg N/ha for each crop (Donahue et al., 1983). On the Regional Bean Inoculation Programme in East Africa (Kenya, Tanzania and Uganda) inoculation of bean with effective *Rhizobia* increased grain yield by 7 to 47% (Kannaiyan, 2003). Sikombe *et al*. (2003) also showed a significant increase with the application of *R. tropici* inoculums and fertilizer application in common bean production.

In some cases, large inoculant rates of elite Rhizobia may be needed to counteract the effect of native Rhizobia (Woomer, 2010). This is necessary in cases where the native Rhizobia are able to enhance nodule formation but are not effective at fixing N. Elhassan *et al*., (2010) showed that the frequency of nodulation and total N content was maximised when Faba bean were inoculated with a strain of Rhizobium. The study also showed that inoculation with a compatible strain of Rhizobium was found to enhance nodulation, dry weight of nodules, nitrogen fixation and total N fixed in alfalfa, cluster bean, field pea and common bean (Elhassan *et al.,* 2010). This showed the importance of compatibility between the Rhizobial strain and the host plant for nodulation and N fixation. Inoculation with non-indigenous strains does not always evoke positive responses. The reason native strains prevent inoculated Rhizobia from infecting the legume could be due to many factors that include: the inoculated strains are ineffective strains; the presence of antagonistic Rhizobia which tend to reduce the number of desirable/ beneficial strains of Rhizobia in the rhizosphere; soil conditions such as acidity and salinity and the presence of large populations of effective indigenous strains of Rhizobia strains (Kannaiyan, 2003). Therefore, co-inoculating Rhizobia with a microorganism that helps the plant tolerate certain conditions could be very beneficial.

2.3.2 Trichoderma

Trichoderma are in the group of a symbiotic fungi known as mycorrhiza. Mycorrhiza refers to a variety of symbiotic associations between plants and fungi that colonize the cortical tissue of roots during the active plant growth stage (Sylvia *et al*., 2005). There are two types of mycorrhiza; ectomycorrhizal and arbuscular mycorrhizal. Arbuscular mycorrhizal are typically associated with angiosperms while

ectomycorrhizal are associated with gymnosperms (APS, 2000). These organisms depend on photosynthates from the shoots of a plant for growth and function. Phosphorus deficiency in particular, stimulates mycorrhiza proliferation of the roots in plants. When there is phosphorus deficiency, there is an increased rate of exudation of reducing sugars and amino acids from the shoot to the root (Johansen *et al*., 1994). The formation of the symbiosis enhances the acquisition of P by plants through colonization of regions that lie beyond the rhizosphere (Clarkson and Grignon, 1991). The supply of photosynthates from the shoot to the symbiosis is considered to be an investment by the plant. This means that the reducing sugars and amino acids that could have been used for plant growth go to the fungus for the uptake of P and other elements such zinc (Z), copper (Cu) and molybdenum (Mo) which are less mobile in the soil (Donahue *et al*., 1983). The extent to which mycorrhiza can improve plant growth depends on plant available P status of the soil. The beneficial effect of mycorrhiza is reduced or eliminated when readily available P is supplied in sufficient quantity (Lee and Wani., 1991). However, increased root infection with increasing P application has also been observed. For example, Lee and Wani (1991) showed that cassava roots showed increased infection by *Glomas manitrotis* with increasing P application up to 200 kg of P/ ha.

Apart from enhancing nutrient uptake, some strains of this fungi have mechanisms against pathogenic fungi and nematodes. They release metabolites such as low molecular weight compounds and antibiotics that are toxic to these fungi and nematodes (Sylvia *et al.,* 2005). Some mycorrhiza like Trichoderma, work against fungal phytopathogens either indirectly by competing for nutrients and space, or by modifying environmental conditions promoting plant disease mechanisms and

antibiosis. They can work directly through mechanisms such as mycoparasitism (Saber *et al*., 2009).

With the promotion of water and nutrient uptake, N – fixation in legumes and the ability to ward off some fungal and nematode pathogens Trichoderma strains can be used as biofertilizers (Saber *et al*., 2009). Most of the beneficial Arbuscular mycorrhiza strains that can be used as biofertilizers belong to *T. harzianum* and *T. hamatumare* strains. Mycorrhiza also enhances the uptake of water (APS, 2000) and promotes nodulation and N fixation in legume by Rhizobia. This is because Rhizobia requires an adequate P supply and a restricted root system leads to poor uptake of soil P (Abudulai *et al*., 2013). For the inoculation to be successful the introduced fungus should; show a certain degree of specificity or preference for the host plant, compete with the indigenous fungi, outnumber indigenous fungi at infection sites on roots and be more efficient than the indigenous fungi in enhancing plant growth (Thompson, 1991). These inoculants can be applied in soils having low levels of Trichoderma. Application of inoculant application can be foliar or as seed or soil treatments. The rate of application depends on the method of application. When applied to soil the rate is 6g of the fungus per kilogram of soil (Akladious and Abbas, 2012) and when applied to seed the rate is 1g of fungus in a kilogram of seed. However, large scale application of these fungi is hindered by inoculum production technologies which require the presence of a plant host. These fungi are obligate symbionts in nature (APS, 2000).

2.3.3 Co-inoculation of bean and groundnut with Rhizobium and Trichoderma

Recognizing the impact that microorganisms have on plant growth has led to the development of inoculants. Most commonly used biofertilizers are soil bacteria of

the genus Rhizobia. African soils contain large populations of compatible but less effective Rhizobia capable of inducing nodulation without providing much benefit to the legume host (Woomer, 2010). This emphasizes the need for inoculation in these soils. However, due to the acid nature and poor fertility of most soils, Rhizobia is best co-inoculated with a mycorrhizal fungus. The fungus solubilises P and /or effectively takes up P and other nutrients from the soil while the bacteria fixes N (Fathollahi *et al*., 2014). While this can have an added energy cost for the host, it can result in striking growth improvement due to enhanced P and N uptake (Sylvia *et al*., 2005). Field grown legumes form tripartite symbiosis with both Rhizobia and arbuscular mycorrhizal fungi. Therefore, co-inoculating microorganisms may have a synergistic effect by providing protection from disease, increasing nutrient uptake or stimulating microbial growth (Sylvia *et al*., 2005).

In particular, *T. harzianum* has been shown to increase the rate of seed germination and emergence, promote earlier flowering and increase flower numbers, growth, height and dry weight and faster rooting of cuttings (Sylvia *et al*., 2005). This mycorrhizal fungus also protects plants from pathogens that cause disease and reduces water stress in plants. Preliminary studies by Abudulai *et al*. (2013) conducted in South Africa to test strains of *T. harzianum* and *Bradyrhizobium japonica* as co-inoculants showed that the two have a synergistic effect and perform better together than individually. The study showed that at 55 days after emergence the plants that were co-inoculated had a 37 % higher growth rate than the singly or un-inoculated plants.

CHAPTER 3: MATERIALS AND METHODS

3.1 Site Location

The study was conducted at the University of Zambia greenhouses. The greenhouses are located at 15^{o} 24' South and 28^{o} 19' 59" East in Lusaka, Lusaka Province of Zambia. During this season mean daily temperature was 21.54^{o}C (December – April) according to the metrological data at the University of Zambia MET station.

3.2 Sample collection and preparation

Soils used in the greenhouse trials were collected from the University of Zambia (Liempe) farm located at 15^{o} 24' South and 28^{o} 28' East. This site is in agro-ecological region II of Zambia with an average annual rainfall of 800 mm to 1000 mm. This site was specifically selected because soils are of the Chelston series which are classified as fine, mixed isohyperthermic Oxic Paleustult soil which are significantly leached of clay and bases (Chirwa and Yerokun, 2012; ISRIC, 1994). Therefore, the area is known to be acidic and has been fallow for a number of years. Soils were collected during the 2013/2014 farming season. Samples were collected from a depth of 0-20 cm at 10 random spots and mixed together to obtain a composite sample. Half a kilogram of the sample was air dried and passed through a 2mm sieve for determination of selected chemical and biological characteristics.

3.3 Soil characterization

Soils were chemically and biologically analyzed for; soil reaction, exchangeable bases, available P, total N, organic matter and soil respiration.

3.3.1Soil reaction

Soil reaction was determined in 0.01 M calcium chloride according to the procedure by Mclean (1976). Air dry soil was passed through a 2 mm sieve and 10 g of this soil was placed in a 50 ml plastic container. A volume of 25 ml of 0.01 M calcium chloride ($CaCl_2$) was added to the soil in the container and the mixture agitated for 30 minutes on a spherical mechanical shaker and allowed to stand for 30 minutes. After calibrating the glass electrode pH meter in pH 4.0 and pH 7.0 standards, the pH of the soil was measured using a glass electrode pH meter.

3.3.2 Available Phosphorus

Available phosphorus was determined using Bray 1 extraction method according to Bray and Kurtz (1945). Three grams of soil that was air dried and passed through a 2 mm sieve was weighed into a 50 ml plastic container to which 21 ml of the Bray 1 extracting solution was added. The suspension was shaken for 1 minute and filtered through a Whatman No. 42 filter paper. An amount of 5 ml of the filtrate was pipetted into a 25 ml volumetric flask. Four milliliters of Ascorbic acid at pH 4.0 were added to the filtrate and distilled water was added to the mark of 25 ml of the volumetric flask. The solution was left to stand for 15 minutes for color to develop. The absorbance was read on a spectrophotometer at 882 nm (Lowry and Lopez, 1946).

$$mg\ of\ \frac{P}{kg} of\ soil = \frac{X\ ppm \times volume\ of\ extractant \times dilution\ factor}{weight\ of\ soil}$$

$$\text{mg of}\frac{\text{P}}{\text{kg}}\ \text{of soil} = \frac{\text{X ppm } \times 0.0211\ \times 5}{0.003\ \text{kg}}$$

3.3.3 Total Nitrogen

Total Nitrogen was determined using the Macro Kjeldahl Method (Kjeldahl, 1883). A gram of soil that was air dried and passed through a sieve was weighed using an electronic balance. The soil was placed in a 500 ml Kjeldahl flask and 10 mls of concentrated sulphuric acid was added. The suspension was swirled for a minute. Three grams of catalyst were then added and the flask with its contents was placed in the digester at 450 ^{o}C for 45 minutes.

The digest was then transferred into 100 ml plastic containers and distilled water was added to make a final volume of 100 ml. An amount of 10 mls of the dilute digest was obtained and distilled with 10 mls of 40 % sodium hydroxide for 5 minutes with boric acid as the indicator collecting the distillate.

The boric acid was then titrated with 0.01 M hydrochloric acid to change the color from green back to the purple color of the boric acid. This volume was the amount of ammonia gas (distillate) collected in the boric acid.

