EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI AND DIFFERENT PHOSPHORUS RATES ON SELECTED SOIL AND GROWTH PARAMETERS OF TWO DRY BEAN CULTIVARS

by

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DECLARATION

I, Maserole Mavis Moila, declare that the mini-dissertation hereby submitted to the University of Limpopo, for the Degree of Master of Science in Agriculture (Soil Science) has not previously been submitted by me for a degree at this or any university. This report is submitted for examination with my approval.

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DEDICATION

This work is dedicated to my adorable son Leoka Edwin Moila and to my beloved parents Mr Mishack Joseph and Mrs Joyce Molatelo Moila. I also dedicated it to my friends and family.

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I thank God almighty, for protecting me throughout the study and giving me the strength to complete this research. If it were not for Him I could not have made it.

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ABSTRACT

Dry bean is one of the most important cash crops and source of protein for small holder farmers. Low yields of dry bean are often reported to be associated with lack of inoculation (Arbuscular mycorrihizal fungi) of seeds prior to planting. Soil phosphorus (P) unavailability is one of the major factors limiting yield of dry bean. Field and Greenhouse experiments were conducted to investigate the response of dry bean cultivars to inoculation and phosphorus application under dry land farming conditions at the Syferkuil experimental farm of University of Limpopo. Both greenhouse and field experiments were carried out as a split-split plot arrangement in randomised complete block design with four replications. Main plot treatment comprised of two dry bean cultivars *VIZ*, red speckled bean and small white haricot. Arbuscular mycorrhizal fungi inoculation levels (inoculated and uninoculated) were assigned in the sub-plot whilst the sub-sub plot was applied with five phosphorus rates at 0, 20; 40; 60 and 80 kg/ ha using single superphosphate (10.5 % P). The data collected were subjected to analysis of variance using statistical software (ANOVA) STATISTIX 10.0.

Dry bean cultivars (red speckled bean and small white haricot bean) were evaluated in a field experiment for their growth, nodulation and yield responses to AM fungi inoculation and different rates of P fertiliser. The results revealed that the red speckled bean had higher number of nodules (45 %), stem diameter 26.96 cm and higher leaf area of 21.05 cm^2 as compared to small white haricot bean. The application of P at the rate of 40 kg/ha produced higher grain yield of 743.47 kg/ha as compared to small white haricot bean with 572 kg/ha. The growth parameters such as yield, soil chemical and biological properties did not significantly respond to inoculation ($P \leq 0.05$). Red speckled bean and small white haricot bean were evaluated in greenhouse experiment for their growth and nodulation on inoculation and phosphorus fertilizer treatment.

In the greenhouse experiment phosphorus fertilizer rate showed significant effect on chlorophyll content, leaf fresh and dry weight. The dry bean cultivars were found to be significant to number of nodules, plant vigour and root dry weight. The red speckled bean showed higher growth parameters as compared to small white haricot bean. The application of Arbuscular mycorrhizal fungi (AM fungi) at varying rates did not have any significant influence on all parameters measured in the experiment on both trials. The study needs to be repeated after 4 to 5 years. The findings of this study concluded that P should be applied at the rate of 40 kg P/ha in order to improve the production of dry bean.

Keywords: Dry bean cultivar, growth parameters, inoculation, grain yield

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

Arbuscular mycorrhizal fungus (AMF) is the type of mycorrhiza in which the fungus penetrates the cortical cells of the roots of a vascular plant. Arbuscular mycorrhizal fungi get their name from their characteristic formation of branching structures called arbuscules within the cortical cells of roots (Bever *et al*., 2001). Arbuscular mycorrhizal fungi are an important component of the rhizosphere microbial community in natural ecosystems (Khakpour and Khara, 2012). The fungi develop symbiotic relationships that improve plant tolerance to drought, resistance to root pathogens and improve post-harvest storage life (Soka and Ritchie, 2014). Networks of AMF hyphae play a crucial role in the formation of stable soil aggregates and a build-up of macropores that allow infiltration of water and movement of air. The arbuscular mycorrhizal fungi form symbiotic associations with plants where phosphorus is limiting (Soka and Ritchie, 2014).

Arbuscular mycorrhizal fungi benefit their host by mobilising phosphate ions around the root zone due to its ability to grow beyond the nutrient depletion zone. The mycelia network of AMF extends into the soil volume and greatly increases the surface area for the uptake of immobile nutrients. The use of AMF greatly reduces the amount of artificial P fertilizers, increases mobility of Calcium (Ca), molybdenum (Mo) and sulphur (S) (Bambara and Ndakidemi, 2010). In South Africa AMF is sold as mycoroot. Mycoroot is a granular product that is applied as a soil or growth medium amendment (Dames, 2011). It contains indigenous strains of mycorrhizal fungi, which form a special symbiotic relationship with the roots of plants, improving plant health and growth.

The deficiency of P, Mo Ca and other important nutrients is a major constraint to common bean (*Phaselous vulgaris L*) production. Their shortages also have marked an effect on phenolic levels in plant tissue. Phenolic compounds influence the rhizosphere by producing exudants that affect microbial population and activity around the roots, alter soil pH which in turn could influence the availability of nutrients (Bambara and Ndakidemi, 2010). Combined use of AMF and phosphorus reduces the

cost of fertilizers (Soka and Ritchie, 2014). Elhassan *et al*. (2010) observed a significant increase in growth and nodulation of faba bean as a result of application of different levels of P combined with bio-fertilizers. Similar technologies that use mycoroot as an inoculant can be applied with different P rates to improve growth and yield of dry beans. The product can be used for container plants, in the garden for ornamentals, vegetables or when transplanting seedlings, trees and shrubs (Dames, 2011). The application is once off for the life of the plant. Once the relationship has established there is no need to inoculate the plants again, unless they have been disturbed.

Dry bean is an important cash crop and source of protein for smallholder (SH) farmers and low income societies (Turuko and Mohammed, 2014). In South Africa, the most common dry bean types produced by the local farmers are the drought tolerant red speckled and small white haricot (DAFF, 2010). The red speckled bean constitutes 65 to 75% of local bean production while the small white haricot beans have about 10 to 20% of the market share. The production of common bean is limited by inadequate phosphorus nutrition. In this study, it is proposed that the use of AMF and different P application rates could improve P availability and crop performance while reducing the application of artificial phosphate fertilizers such as single super phosphate and the dangers that such fertilizers pose to the environment. The proposed low cost production initiatives could trigger an increase in area planted to dry bean, as well as its productivity levels.

1.2 Problem statement

South African soils are generally deficient in phosphorus (P) which is one of the most essential macronutrients for growth and root development (Tairo and Ndakidemi, 2013). The cost of production of high P demanding crops such as dry bean is high due to the high cost of artificial fertilizers (Liebenberg, 2002). This has had multiple effects on the demand chain with consumers paying more than they should and ways of increasing production without depending on inorganic fertilizers should be sought. The use of plant growth promoting micro-organisms could also improve the quality of the soil, boost yields and maintain crop productivity (Zafar *et al*., 2011). The use of AMF has a potential to improve crop growth and development in dry bean and increase availability of immobile nutrients such as phosphorus (Faboodi *et al.,* 2011).

1.3 Motivation of the study

Most of smallholder (SH) farmers in South Africa are resource-poor and cannot afford the phosphorus fertiliser inputs that are required to increase the yield of crops on infertile soil (Maingi *et al.,* 2006). This often leads to food insecurity. Arbuscular mycorrhizal fungi benefit their host by increasing uptake of relatively immobile phosphate ions due to the ability of the fungi to grow beyond the phosphate depletion zone that quickly develops around the roots (Khakpour and Khara, 2012). It also forms symbiotic association with plants under conditions of P limitation (Soka and Ritchie, 2013). Phosphorus application and mycorrhizal inoculation led to an increase in seed yield components and significantly increased growth parameters and improved nodulation of bean (Elhassan *et al*., 2010). Identifying the appropriate recommended P fertiliser application rates in combination with the use of AM fungi on dry bean seeds will increase plant available P. Most dry bean crops grown by SH farmers are without irrigation in low rainfall areas. The use of AM fungi may enhance the resilience of the crop to in-season dry spells. The technology could be used to sustain production of dry bean. Using the results from this study, recommendations on better management of common bean could be made, particularly under low input conditions.

1.4 Purpose of the study

1.4.1 Aim

The aim of this study was to investigate the effect of AM fungi applied in combination with P fertiliser rate on growth and yield of two dry bean cultivars

1.4.2 Objectives

The objectives of the study were to determine:

(i). The effect of AM fungi on growth responses (grain yield, number of nodules/ plant and number of pods/plant) of the small white haricot and red speckled bean cultivars.