% N was calculated using the formula:

$$\%N = \frac{(Vs - Vb) \times \text{Molarity of Acid} \times \text{Dilution Factor} \times 14.01 \frac{g}{mol} \times 100\%}{\text{Dried plant sample used(g)}}$$

Where Vs = Titer, Volume of standard acid for sample

Vb = Titer, Volume of standard acid for blank

$$\%N = \frac{(Vs - Vb)\text{litre} \times 0.01 \frac{mol}{litre} \times 10 \times 14.01 \text{ g/mol} \times 100}{\text{Dried plant sample used (g)}}$$

$$= \frac{\left[\frac{Vs-Vb}{1000}\right] litre \times 0.01 \frac{mol}{litre} \times 10 \times 14.01 \frac{g}{mol} 100}{1g}$$

Where Vs and Vb are in ml

% N = (Vs – Vb) x 0.1401

This same procedure was used to determine % N in plants.

Amount of N per plant = weight of plant biomass x % N

3.3.4 Organic Matter

The Walkley and Black method (Walkley, 1934) was used to determine the amount of organic matter in the soil

A gram of soil was placed in a 500 ml wide mouth Erlenmeyer flask. A volume of 10 ml of 1 N potassium di – chromate was added and the suspension swirled gently before 20 ml of sulphuric acid was added rapidly. The suspension was swirled more

vigorously for a minute then allowed to stand for 30 minutes. Distilled water (150 ml) and 10 drops of Diphenylamine indicator were added and then titrated with 0.5 M Iron II Sulphate.

The percentage of organic matter was calculated using the formula below.

$$\%C = \frac{4(\text{Volume of } FeSO_4 \text{ used for the blank} - \text{Volume of } FeSO_4 \text{ used for sample})}{\text{Volume } FeSO_4 \text{ used for the blank}}$$

% OM = % C X 2

3.3.5 Exchangeable Cations

Exchangeable cations were determined using the Ammonium acetate extract at pH 7.0 according to the method of Thomas (1982).

Ten grams of soil that was air dried and passed through a 2 mm sieve was weighed in a 250 ml Erlenmeyer flask. Then 50 g of ammonium acetate at pH 7.0 was added and the mixture was shaken for 30 minutes on a spherical mechanical shaker. The suspension was then filtered using No. 42 Whatman filter paper. Potassium and sodium were measured in this filtrate using the Atomic Absorption Spectrophotometer. Ten milliliters of the same filtrate were pipetted into a 25 ml volumetric flask to which 5 ml of 5000 mg/l of Strontium chloride was added and distilled water was used to fill to the volume of 25 ml. The solution was used to determine the amount of calcium and magnesium in the soil using the atomic absorption spectrophotometer. The formula below was used to calculate the concentrations of the cations in the soil.

$$\frac{Cmol}{kg} = \frac{Reading\ \left(\frac{mg}{l}\right) \times Volume\ of\ extract(l) \times Dilution\ Factor}{Mass\ of\ soil(kg) \times Equivalent\ weight\ of\ cation\left(\frac{mg}{cmol}\right)}$$

3.3.6 Soil Respiration

3.3.6.1 Soil Respiration

Determination of soil respiration was based on the incubation method of Jenkinsen and Powlson (1976).

A weight of 50 g of moist soil was obtained in an incubation container. A small beaker consisting of 5 ml of 0.2 M potassium chloride was placed in the incubation container that had the soil. The potassium chloride was a base trap for the carbondioxide produced by the microbes. The incubation container was sealed with a rubber lid and placed in a dark place for 7 days. After 7 days, 2 drops of phenolphthalein indicator were added to the potassium chloride in the beaker. The solution was then titrated with 0.1 M hydrochloric acid until the color change (pink to colorless) then 2 drops of methyl orange were added to the solution and again titrated with 0.1 M hydrochloric acid until the color change (orange to red). Soil respiration was the carbon dioxide – carbon evolved from the soil by the microbial biomass. Soil respiration was estimated in mg C/ g soil per day.

$$mgC/g\ soil\ per\ day = \frac{(a-b) \times 10 \times concentrationof\ titrant \times 1.2 \times 10}{number\ of\ incubation\ days}$$

a = amount of HCl titrated by sample

b = amount of HCl titrated by blank

3.4 Greenhouse experimentation

Duplicate experimental sets were set up with 5 kg of soil in each pot. Common bean and groundnut were grown in pots during the 2013/14 growing season.

3.4.1 Treatments and Experimental Design

A completely randomized designed (CRD) experiment was set up with five (5) treatment with 4 replicates. The treatments were as follows:

1) A single inoculation of *Rhizobium tropici*
2) A single inoculation of *Trichoderma harzianum*
3) Co-inoculation of *T. harzianum* and *R. tropici*
4) A fertilizer application of inorganic Nitrogen and Phosphorus
5) Control (un-inoculated)

The bacteria *Rhizobium tropici* was added in the form of Biofix legume inoculant. Biofix is manufactured through artificially culturing nitrogen fixing bacteria in a controlled environment. Biofix which consists of *Rhizobium tropici* was developed in Kenya by MEA limited in Nairobi for local soils and is environmentally friendly. It is able to deal with low pH, high temperature and water stress (Orrillo *et al.*, 2012). Which results in faster growth and higher grain yield in bean, soya bean, groundnut and cowpea and other pasture and tree legumes. This bacterium is best suited for acidic soils (de Bruijn, 2015).

The fungus *Trichoderma harzianum* (T 22) was applied in the form of Eco-T, an inoculant produced in Switzerland for the control of crop root diseases and for

enhanced plant growth. This inoculant has been tested in several field trials and on different types of soils.

For the single inoculation of seeds, inoculation was done just before planting at the rate of 1 g/kg of seed for *T. harzianum* and 100 g/15kg of seed for *R. tropici*. For the co – inoculation both inoculants were added to the seed at the recommended rates as above. Fertilizer application was done at the rate of 100 kg of N/ha and 80 kg of P_2O_5/ha. Three seeds were planted per pot and thinning of plants was done 14 days after emergence (DAE) to one plant per pot.

Two sets of plants were grown. The first set was grown up to 51 DAE and the second set of plants was grown to maturity of the respective crops. This was until the crops reached senescence of their growth stage.

3.5 Data Collection

In order to assess the following, parameters were measured and data collected

3.5.1. Number of nodules, nodule effectiveness and tissue nitrogen

The first set of plants was harvested at 51 DAE. The above ground biomass was rinsed and dried. The below ground biomass was rinsed to remove soil particles. Number of nodules per plant was determined by physically counting them on the roots. The effectiveness of nodules was determined as a percentage by selecting 4 random nodules and determining the effectiveness of each nodule. The effectiveness was measured by cutting the nodules open to expose the colour inside. Pinkness or redness represented effective and white, brown or green colors represented non-

effective nodules (Smarrt,1994). Total nitrogen was determined in the above ground biomass using the modified Kjeldahl method (Kjeldahl, 1883).

3.5.2. P uptake.

The above ground biomass of the second set of plants was harvested at maturity. Plants were cut just above the soil level, rinsed and dried. Dried plant samples were crushed and a gram was obtained for analysis for total P using the Dry Ashing method (Jones, 1984).

Determination of P using the modified Dry Ashing method (Jones, 1984)

The sample was dried for 16 hours at 70 – 80 ^{o}C and cooled in a desiccator. The dry sample was ground and 1 g of sample was obtained and placed in a crucible and then placed in a furnace at 500 ^{o}C for 2 hours. The ash was cooled in a desiccator and dissolved in 20 ml of 1.0 N nitric acid. The solution was then diluted with water in a 100ml container to the mark.

Colour was developed using the Lowey and Lopez method (1946) and absorbance read at 882 nm on the spectrophotometer.

3.5.3 Biomass and Grain Yield; Both biomass and grain yield were measured

The above ground biomass was harvested for common bean and groundnut. For common bean, this also included the weight of the pods. The pods for common bean and groundnut were both weighed shelled to obtain the grain yield.

3.6 Statistical Analysis

Effects of the different treatments were analysed using analysis of variance (ANOVA) with SAS version 9.0. In order to separate the means, the Least Significant Difference method was used and correlation analysis was used to establish relationships among measured variables. All statistical analyses were done at 95 % confidence limit.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Initial soil characteristics at planting

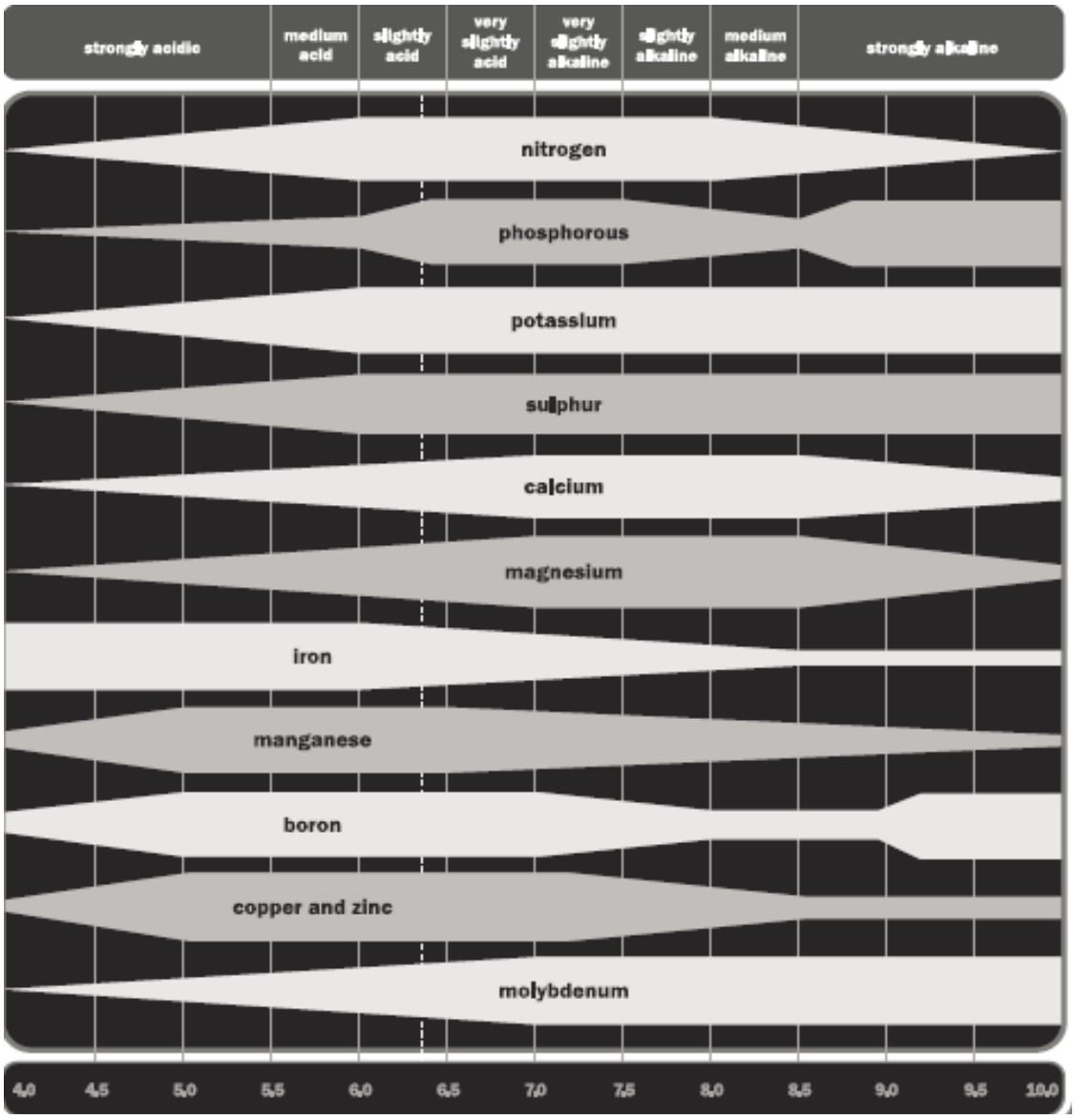
Results of initial soil characterization are presented in Table 3 below. The soil used in this study had a pH of 5.58. This level of pH is interpreted as acidic according to Whalen and Sampedro (2010). Groundnut and common bean grow well at pH 6.0 – 7.5 and 5.9 – 7.0, respectively; which is a neutral soil pH range as was observed by Vara Prasad *et al.*, (2011).