(ii). The effect of P rates on growth responses (grain yield, number of nodules/ plant and number of pods/plant) of the small white haricot and red speckled bean cultivars

(iii). The interaction of AMF and P rates has effect on growth parameters of dry bean

(iv). The effect of AM fungi on soil chemical, soil biological properties and phosphorus mobilisation.

1.5 Null Hypotheses

(i). The effect of AM fungi has no effect on growth responses (grain yield, number of nodules/ plant and number of pods/plant) of the small white haricot and red speckled bean cultivars.

(ii). The P rates have no effect on growth responses (grain yield, number of nodules/ plant and number of pods/plant) of the small white haricot and red speckled bean cultivars

(iii).The interaction of AMF and P rates has no effect on growth parameters of dry bean

(iv). The AMF have no effect on soil chemical, soil biological properties, dry bean performance and P mobilization.

CHAPTER 2
LITERATURE REVIEW

2.1 Dry bean origin, description and benefits

Dry bean (*Phaseolus vulgaris*) belongs to the family Fabaceae, which is believed to have originated from Central and Southern America (Wortmann, 2006). They are the second most important legumes in the world after soya beans (DAFF, 2010). Dry beans are warm annual legumes with upright, bush as well as creeping type or indeterminate growth habit. The crop grows optimally at temperatures of 18 to 24 °C and performs best on sandy loam, sandy clay loam or clay loam with good drainage and clay content between 15 and 35 %. Soil with pH of 5.8 to 6.5 is considered to be the best (Liebenberg, 2002). Dry bean is a major vegetable legume grown and consumed in Southern Africa with high levels of starch, protein (15-25%) and dietary fibre. Dry bean is an excellent source of potassium, selenium, molybdenum, thiamine, vitamin B6 and folic acid (Turuko and Mohammed, 2014). It is also used as food consumption and the green unripe pods are also consumed as vegetables. Dry bean is one of the most important cash crops and source of protein for supplements low-income societies and farmers in many low-land and mid-altitude zones (DAFF, 2010).

2.2 Production level in South Africa

Worldwide statistics on dry beans are difficult to collect, as the various *Phaseolus* and *Vigna* species are often lumped together. According to FAO (2013), dry beans production was about 23 million tons in 2012, cultivated on 29 million hectares. Myanmar, India, Brazil, China, USA, Mexico and Tanzania represented two-thirds (2/3) of the world production of dry beans while China was the main producer of fresh beans. According to DAFF (2010), South Africa produces only 75% of the dry beans consumed in the country, the remainder is imported. A continuous effort is being made to obtain higher production per unit area in order to increase profitability and to meet the ever-increasing demand for food, especially proteins. According to Agricultural Statistics (SA) (2009), distribution of dry bean production in South Africa for the 2007/08 production season shows that Mpumalanga, Free State and Limpopo provinces produce greatest volumes. These are followed by Kwazulu-Natal and the Gauteng province. Of all Provinces in South Africa, Free State produces the largest

quantity, with about 36 % of the total harvest, followed by the Mpumalanga with 25 % while KwaZulu-Natal produces 10%, Limpopo produces 10 % whereby Thabazimbi and Koedoeskop are the main production areas and the North West 7 % and Gauteng produce 9 %.

2.3 Plant description and characteristic of dry bean cultivars

Dry bean (*Phaseolus vulgaris* L.) is a major grain legume consumed worldwide for its edible seeds and pods (DAFF, 2010), belongs to the family Fabaceae and considered as an important crop world-wide. Dry bean originated in Central and South America (Liebenberg, 2002). It is now widespread and cultivated as a major food crop in many tropical, subtropical and temperate areas of the Americas, Europe, Africa and Asia (Wortmann, 2006). It is one of the most important cash crops and source of protein for smallholder farmer. Dry bean is also suitable protein supplements for low income societies (DAFF, 2010). It is used as food stuff and green unripe pods conserved as vegetable.

Dry bean is highly preferred by most farmers because it has an early maturity and drought resistance which enable farmers to get income to purchase food (Turuko and Mohammed, 2014). There are two plant types of beans: erect herbaceous bushes, up to 20-60 cm high and twinning, climbing vines from 2 to 5 m long. The stems are rather slender and pubescent. The flowers of dry bean are arranged in pairs or solitary along the rachis, white to purple and typically *papillonaceous* (Wortmann, 2006).