Table 3: Characteristics of soil at planting

Soil reaction (pH)	**Plant available P (mg/kg)**	**Exchangeable cations (cmol/kg)**			**Total N (%)**	**Organic matter (%)**	**Soil respiration (mg C/kg.day**
		Ca	**Mg**	**K**			
5.58 *acidic	13.9 inadequate	2.37 Adequate	1.22 Adequate	0.345 Adequate	0.0168 adequate	1.79 Low	0.978 very Low activity

*Critical limits for legume growth based on Fairhurst (2012).

At a neutral soil pH, most plant nutrients are available for root uptake (Donahue *et al.*, 1983). Some major plant nutrients such as N, K and S appear to be less affected directly by soil pH than many others, but still are affected to some extent. Phosphorus however, is directly affected, at alkaline pH values, P quickly reacts with Ca and Mg to form an insoluble compound of $(Ca_3(PO_4)_2$. At acid pH values P quickly reacts with Aluminum (Al) and Iron (Fe) to again form a less soluble compound of $Al(PO_3)$ and $Fe(PO_3)$ (Thomas, 2010).



Source: Ketterings et al., (2008)

Chart 1. Chart giving nutrient availability due to pH change

At pH 5.58, nutrients such as P and calcium are less available for uptake to the plant. At this soil pH plant available P ($HPO_4^{-2}$and $H_2PO_4^-$) reacts with the excess Al^{3+} to form $Al(PO_3)$ that cannot be taken up by plants. Smyth and Cravo (1990) showed that common bean and groundnuts need larger amounts of P compared to other crops. This is because the process of biological nitrogen fixation in legumes requires an adequate supply of P. The P content in the soil used in the experiment was below critical levels. However, there is large variations among major crops in the quantity

of P required to achieve optimal levels of growth in different soils for different regions (Smyth and Cravo, 1990). According to Smyth and Cravo (1990), the critical level of P are 9 and 13 mg/kg for cereal and legumes, respectively, based on the Bray 1 extraction method. A more recent study shows that the critical level is 15 mg/kg based on the same method (Fairhurst, 2012).

Most soil microorganisms prefer a near neutral pH range of 6 – 7 (in water) because the availability of most soil nutrients is greatest in this pH range (Whalen and Sampedro, 2010). The growth and activity of these microorganisms relies on the manipulation of nutrients and substrate in soil environment. Some studies have suggested that, carbon and nitrogen ratio in addition to the ambient pH are the main environmental factors influencing colonization of Trichoderma *sp.* (Gao *et al.,* 2007). Singh et al (2014) confirmed that Trichoderma *sp*. colonization is most favoured in an acid condition and that the most favorable condition range is between pH 5.5 and 7.5. This range produces a total dry weight of mycelium varied between 1.41 and 1.35g. Strain-specific symbiotic potency varies at different soil pH levels, where strains that are outcompeted under neutral pH conditions become dominant under lower pH conditions of less than 5.5. Although most Rhizobia *sp*. thrive at neutral conditions *R. tropici* and *R. loti* tend to be highly tolerant to acidic conditions and thrive at soil pH of less than 5 (Ferguson *et al.,* 2013). Therefore, at the soil pH of 5.58, *T. harzarium* and *R. tropici* should be able to thrive and effectively outcompete and infect the two legumes successfully.

Nitrogen is a very important nutrient used for growth, nodulation and other metabolic processes in legumes. The amount of nitrogen (N) in the soil was adequate for nodule formation but not in excess to prevent nodulation of the legumes. The

minimum amount of N in the soil required for nodulation to take place is 0.001 % total N (Woomer, 2010). Nonetheless, legumes grow best if there is some mineral N available at the time of nodule formation. Therefore, a small amount of starter nitrogen (10 to 30 kg per ha) at planting may increase total BNF over the crop's lifetime. On the other hand, calcium levels were below the optimum levels of0.5 $cmol^{+}$/kg required for legume production (Metson, 1971). Acid soils typically have low levels of bases due to leaching; calcium levels were low in this soil (Figure 2). As a result, liming should be an important activity in legume production as it not only increases soil pH but also increases the amount of Ca and Mg in the soil.

Apart from soil Organic matter, soil pH is a major factor affecting soil respiration. Soil organisms obtain energy for growth and activity from organic matter and soil pH determines the availability of nutrient that are required for growth and activity of the soil organisms. Soil respiration is the major carbon dioxide emission from soil released into the atmosphere (Liang *et al.,* 2014). The respiratory activity of plant roots and their mycorrhizal fungi in soil are estimated to be from 10 to 90 % of the total Soil respiration (Hanson *et al.,* 2000). Contribution to total soil respiration by roots is commonly higher during the growing season and is lower during the dormant periods of the year.

4.2 Effect of inoculation and fertilizer application on nodulation in common bean and groundnut plants

4.2.1 Effect of inorganic fertilizer and microbial inoculants on nodulation and nodule effectiveness in groundnut

It was observed that there were no significant effects of number of nodules that ranged from 309 to 395 per plant in groundnut. The highest number of nodules was

observed in plants inoculated with both Rhizobium and Trichoderma while the least was where inorganic N and P had been applied.

Nodule effectiveness ranged from 0 to 87.5 % and showed significant differences across amendments (Figure 1). Nodules from the inorganic N and P fertilizer amendment had the least (0 %) while the dual inoculation had the highest (87.5 %) effectiveness. Statistically, the inorganic fertilizer amendment (0 %) results did not differ from the inoculation with Rhizobium alone (26.3 %); while the dual inoculation (87.5 %) did not differ from inoculation with Trichoderma alone (75 %) and the unamended controls (75 %) in terms of nodule effectiveness.

Studies have shown that number of nodules in groundnut can range from 82 to 252 nodules per plant (Mweetwa *et al.,* 2014) with or without Rhizobium inoculation and 134 to 165 nodules per plant with and without N inorganic fertilizer application (Nambiar *et al.,* 1982). The number of nodules in this study were much higher with or without inoculation or N fertilizer application, when compared to the earlier studies of Nambiar *et al*. (1982) and Mweetwa *et al*. (2014). However, Graham and Scott. (1984) had recorded 400 of nodules per plant without N fertilizer application or inoculation. Their results corroborate our findings in this study.

The soils in the current study showed inadequate levels of P at 13.9 mg/kg, below the critical level of 15 mg/kg P for plant growth Fairhurst (2012). Nodulation in legumes is affected by mineral N, levels of available P and Mo, soil reaction (soil pH) and the type and vigor of the legume (Sultenfuss and Doyle, 1999). Qiao *et al*. (2007) showed that nodule formation and functioning is directly affected by the availability of phosphorus to the plant.

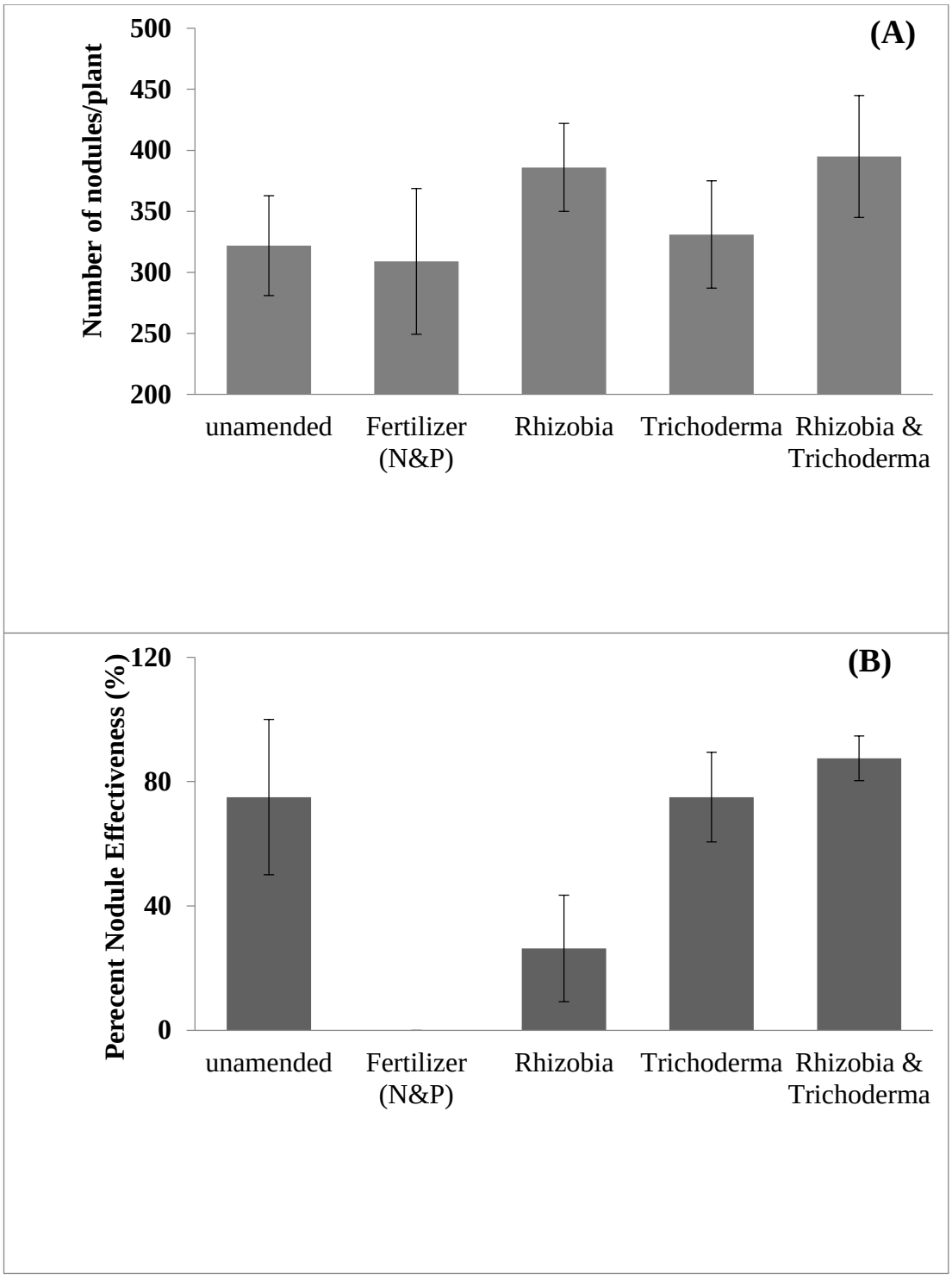


Figure 1. Number of nodules and nodule effectiveness of the groundnut plant in soils amended with inorganic fertilizer, *Rhizobium* and *Trichoderma* A: Number of nodules per plant, B: Percent nodule effectiveness

Nitrogen fixing legumes usually require more phosphorus than most other plants. Phosphorus plays a major role in nodule initiation (Tisdale *et al*., 1985). Several

studies in groundnut and other legumes have shown the significance of P in increased nodulation and yield (Qiao *et al*. 2007; Kamara *et al*.,2011; Mweetwa *et al*.,2014). In addition, high levels of P are required to meet the high cost of N_2 fixation in the form of ATP; the ATP is required to build and maintain functioning of nodules. A total of 16 ATPs are required in the reaction for the binding and dissociation of the Iron (Fe) protein and the Molybdenum (Mo) Fe protein in the nitrogenase complex during the reduction of atmospheric N to NH_3 (Whalen and Sampedro, 2009). However, most soil microorganisms and plants prefer a near neutral pH range of 6 to 7 because the availability of most nutrients is best at this pH range (Whaten and Sampedro, 2010).