Department of Agriculture forestry and fisheries (2010) shows that red speckled and small white haricot bean are the most important legumes that are produced by the local farmers because they are drought tolerant. Red speckled bean has a local production of between 65 and 75 % while the small white haricot bean has about 10 to 20% of local bean local production. The production of common bean is limited by phosphorus fertilizer. Therefore, there is a need to investigate the effect of AM fungi applied in combination with phosphorus fertiliser rates on growth and yield of two dry bean cultivars.

2.4 Effect of arbuscular mycorrhizal fungi (AMF) on nodulation, crop growth and yield components

The mycorrhiza is the term that is rooted from two Greek words myco meaning fungi and rhiza meaning root and in reality it means symbiosis between a fungus and roots (Krishnakumar *et al*., 2013). Arbuscular mycorrhizal fungi (AMF) get their name from their characteristic that is formed from branching of the structures called arbuscules within the cortical cells of roots (Bever *et al*., 2001). Arbuscular mycorrhizal fungi play the vital part on the vegetation restoration because of symbiosis with plant root, they can also facilitate mineral absorption by the host plant and they can also improve soil structure, affect the population structure and preserve species diversity (Bothe *et al*., 2010). The ecto and endo are the major types of mycorrhizae. The ecto mycorrhizae are characterised by the extra cellular fungal growth in the root cortex while the endo mycorrhizae characterised by inter and intra cellular fungal growth in the root cortex (Krishnakumar *et al*., 2013).

The AMF are intimately associated with the roots of most flowering plants, herbs vegetables and agricultural crops. They form symbiotic relationship with the plants providing them with nutrients from the soil through better exploitation of the soil environment (Dames, 2011). Improves root development and increase the uptake of nutrients and water. The application of AMF into the soil improves the availability of phosphorus and other immobile nutrients such as iron and zinc. In some studies the mycorrhization status decreases with increased in concentration of P.

According to Yadav and Aggarwal (2014), soil with higher P content could have decreased mycorrhizal colonisation levels and as a consequence, the effect of AMF on plants might barely been less pronounced. Similarly, Pharudi (2010) reported that the non-significant responses of micorrhizal plants to increases in phosphorus levels could be due to the facts that mycorrhizal associations tend to decrease with increasing background soil phosphorus.

According to Dash and Gupta (2011), the AM fungi are important rhizospheric micro-organisms and they increase the plant uptake of nutrients especially relatively immobile elements such as phosphorus and zinc. According to Fatima *et al*. (2012), inoculation of the common bean plant with an AMF fungi resulted in a significant

increase on nodulation and plant growth compared to plants without inoculation. The taxonomy of these fungi is based on the discrete characters of the spore subcellular structure, which can vary from simple to very complex for a single multinucleate cell (Morton and Benivenga, 1994).The application of AMF into the soil improves the availability of phosphorus; other immobile nutrients such as iron and zinc. According to Fatima *et al.* (2012) inoculation of the common bean plant with an AMF resulted in a significant increase on nodulation and plant growth compared to plants without inoculation. The nodulation of beans is strongly affected by the AMF, enhancing the number of nodules and the dry weight per plant. Al-Amri (2013) found that the relative chlorophyll content, leaf area and Mg content of the mycorrhizal plants were significantly higher than that of the non-inoculated plants grown.

Application of Mycorrhiza along with bacteria significantly increased leaf chlorophyll content (Mehrvarz *et al*., 2008). Seeds inoculated by bio-fertilizer strains performed better than the control with no fertilizer (Moradi *et al*., 2015). Yadav and Aggarwal (2014) significantly observed that plants inoculated with AMF increase the shoots length and root length, dry weight of shoot and root, total number of nodules and dry weight of nodule to control. Fatima *et al.* (2012) reported that application of AMF improved growth parameters such as plant height, canopy volume, mean leaf area and number of new shoots per plant but had no effect on trunk diameter, number of leaves per new shoot and new shoot diameter.

Inoculation with AMF and Phosphate Dissolving Bacteria (PDB) as solely or dual under the applied three phosphorus mineral level supplies enhanced phosphorus and micro-nutrients availability in soil, consequently tended to increase their uptake by bean plants (Zaki and Radwan, 2006). Smallholder farmers are generally resource-poor and cannot afford expensive phosphorus fertilisers so using AMF can assist in solving this problem. There is limited or no local research done on growth, nodulation and yield response of dry bean in the small holder farming sector. It is important to explore the potential of introducing the use of AMF together with optimum phosphorus fertilizer rate to smallholder farmers in Limpopo Province.