The results in Figure 1 showed that the single inoculation with Rhizobium and Fertilizer application amendments significantly reduced the effectiveness of nodules, while there was no statistical increase in the dual inoculation and single inoculation of Trichoderma. The single inoculation of Rhizobium and the N and P fertilizer amendment showed a significant decrease in the percent effectiveness of nodules. Nodule effectiveness is affected by not only the amount of N and P in the soil which determine the symbiosis of Rhizobium and Trichoderma with the plant (Sylvia *et al.*, 2005), but also by the specificity of the Rhizobium strain to its plant host. The current study showed that an adequate amount of N and P provided by the N and P fertilizer amendment reduced nodulation and nodule effectiveness. Both the amendments that contained the inoculation of Trichoderma showed no significant difference from the unamended. This shows that *R. tropici* is a good competitor for nodule occupancy in an acid environment (Moron *et al.*, 2005).

Soil acidity reduces both plant growth and nodulation in legume production (Rossum *et al.*, 1994). Besides proton toxicity other factors associated with low pH causing

these constraints are aluminum and manganese toxicity and calcium, magnesium, molybdenum and P deficiency (Foy, 1984). However, effects of soil acidity differ among groundnut cultivars and Rhizobium strains. The Rhizobium strain that was used in this study was *Rhizobium tropici* which is a fast-growing bacterium that is able to adapt to acidic environments. A Study by Rossum *et al.* (1994) showed that one strain of Rhizobium performed well in a neutral environment while another strain performed well in an acid environment. Even though the Rhizobium strain in the current study was able to adapt to acidic environments there were no significant differences that were observed across amendments in terms of number of nodules per plant in the groundnut crop. This could be attributed to the fact that the indigenous Rhizobium were more effective in infecting the crop than the inoculated Rhizobium strain. Rossum et al., (1994), also observed that the indigenous strain was more effective than the inoculated.

African soils are known to contain large populations of compatible but less effective *Rhizobia* capable of inducing nodulation without providing much benefit to the plant host (Woomer, 2010). This fact was not observed in the current study because even though there were no significant differences across amendments for number of nodules, there were significant differences in terms of nodule effectiveness.

4.2.2 Effect of N and P fertilizer, *Rhizobium tropici* and *Trichoderma harzianum* on number of nodules per plant and nodule effectiveness in common bean

The number of nodules across amendments ranged from 0 to 228 nodules per plant in common bean, with the inorganic N and P fertilizer amendment yielding no nodules and the dual inoculation of Rhizobium and Trichoderma yielding the highest number of nodules per plant. Number of nodules per plant was significantly different across

amendments. The single inoculation of *Rhizobium* and *Trichoderma* increased number. of nodules by 338 and 320 % respectively even though the single inoculations of Rhizobium and Trichoderma showed no statistical increase in number of nodules. The N and P fertilizer amendment showed no significant reduction in the number of nodules

However, the dual inoculation showed a significant increase in the number of nodules per plant.

Nodule effectiveness in this study ranged from 25 % to 88 %, where the lowest percentage was observed in the single inoculations of Rhizobium and Trichoderma and the highest percentage was observed in the dual inoculation. The single inoculations of Rhizobium and Trichoderma showed no significant differences from the unamended. However, the dual inoculation of Rhizobium and Trichoderma showed a significant increase from the unamended controls in both the number of nodules per plant and nodule effectiveness. These results were similar to the findings of Tafini *et al.* (2012) who showed that co-inoculating a fungus with *R. tropici* resulted in a significant increase in nodulation compared to the plants without inoculation.

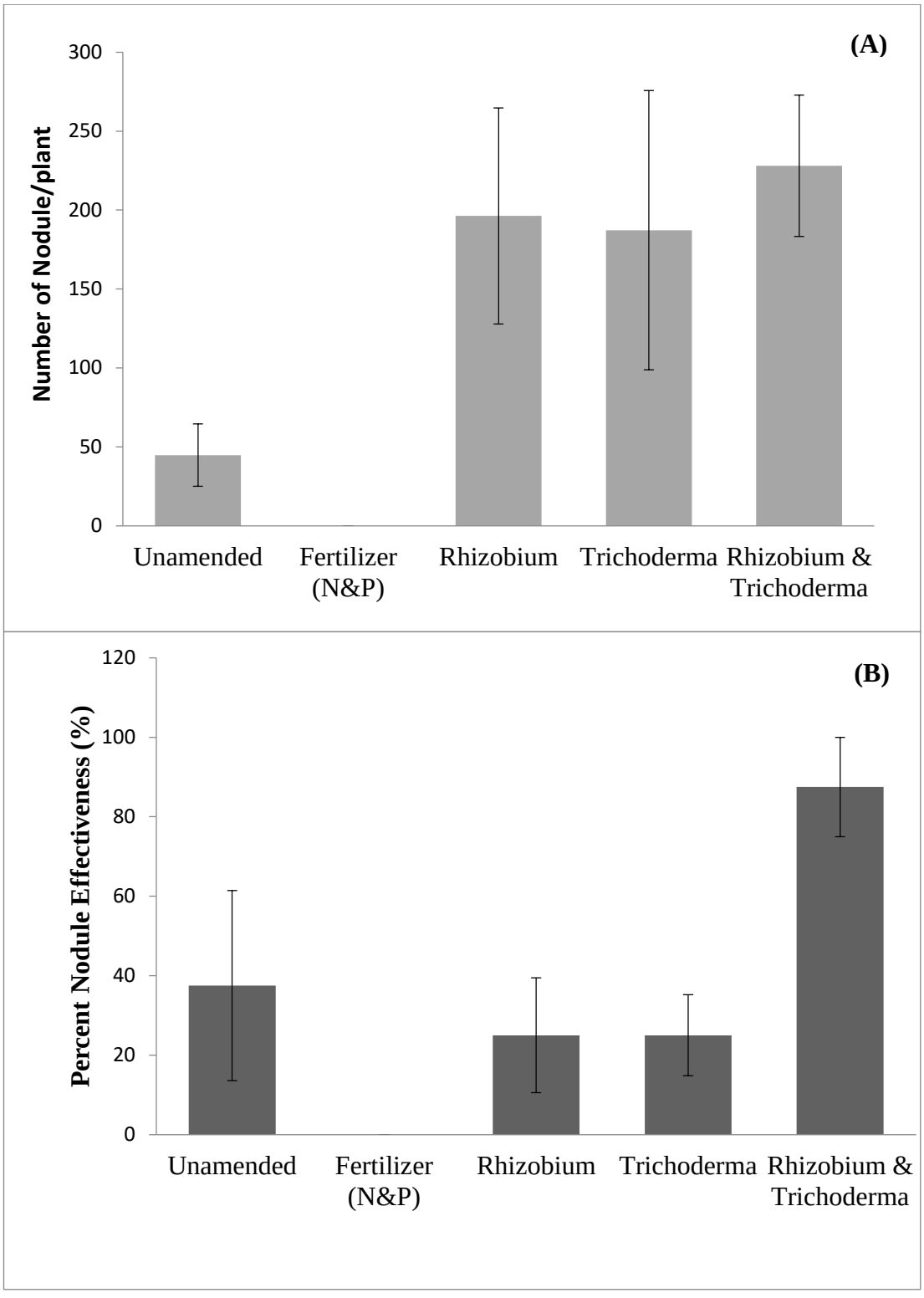


Figure 2. Effect of N and P fertilizer, Rhizobium and Trichoderma on number of nodules per plant and nodule effectiveness 51 days after emergence in common bean A: Number of nodules and, B: Percent nodule effectiveness

Trichoderma was able to supply an adequate amount of P to the plant and it was put to efficient use by the *R. tropici*, an effective strain of Rhizobium in common bean (Sikombe *et al.,* 2003). This suggests that Trichoderma and Rhizobium have synergistic effects in terms of increasing nodulation and nodule effectiveness in common bean. The Trichoderma increases number of nodules by increasing infection sites through the extension of the root system of the plant. The fungus also enhances the uptake of P required for nodule initiation and function. Due to the fact that this Rhizobium is tolerant to acidic conditions and there was anadequate supply of P to the plant by the Trichoderma resulting in increased nodule effectiveness.

4.3 Nitrogen and Phosphorus accumulation in groundnut and common bean due to N and P fertilizer application and Rhizobium and Trichoderma inoculations

Nitrogen and P are among the major nutrients that plants require. These nutrients are required in large amounts. Nitrogen is the principle constituent of plants; it accounts for at least one half of the total number of ions absorbed (Tisdale *et al.,*1985). Phosphorus plays a significant role in plant metabolism and legume nodulation through its ability to enhance root development and proliferation. The uptake of P ions depends predominantly on diffusion which makes the ion more dependent on aspects of crop root activity (Archer, 1988). The symbiotic relationship of plants with a fungus such as Trichoderma is able to increase crop root activity and therefore, increase the uptake of P. Infection and growth of Trichoderma are stimulated by P deficiency in the soil. The extension of roots through the hyphae produced by the fungi provides a larger surface area for the uptake of P. In legumes, an increase in the uptake of P increases N fixation by Rhizobia and thereby increases the amount of N accumulated by the legume.

4.3.1 Nitrogen and Phosphorus accumulation in groundnut due to N and P fertilizer application and inoculations of Rhizobium and Trichoderma

The amount of P accumulated in the groundnut plants was significantly different across amendments. The amount of P accumulated ranged from 50.3 to 120 mg of P/ plant. The inorganic N and P fertilizer amendment showed the largest P accumulation while the dual inoculation showed the least.