2.5 Effect of AMF and phosphorus fertiliser on macronutrients and micronutrients

Macronutrients are essential elements that are required in large amounts for plant growth. The main macronutrients are nitrogen, potassium and phosphorus. Arbuscular mycorrhizal fungi are widespread and agronomically important on plant symbiont and often stimulate plant uptake of nutrients such as P, Zn, Cu, and Fe in deficient soils (Krishnakumar *et al.,* 2013). Arbuscular mycorrhizal fungi plays a vital role in increasing the P uptake by producing oxalic acid and phosphatase enzyme which has high completion constants for Calcium, Iron and Aluminium (Krishnakumar *et al*., 2013). AM symbiosis enhance plant growth by increasing plant access to immobile mineral ions mainly phosphorus and Zinc, improving physical conditions and by binding heavy metals into roots that restricts their translocation into shoot tissues.

Mycorrhizae could increase the phosphorus and the micronutrient uptake especially Zn and Cu uptake (Makoi and Ndakidemi, 2009). Results have revealed that dual inoculation with AMF and rhizobium enhanced nitrogen, phosphorus, zinc, iron and copper in plants but the effects were different between fungal and bacterial treatments (Dash and Gupta, 2011). According to (Hajiboland *et al*., 2009) AMF colonisation had a significant effect on uptake of P and Zn. There were no significant differences for Cu concentration among AMF treated and untreated control (Ali *et al.,* 2002).

2.6 Effect of phosphorus fertilizer on growth of dry bean and on selected soil properties

Phosphorus (P) deficiency/unavailability is one of the major factors limiting yield of crops (Shabbir *et al*., 2013). The deficiency of phosphorus may also limit nitrogen fixation through its effects of growth and nodule formation, nodule functioning and host plant growth. The study conducted by Zafar *et al.* (2011) to investigate the influence of integrated phosphorus supply on growth, nodulation, yield and nutrient uptake in *Phaseolus vulgaris* indicated that two mineral phosphorus fertilizers at rate of 60 kg P/ha diammonium phosphate and triple super phosphate increased plant height, number of nodules per plant, nodule fresh weight and nodule dry weight.

Application of the correct level of fertilizer is necessary to achieve maximum yield of common bean crop. Turuko and Mohammed (2014) found that the application of 20

kg P/ ha significantly increased dry matter yield and yield components. Phosphorus application and inoculation (AMF) led to an increase in seed yield components and significantly increased growth parameters and improved nodulation of Faba bean (Elhassan *et al*., 2010). El-Gizawy (2009) recorded highest plant height, number of branches, 100 seed weight and seed yield per plant of Faba bean (*Vacia faba*) with application of 30 kg P/ha. Mehrvarz *et al*. (2008) found no significant effect of phosphorus fertilizers, bacterial strains and mycorrhiza treatment and their interaction effects on plant height of maize.

Ayub *et al.* (2013) significantly found an increased in number of leaves per plant, steam diameter, plant height and number of branches per plant of cluster bean by the influence use of phosphorus solubilizing bacterial inoculation and phosphorus application at the rate of 37.5 kg P/ha. Similarly, Shabbir *et al*. (2013) significantly observed an increase in plant height, number of leaves per plant, dry matter yield and green forage yield at application 60 kg P/ha and phosphorus solubilizing bacterial inoculation. The highest number of pods per plant, pod length and pod circumference of French bean were observed with the treatment that received 60 kg P/ha as compared to 0 and 40 kg P/ha (Shabbir *et al*., 2013).

Malik *et al.* (2006) observed that application of P at the rate of 120 kg P/ha enhance the plant height, leaf area index, number of pods per plant, number of seeds per pods and 1000-seed weight of soya bean. Similarly, Masood *et al.* (2011) observed that seed inoculation along with 100 kg P/ha phosphorus application enhances the plant height number of grain per cobs, 1000-grain weight, grain and biological yield of maize. Nevertheless, Liebenberg (2002) reported that phosphorus is not normally a yield-restrictive factor and under commercial production the yield responses to phosphorus fertilization are not affected in dry bean. However, under subsistence production, where small quantities of fertilizer are applied, phosphorus can be a yield limiting factor. This suggests that phosphorus requirements for maximum yield production may vary in terms of nutritional status of the soil. The appropriate recommended fertiliser rate for dry bean production needs to be created in order to increase yield.

Nutrient availability can have major effect on arbuscular mycorrhizal colonization. It is well known that high P levels in soil inhibit mycorrhizal development and root colonization (Shabbir *et al*., 2013). Valentine *et al.* (2001) reported that the AMF

infection depended on both P supply and the availability of other nutrients, and plants grown at low P with high concentrations of other nutrients had the highest AMF infection, and a higher biomass due to an enhanced maximum net photosynthetic rate. Nursu'aidah (2014) reveal that greater nodulation, higher pods per plant and identical seed yield can be a good indicator to avoid use of chemical fertilizer for both crops production.

2.7 Roles of humic and fulvic acids on soil microbial activity

Humic substances are end products of microbial decomposition and chemical degradation of dead biota in soils. Humic substances also play a vital role in soil fertility and plant nutrition. Plants grown on soils which contain adequate humic acid and fulvic adds are less susceptible to stress, are healthier, produce higher yields; and the nutritional quality of harvested foods and feeds are superior (Pettit, 2004). When plant residues are returned to the soil various organic compounds undergo decomposition. Non-humic organic molecules are released directly from cells of fresh residues, such as proteins, amino acids, sugars and starches (FAO, 2005).

Humic acids comprise a mixture of weak aliphatic (carbon chains) and aromatic (carbon rings) organic acids which are not soluble in water under acid conditions but are soluble in water under alkaline conditions. Fulvic acid is considered to be the soil organic fraction that is soluble in both alkali and acid. Humic substances retain nutrients available on demand for plants, it also improved fertilizer efficiency, improved nutrient uptake, particularly of P and Ca and stimulation of beneficial soil life into the soil (FAO, 2005). Similarly, Gryndler *et al*. (2005) reported that the humic substances, such as fulvic acids that result from the decomposition of organic fertilizers, adsorb free cations from the soil solution and may favour the physiological functions of the fungal mycelia (absorption and transport).

According to FAO (2005) soils in cooler climates commonly have more organic matter because of slower mineralization (decomposition) rates. Humic acid increases nutrient uptake to the plants and drought tolerance. Also increases the microbial activity in the soil, making it an excellent root stimulator. It also increases the availability of nutrients in fertilizers and in those already existing in the soil. The humic acid it also help to lower the pH of the soil to a more neutral level and will flush high levels of salts out of

the root zone, all of which will help to promote better plant health and growth (Mikkelsen, 2005).

2.8 Link between the phosphorus solubilizing bacteria and Arbuscular mycorrhizal fungi

Soil microorganisms are able to solubilize phosphate ions from sparingly soluble inorganic or organic P compounds in *vitro* (Chandrasekeran and Mahalingam, 2014). Phosphorus is the least mobile nutrient element in plant and soil compared to other essential macronutrients. Phosphorus solubilizing microorganisms play role in P nutrition by enhancing its availability to plants through release from inorganic and organic soil P pools by solubilisation and mineralization (Thakur *et al.*, 2014). These microorganisms have the ability to solubilize; mineralize P from inorganic; organic pools of total soil P and making the element available for plants (Gyaneshwar *et al.*, 2002). Phosphate solubilizing microorganisms refer to a group of soil microorganisms that as components of phosphorus cycle, can release it from insoluble sources by different mechanisms (Mehrvarz *et al*., 2008).

Arbuscular mycorrhizal fungi (AMF) improve the absorption of P and other nutrients by plants increasing the contact surface and the explored soil volume (Chandrasekeran and Mahalingam, 2014) and possibly facilitating nutrient transport among plants. The microbiologically solubilized phosphate could, however be taken up by a mycorrhizal mycelium, thereby developing a synergistic microbial interaction. Phosphorus solubilizing bacteria play a role in phosphorus nutrition by enhancing its availability to plants through release from inorganic and organic soil P pools by solubilization and mineralization (Ahmed and El-Abagy, 2007). Inorganic forms of P are solubilized by a group of heterotrophic microorganisms excreting organic acids that dissolve phosphatic minerals and/or chelate cationic partners of the P ions, i.e. phosphatase (PO_4^{3-}) directly, releasing P into solution.

Microorganisms enhance the P availability to plants by mineralising organic P in soil and by solubilizing precipitated phosphates. Bacteria are more effective in phosphorus solubilization than fungi (Yousefi *et al*., 2011). Phosphorus solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to

phosphate, the latter being converted to soluble forms. Phosphorus solubilizing bacteria mainly *Bacillus, Pseudomonas and Enterobacter* are very effective for increasing the plant available P in soil as well as the growth and yield of crops (Ahmed and El-Abagy, 2007).