Phosphorus accumulation in inoculated groundnut plants showed no significant differences from the unamended. These results were incongruent with those of Guru *et al*. (2011) who reported increased P content and N content in tomato plants co-inoculated with an AM fungus and a bacterium. This observation could be because legumes tend to have a greater demand for P compared to other crops such as tomatoes. For most crops, an amount of 9 mg of P/kg of soil is the critical level for production, while 13 mg of P/ kg of soil is the critical level for legumes (Smyth and Cravo, 1990).

In legumes, P is required for metabolic processes and nodule initiation and function as well. In addition, P is required for growth and respiration reactions of the microorganisms that are in symbiosis with the plant (Mus *et al.*, 2016). Therefore, an increase in the number of microorganisms that are in a symbiotic relationship with the plant increases the distribution and use of the P by the plant.

The amount of N accumulated at harvest in the groundnut crop showed no significant differences across amendments but ranged from 920 to 1618 mg of N /plant. The N and P fertilizer amendment had the highest total N accumulated while the single inoculation of *Trichoderma* had the lowest N accumulated.

Table 4: Nitrogen and phosphorus accumulation in groundnut plants supplied with inorganic N and P fertilizers and inoculated with Rhizobium and Trichoderma at planting.

Amendment	**Total Nitrogen (mg/plant)**	**Total Phosphorus (mg/plant)**
Unamended	1388a	58.5b
Fertilizer (N & P)	1618a	120.0a
Rhizobia	1388a	64b
Trichoderma	920a	56.3b
Rhizobia & Trichoderma	1148a	50.3b
LSD	760	25.2
CV%	34.07	23.95
p-value	0.3882	0.0002

Inorganic N and P fertilizer amendment showed the highest amount of N and P accumulated in the groundnut crop among all amendments. This is due to the fact the inorganic fertilizer provided adequate amounts of N and P in readily available forms for uptake by plants. Not only where these two nutrients available to the plant but the cost of their uptake was low compared to the cost the plant would pay when in symbiosis with microorganisms. The phosphorus accumulation in the plant biomass of the inoculated groundnut plants showed no significant differences from the unamended. Therefore, even though the dual inoculation showed an increase in nodule effectiveness, this did not translate into an increase in the amount of P and N accumulated. In the amount of P accumulated among amendments the dual inoculation had the lowest amount of P accumulated. These results were not the same as those of Guru *et al*. (2011) who reported that the co-inoculation of an AM fungus and a bacterium increased P content and N content by 4% in tomato plants. However, legumes tend to demand a greater amount of P as compared to other crops.

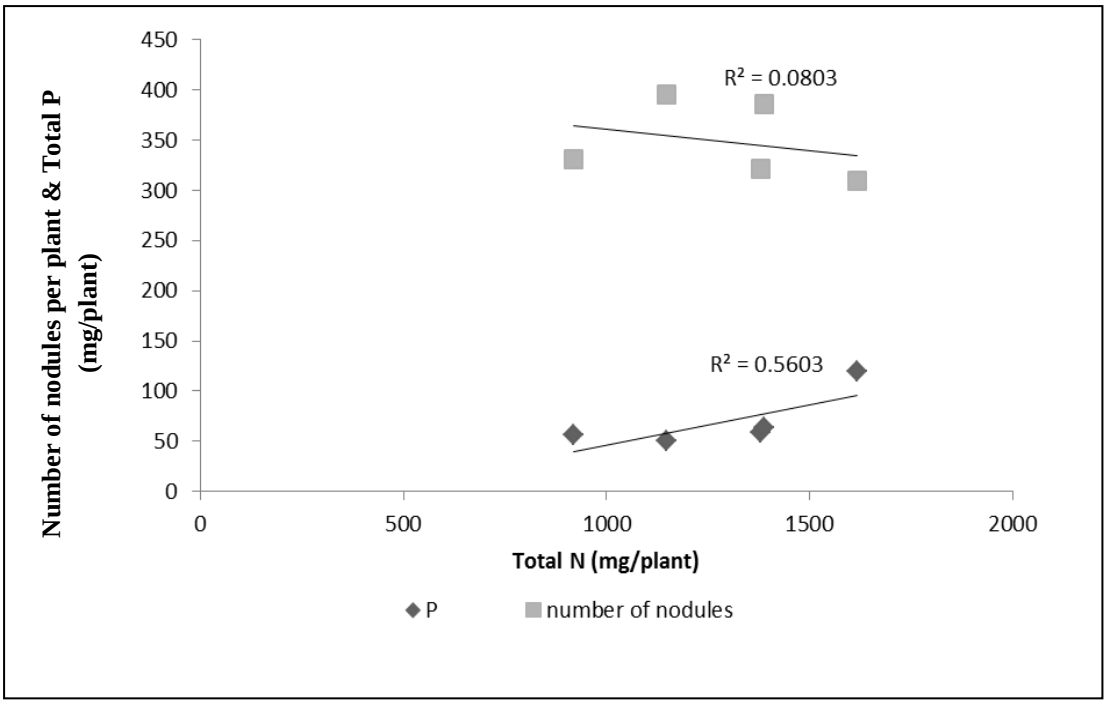


Figure 3. Relationship between P accumulation at harvest, number of nodules per plant at 51 days after emergence and N accumulation in plant at harvest

The relationship between number of nodules and N accumulated was a negatively weak relationship with an R^2 of 0.0803. However, the relationship between the P accumulation and N accumulation showed a positive relationship with an R^2 of 0.5603.

The positive relationship between the N and P accumulation showed that there is an interaction between N and P uptake in legumes. The evidence that N promotes P uptake by the plant is that N increases shoot and root growth, alters plant metabolism and increases solubility and availability of phosphorus. On the other hand, the weak relationship between number of nodules per plant and nitrogen accumulation shows that bacterium – legume strain relationship is very specific. This suggests that there can be cases where these partnerships maybe considered parasitic resulting from the failure to form effective symbiosis. In such relationships, the microorganisms benefit

from a continuous carbohydrate supply while they fix little or do not fix nitrogen for the host plant (Allito *et al.*, 2014).

4.3.2 Nitrogen and Phosphorus accumulation in common bean due to N and P fertilizer application and inoculation of Rhizobium and Trichoderma

Inorganic N and P amendment resulted in the most N and P accumulated in the common bean crop, while the unamended had the lowest amount of N and P accumulated. The amounts of the accumulated N and P ranged from 780 mg of N/plant and 110 mg of P/ plant to 90 mg of N/plant and 8.89 mg of P/plant, respectively. The inoculated amendments did not differ significantly from the unamended with respected to the accumulated N. With regard to P accumulation, the highest levels were associated to the inoculation treatment with sole Rhizobium.

Table 5: Nitrogen and Phosphorus accumulation in common bean plants supplied with inorganic N and P, and inoculated with Rhizobium and Trichoderma at planting

Amendment	**Total Nitrogen (mg/plant)**	**Total Phosphorus (mg/plant)**
Unamended	90 b	8.89 c
Fertilizer (N & P)	780 a	110 a
Rhizobia	160 b	30 b
Trichoderma	170 b	15.7 bc
Rhizobia &Trichoderma	140 b	26.8 b
LSD	192	14.9
CV %	24.17	47.84
p-value	0.034	0.0001

The N and P accumulation in common bean showed a significant increase in the plants that were amended with N and P fertilizer. The presence of inorganic N and P that were both abundant and readily available to the plant resulted in more accumulation in the plant.

An increase in the amount of P accumulated in common bean that were amended with *Rhizobium* showed an increase in the effectiveness of nodules (Sylvia *et al.,* 2005). These results were similar to Tafini *et al.* (2012) who had observed an increase in the amount of P accumulated as a result of co-inoculation with mycorrhiza and Rhizobium. However, the amount of fixed N in the single inoculation of Rhizobium and the Dual inoculation was not enough to significantly increase the N accumulated in the plants with these amendments. Since the N fixed by the bacteria is in the form of NH_4-N this was able to stimulate the uptake of P (Tisdale *et al.,* 1985) increasing the amount of P accumulated in these plants. A previous study by Kanniaiyan, (2003) reported that failure to obtain the desired inoculation response was due to; the presence of native ineffective strains which could not be displaced by the inoculated strains, the presence of microorganism antagonism to Rhizobium which reduce the number of Rhizobium in the rhizosphere, soil conditions such as acidity, alkalinity, application of pesticides and herbicides, the presence of ineffective native Rhizobium strains in large amounts and a very low dose of the microorganism during inoculation. Any of these factors could have affected the amount of N fixed by the inoculated amendments. However, during the experiment the plants were sprayed with a fungicide for prevention and cure of a fungal disease, this could have affected colonization of both the Rhizobium and Trichoderma inoculant.

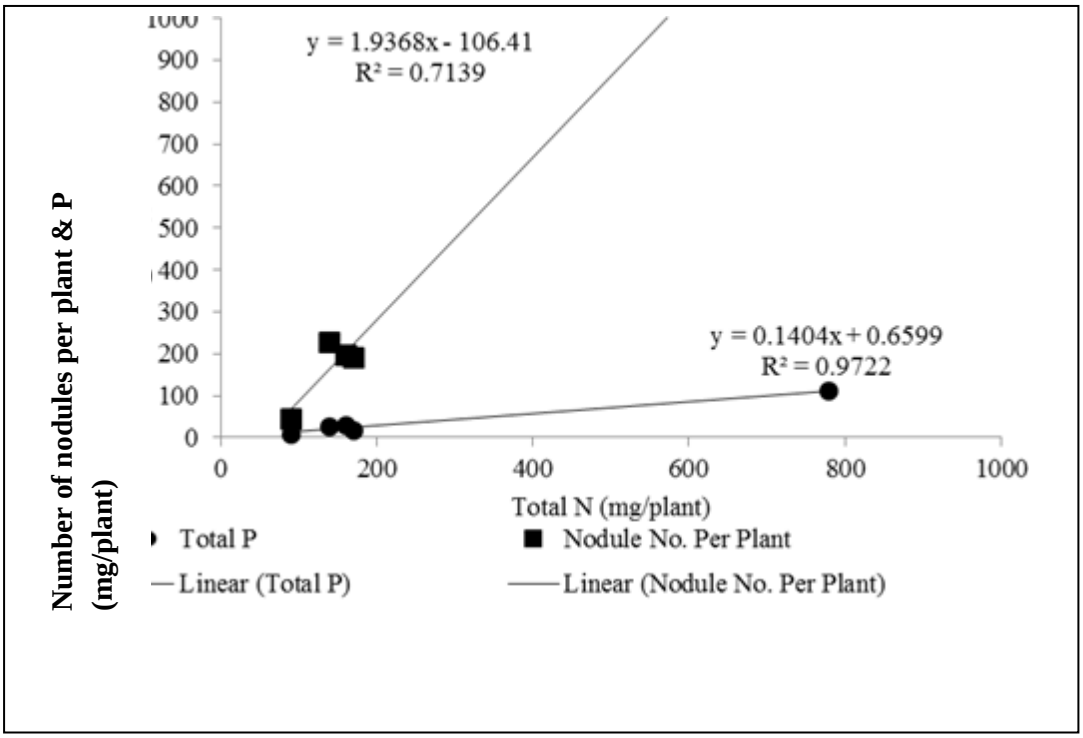


Figure 4. Relationship between P accumulated at harvest, number of nodules per plant at 51 days after emergence and nitrogen accumulation in plant at harvest in common bean

Nitrogen accumulation was strongly and positively correlated with P accumulation (R^2=0.97). An increase in the accumulation of N would lead to an increase in P. This is why it is important that soils have at least an amount of 0.001 % of N in the soil (Woomer, 2010) in order for nodulation to be stimulated in legume production. The uptake of P is stimulated by the uptake of N, the increased uptake of P is able to stimulate N fixation through energy production and growth of the Rhizobium.

The results also showed a strong and positive relationship between N accumulated and number of nodules per plant (R^2=0.71). These results imply that an increase in the number of nodules will determine the amount of N fixed. Even though the dual inoculation showed an increase in number of nodules it did not show the same significant increase in the N fixed. However, the increase in number of nodules did

not lead to an increase in N accumulated but the results showed an increase in P accumulated. This means that for nodulation to occur there has to be an increase in the amount of P uptake by the legume plant. This agrees with Qiao *et al.* (2007) who showed that nodule formation and functioning is directly affected by the availability of P.

4.4 Biomass and grain yield of common bean and groundnut due to an application of Inorganic N and P fertilizer and inoculations of Rhizobium and Trichoderma

In the production of legumes, N is important in leaf photosynthesis, radiation use efficiency and biomass accumulation (Dakora *et al.,* 1987). Phosphorus affects legume yields through optimum nodulation during the early growth stages of legumes (Musandu and Joshua, 2001). Nitrogen and P are also important as plant metabolism components of chlorophyll and amino acids as well as energy storage and transfer, cell division, cell enlargement and several other processes in the plant that determine yield.

4.4.1 Biomass and grain yield of groundnut supplied with inorganic N and P and inoculated with Rhizobium and Trichoderma

The estimated grain yield ranged from 5.22 to 8.42 t/ha with the single inoculation of Trichoderma having the lowest estimated grain yield and inorganic N and P fertilizer amendment having the highest grain yield. Estimated biomass yield ranged from 9.07 to 15.6 t/ha with the dual inoculation having the lowest biomass yield and inorganic N and P fertilizer amendment having the highest biomass yield. The inorganic N and P fertilizer amendment significantly increased the amount of grain yield and biomass yield in the groundnut crop. The inoculated amendments did not show any significant difference from the unamended in terms of grain yield and biomass yield.

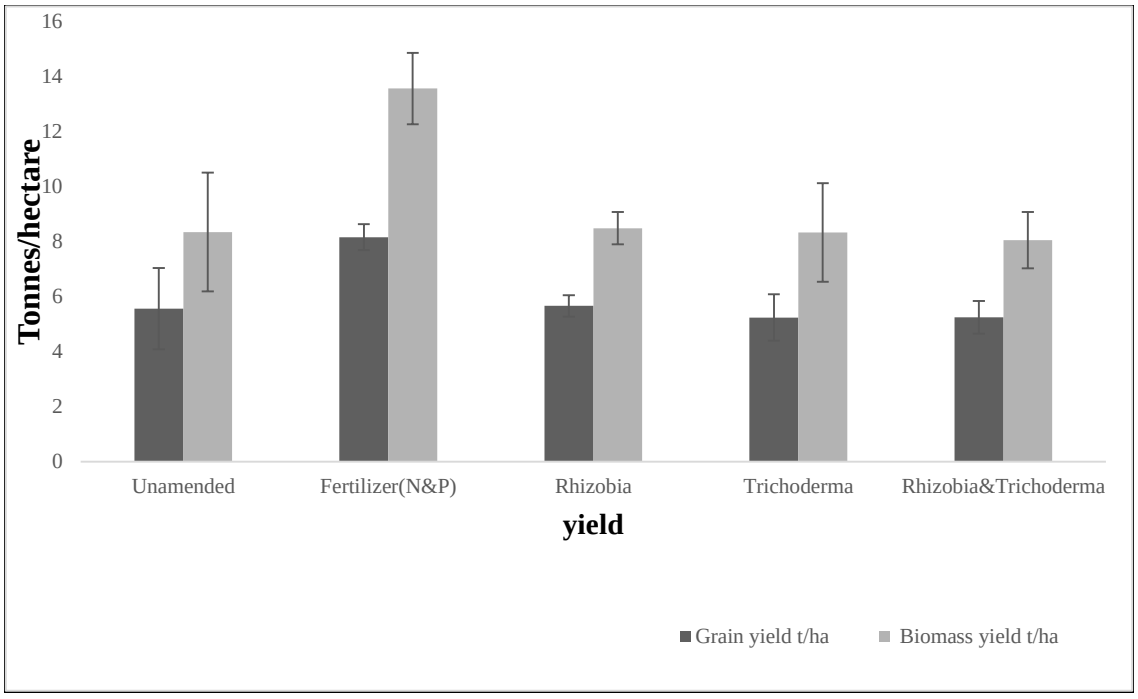


Figure 5. Estimated biomass and grain yield (t/ha) of groundnut with inorganic N and P fertilizer and inoculations of *Rhizobium* and *Trichoderma*

These findings are similar to Latif *et al*. (2014) who showed that N application resulted in increased dry matter yield and that P application resulted in increased grain yield. The fact that plants could take up N and P with little cost compared to the cost that is incurred when the plant is in symbiosis with the *Rhizobium* and *Trichoderma*, allows the plant to use the N and P accumulated more efficiently thereby increasing grain yield and biomass yield.

The inoculated amendments showed no significant differences from the unamended. These results agree with previous studies in several legumes that have shown that a majority of nodules maybe occupied with moderately effective strains from the inoculants rendering more effective indigenous soil strains incapable of establishing themselves. This results in unimproved or even reduced N fixed and biomass yield (Singlton and Tavares, 1986).

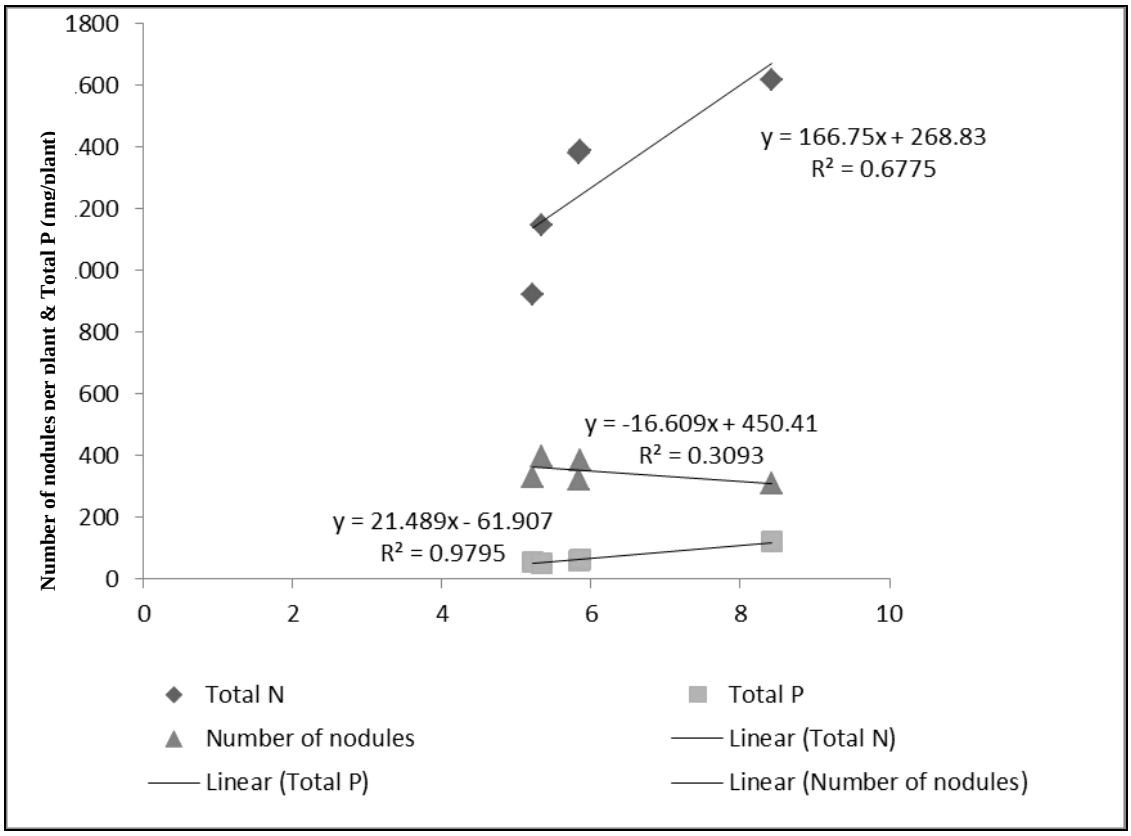


Figure 6. Relationship between accumulated N and P (mg/plant), number of nodules per plant and grain yield

The results in this study showed a very strong positive relationship between P accumulation and estimated grain yield (R^2=0.98), it also showed a positive relationship between N accumulated and grain yield and N accumulated (r^2= 0.68). However, the relationship between the number of nodules per plant and the grain yield showed a weak negative relationship of R^2= 0.31.

The current study showed that grain yield is greatly affected by the uptake of P in groundnut. These results agree with Ncho *et al.* (2013) who had determined that availability of P was found to be critical to groundnut production compared to N because the application of P alone increased grain yield.

Number of nodule per plant had a negative relationship with the grain yield. This shows that the indigenous *Rhizobium* that had infected the groundnut crop was not

effective in N fixing. As a result, the legume formed a symbiotic relationship with the indigenous *Rhizobia* with no gain or benefit from this relationship as was stated earlier.

Applying inorganic N and P to the soil at planting resulted in the highest biomass and grain yield compared to inoculated amendments. The unamended had the lowest grain yield and biomass yield in groundnut. The values of the grain yield and the biomass yield ranged from 0.35 to 4.36 t/ha and 0.79 to 6.69 t/ha, respectively.

4.4.2 Biomass and grain yield of common bean due to N and P fertilizer application and inoculation of Rhizobia and Trichoderma

Even though the inoculated amendments showed percentage increases of 314, 163 and 277 % of Rhizobium, Trichoderma and the dual inoculation respectively, these amendments showed no significant differences from the unamended in terms of grain yield. However, biomass yield of the inoculated amendments Trichoderma, Rhizobium and the dual inoculation increased by 23, 187 and 230 % respectively. The study also showed a significant increase in biomass yield with N and P fertilizer amendment, Rhizobium and the dual inoculation. The single inoculation of Trichoderma showed no significant difference from the unamended.

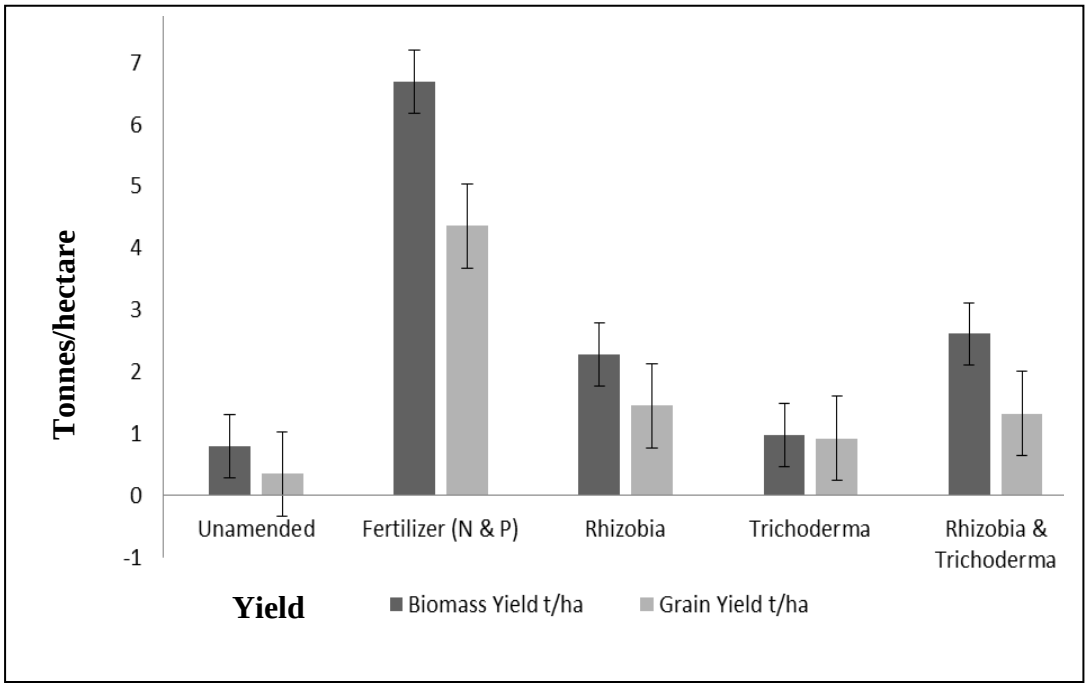


Figure 7. Biomass and grain yield (t/ha) of common bean due to N and P fertilizer Rhizobium and Trichoderma application

Similar results were observed by Otieno *et al.* (1997) and Mungai and Karubia (2010) where biomass yield and grain yield increased significantly in common bean due to N and P fertilizer application. P and N as plant nutrients are important in plant metabolism as components of chlorophyll and amino acids and several other processes that affect plant yield (Whalen and Sampedro, 2010). This explains why plants with the highest amount of N and P also had the highest grain yield and biomass yield.

The current study also showed that the single inoculation of Rhizobium and the dual inoculation significantly increased biomass yield. These results differed from Mweetwa *et al*. (2014) who had shown non- responsiveness of biomass yield to the inoculation of Rhizobium to groundnut, cowpea and soya bean. These differences could be due to the fact that *R. tropici* was originally recovered from common bean nodules that grow in an acid soil in Colombia and Brazil (Romero, 2003), making the

bacteria more specific to infecting and being effective in common bean than other legumes.

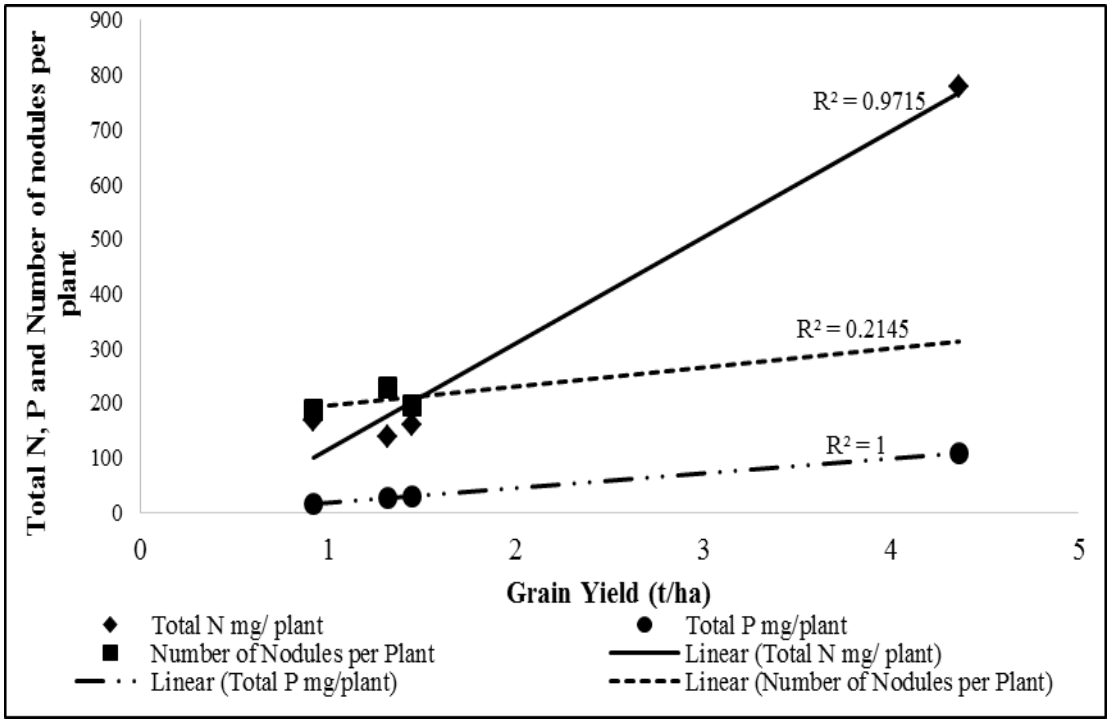


Figure 8. Relationship between accumulation N and P (mg/plant), number of nodules per plant and grain yield

Grain yield positively correlated with the amount of P and N accumulated in common bean. The r^2 values of the correlations of N and P accumulation were 0.97 and 1 respectively. These results agree with Manske *et al*. (2001) who had also shown that uptake of P explained 71 – 80 % of the variation in grain yield in wheat production.

There was a positive and weak correlation of number of nodules with grain yield. These results were consistent with the results of groundnut that also showed a weak but negative correlation between grain yield and number of nodule per plant. This can be explained by the fact that even if a plant has a large number of nodules it does not mean that those nodules are fixing N.

4.5 Changes in selected soil chemical properties due to N and P fertilizer amendment, Rhizobium and Trichoderma in common bean and groundnut after harvest.

4.5.1 Changes in soil reaction and selected exchangeable bases due to N and P fertilizer, Rhizobium and Trichoderma application in groundnut

The After-Harvest Soil (AHS) of the different amendments in groundnut showed significant differences in soil reaction and the amount of magnesium in the AHS. The soil pH values change ranged from 5.79 with the single inoculation of Rhizobium to 6.27 with the dual inoculation. The single inoculation of Rhizobium amendment significantly reduced the pH in AHS in groundnut but it was not significantly different from the N and P fertilizer. The amount of magnesium in the AHS ranged from 1.05 to1.62 cmol/kg of soil, with the single inoculation of Trichoderma having the highest amount and the dual inoculation having the lowest amount of magnesium.

These results showed a general increase in the soil pH from its originally 5.58. The results also showed a general increase in the amounts of calcium in the AHS but did not show any significant differences across amendments. The increase of the calcium in the soil could have been the reason for the general increase in free calcium (Ca^{++}). However as for the reason to this increase in calcium ion in the AHS, further investigation on groundnut uptake and exudation of ion need to be carried out.

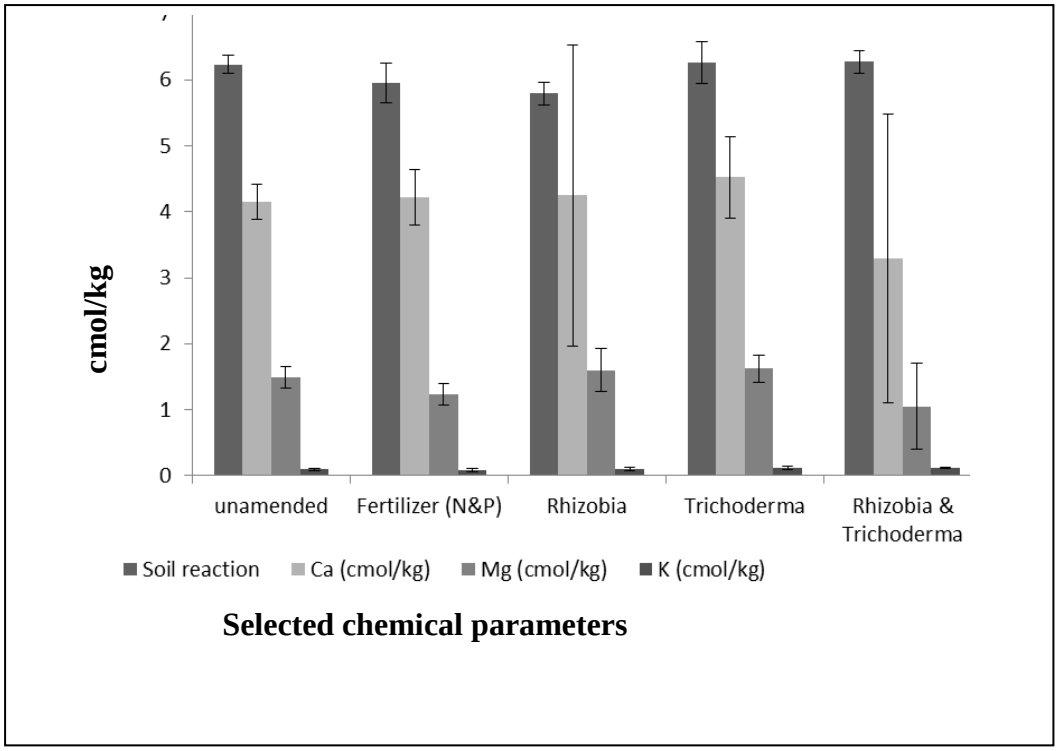


Figure 9. Changes in selected soil chemical properties due to N and P fertilizer, Rhizobium and Trichoderma amendments in groundnut plant after harvest soil

The single inoculation of Rhizobium showed a significant decrease in the soil pH in the AHS. This reduction in pH as reported by Jarvis and Robson (1983) is due to an increase in the uptake of excess cations that reduce the pH of the soil in the plants rhizosphere.

4.5.2 Changes in soil reaction and selected exchangeable bases due to N and P fertilizer, Rhizobium and Trichoderma application in common bean

Soil pH declined from the initial 5.58 to 5.35, 5.11, 4.39, 5.19 and 4.18 in the unamended soil, soil amended with inorganic fertilizer, Rhizobium, Trichoderma and the dual inoculation. The soil pH and magnesium content in the AHS was lower in the single inoculation of Rhizobium and the dual inoculation.

Common bean showed a general reduction in soil pH in the AHS. This shows that the common bean crop absorbed excess cation over anions which lowered the pH of

the plants rhizosphere. Hedley *et al*. (1983) stated that plants absorb excess cation over anions lowering the pH of the rhizosphere, which in turn affects the solubility of P in the soil and its uptake by the plant. This could explain why the inoculation may not have been successful in efficiently increasing grain yield and biomass. The excess uptake of cations by the common bean plant causes an increase in the release of hydrogen ions by the proton antiports, which then reduces the soil pH.

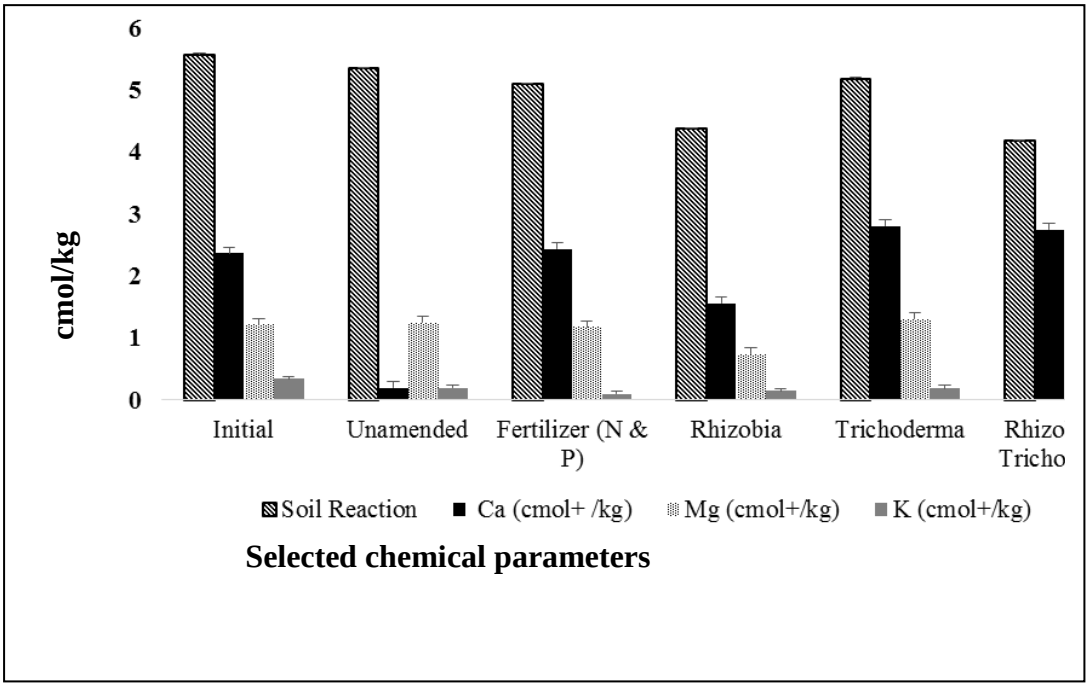


Figure 10. Changes in selected soil chemical properties due to N and P fertilizer Rhizobium and Trichoderma application to common bean

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The presence of adequate N and P in the soil reduces nodulation in both common bean and groundnut, but it also significantly increases grain and biomass yield.

The dual inoculation of Rhizobia and Trichoderma significantly increases nodule effectiveness in both common bean and groundnut. However, it does not significantly increase nodule number per plant in groundnut. This therefore, may lead to the conclusion that *R. tropici* may not be an effective competitor to the Rhizobium- groundnut symbiosis as compared to indigenous Rhizobia strains in the soil.

Even though Trichoderma is known for its ability to increase the uptake of P from the soil, the single inoculation of Trichoderma did not prove to be so in the groundnut crop and common bean plants.

Co-inoculation of *T. harzianum* and *R. tropici* on groundnut and common bean increases the effectiveness of nodules in a slightly acidic soil. However, this does not translate to an increase in biomass and grain yield when compared to fertilizer application.

5.2 Recommendations

Further research could be done with different levels of fertilizer application with the co-inoculation to determine the amount of fertilizer needed to promote nodulation. This will provide the microorganisms with the nutrients needed for nodule production and without preventing nodulation due to excess N in the soil.

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APPENDICES

APPENDIX A1

Soil characterization of selected soil parameters after planting

Treatment	Organic matter %	pH	Available P mg/kg	Total N %	C/N Ratio	Microbial biomass mg/kg/day	Soil respiration mg/kg/day
Rhizobium	2.06^{A}	4.39^{B}	8.26^{B}	0.0133^{A}	79.9^{A}	1.17^{A}	1.67^{A}
Rhizobium +Trichoderma	2.22^{A}	4.18^{B}	8.64^{B}	0.0130^{A}	95.5^{A}	1.08^{A}	1.5^{A}
Trichoderma	2.08^{A}	5.19^{A}	4.98^{B}	0.0112^{A}	93.4^{A}	1.58^{A}	1.25^{A}
Non-inoculated	2.12^{A}	5.11^{A}	17.96^{A}	0.0123^{A}	96.0^{A}	0.918^{A}	1.84^{A}
Control	2.02^{A}	5.35^{A}	4.060^{B}	0.0126^{A}	91.4^{A}	1.17^{A}	2.59^{A}
LSD	0.328	0.504	5.96	0.0059	47.3	1.52	1.44
CV	2.13	2.13	2.13	2.13	2.13	2.13	2.13
PV	0.05	0.05	0.05	0.05	0.05	0.05	0.05

APPENDIX B1

Analysis of variance for average phosphorus uptake in plants in above ground biomass of common bean

Source	DF	Squares	Mean Square	F Value	PrF
				17.07	0.0001
Method of inoculation	4	0.01014861	0.00253715		
Error	15	0.00222884	0.00014859		
Corrected Total	19	0.01237744			

CV (%) 65

P value 0.05

APPENDIX B2

Analysis of variance for average phosphorus content in common bean

Source	DF	Squares	Mean Square	F Value	Pr> F
				10.2	0.0004
Method of inoculation	4	0.00438	0.00109		
Error	15	0.00164	0.000109		
Corrected Total	19	0.00603			

CV (%) 53

P value 0.05

APPENDIX B3

Analysis of variance for total phosphorus uptake in common bean

Source	DF	Squares	Mean Square	F Value	Pr> F
				69.08	.0001
Method of inoculation	4	0.02713875	0.00678469		
Error	15	0.00147316	0.00009821		
Corrected Total	19	0.02861191			

CV (%) 25.8

P value 0.05

APPENDIX C1

Analysis of variance for averages of number of nodules in common bean

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
				3.47	0.0337
Method of inoculation	4	165721.5000	41430.3750		
Error	15	178922.2500	11928.1500		
Corrected Total	19	344643.7500			

CV (%) 83.21224

P value 0.05

APPENDIX C 2

Analysis of variance for averages of percent effectiveness of nodules in common bean

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
				5.03	0.0090
Method of inoculation	4	16750.00000	4187.50000		
Error	15	12500.00000	833.33333		
Corrected Total	19	29250.00000			

CV (%) 82.47861

P value 0.05

APPENDIX C 3

Analysis of variance for averages for Total N content in `common bean

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
				20.62	0.0001
Method of inoculation	4	1.33266179	0.33316545		
Error	15	0.24233034	0.01615536		
Corrected Total	19	1.57499213			

CV (%) 47.84361

P value 0.05

APPENDIX D 1

Analysis of variance for averages for plant biomass in common bean

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
				49.29	0.0001
Method of inoculation	4	90.91722720	22.72930680		
Error	15	6.91727900	0.46115193		
Corrected Total	19	97.83450620			

CV (%) 25.45952

P value 0.05

APPENDIX D 2

Analysis of variance for averages for grain yield in common bean

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
				11.97	0.0001
Method of inoculation	4	38.75282280	9.68820570		
Error	15	12.14249300	0.80949953		
Corrected Total	19	50.89531580			

CV (%) 53.48802

P value 0.05

APPENDIX D 3

Analysis of variance for averages of Total Plant Biomass in common bean

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
				49.92	0.0001
Method of inoculation	4	246.9367588	61.7341897		
Error	15	18.5497780	1.2366519		
Corrected Total	19	265.4865368			

CV (%) 25.56786

P value 0.05

APPENDIX E 1

Analysis of variance for averages of phosphorus content in above ground biomass in groundnut

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
				35.46	0.0001
Method of inoculation	4	0.00062079	0.00015520		
Error	15	0.00006566	0.00000438		

Corrected Total	19	0.00068645			

CV (%) 24.17294

P value 0.05

APPENDIX E 2

Analysis of variance for averages of phosphorus in seed in groundnut

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
				8.00	0.0012
Method of inoculation	4	0.00805425	0.00201356		
Error	15	0.00377466	0.00025164		
Corrected Total	19	0.01182890			

CV (%) 25.91838

P value 0.05

APPENDIX E 3

Analysis of variance for averages of total phosphorus content in groundnut

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
				11.67	0.0002
Method of inoculation	4	0.01306570	0.00326642		
Error	15	0.00419951	0.00027997		
Corrected Total	19	0.01726520			

CV (%) 23.95115

P value 0.05

APPENDIX F 1

Analysis of variance for average of Plant Biomass in groundnut

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
				8.37	0.0009
Method of inoculation	4	36.95867400	9.23966850		
Error	15	16.55809800	1.10387320		

Corrected Total	19	53.51677200			

CV (%) 23.29093

P value 0.05

APPENDIX F 2

Analysis of variance for averages of grain yield in groundnut

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
				9.84	0.0004
Method of inoculation	4	27.51155680	6.87788920		
Error	15	10.48821600	0.69921440		
Corrected Total	19	37.99977280			

CV (%) 13.63739

P value 0.05

APPENDIX F 3

Analysis of variance for averages of Total Biomass in groundnut

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
				10.87	0.0002
Method of inoculation	4	125.3147308	31.3286827		
Error	15	43.2322380	2.8821492		
Corrected Total	19	168.5469688			

CV (%) 15.95183

P value 0.05

APPENDIX I 1

Analysis of variance for averages of number of nodules in groundnut

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
				0.70	0.6054
Method of inoculation	4	24487.7000	6121.9250		
Error	15	131635.5000	8775.7000		

Corrected Total	19	156123.2000			

CV (%) 26.90371

P value 0.05

APPENDIX I 2

Analysis of variance for averages of nodule effectiveness in groundnut

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
				6.03	0.0043
Method of inoculation	4	22730.00000	5682.50000		
Error	15	14143.75000	942.91667		
Corrected Total	19	36873.75000			

CV (%) 58.21222

P value 0.05

APPENDIX I 3

Analysis of variance for Total Nitrogen content in groundnut

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
				6.06	0.0042
Method of inoculation	4	0.34900870	0.08725218		
Error	15	0.21609150	0.01440610		
Corrected Total	19	0.56510020			

CV (%) 34.06909

P value 0.05