2.9 Work not yet done on the research problem

Most of the smallholder farming sector in Limpopo province are located on infertile soil where nutrient deficiencies, such as nitrogen and phosphorus, limit crop production. One of the causes of declining soil fertility is continuous cropping without the use of either organic or inorganic fertilizers (Mabapa *et al*., 2010). Many soil microorganisms such as AM fungi are able to solubilise phosphate ions from sparingly soluble inorganic or organic P compounds. The use of AM fungi as bio-fertiliser will help in improving the production of the dry bean.

However, there are no research studies indicating the recommended phosphorus fertiliser rate in combination with AMF for dry bean production under dry land conditions in Limpopo province. Identifying the appropriate recommended P fertilizer rate in combination with AM fungi will help smallholder farmers who are resource poor to be able to improve dry bean yield.

2.10 Addressing issue

The study was conducted at two sites which were field and greenhouse experiments. All the experiments were carried out in split split design, where the main aim of the study was to investigate the effect of AM fungi applied in combination with phosphorus fertiliser on growth and yield of two dry bean cultivars. Identifying the appropriate recommended P rate in combination with AM fungi will be more useful to the smallholder farmers who cannot afford expensive inorganic fertiliser. The application of phosphorus fertiliser rate at the recommended rate will reduce food insecurity and improve yield of dry bean.

CHAPTER 3
MATERIALS AND METHODS

3.1 Site Description

The experiment was conducted at two different sites. The first experiment was carried out inside the greenhouse at the Horticultural Unit of the University of Limpopo (23° 53'10" S, 29° 44' 15"E). The second experiment was conducted at the University of Limpopo experimental farm, Syferkuil (23◦ 59' 35" S, 29◦ 33' 46" E) near Mankweng in the Capricorn district of the Limpopo Province. The climate of the area is classified as semi-arid and receives annual average rainfall ranging from 400 mm to 600 mm that falls predominantly in summer. The soil at the farm is sandy loam belonging to the Hutton form.

3.2 Experimental design, treatments and layout

3.2.1 Greenhouse experiment

Greenhouse experiment was carried out using soil from the Syferkuil farm. Soil for pots was collected at a depth of 0-30 cm and sieved through a 5 mm mesh screen to remove stones and twigs. Soil was tested for P availability before planting. The field experiments were carried out as split split-plots arranged in a Randomized Complete Block Design (RCBD) with four replications. The treatment factors were: (i) Main plot factor – dry bean cultivars (red speckled and small white haricot beans), (ii) Sub-plot factor – Arbuscular mycorrhizal fungi inoculation levels (with and without inoculation) and (iii) Sub-sub plot factor - phosphorus fertiliser rates using single superphosphate (10.5 % P) at 0, 20, 40, 60 and 80 kg P/ha.

The Kranskop cultivar was used for red speckled bean while the Teebus was used for the small white haricot bean. The rates were based on the optimum recommended rate of 45 P kg/ha (Nndwambi *et al.,* 2015). The whole soil was steam sterilized before planting. The phosphorus fertilizer rate and AMF was applied near the seeds inside the pot. The Mycoroots^{TM} product was used for inoculation (Dames, 2011). Pots Measuring 25 cm diameter and volume of 393 cm^3 were used where four dry bean seeds were sown per pot and later thinned to two plants at two weeks after emergence.

Weeds were controlled by hand picking. The pots were watered to field capacity before planting the seeds. Soil was saturated and watered gently after covering the seeds.

3.2.2 Field experiment

The experiment was laid in a split-split plot design fitted into a Randomized Complete Block Design (RCBD) with four replications. The main plot factor consisted of two dry bean types (red speckled and small white haricot bean), the subplot factor was AMF consisting of two levels (without AMF and with AMF), the sub-subplot were four P rates (0, 20; 40; 60 and 80 kg/ ha). Each treatment was laid out in a 3 m × 3 m plot. Each plot consisted of 5 rows with inter-row spacing of 60 cm and intra-row spacing of 15 cm. The AMF and phosphorus fertiliser were applied on banding furrow during planting. Only 5 g of AMF was weight and applied to that furrow where 4-5 seeds are planted (Dames, 2011). The soil was watered gently after covering the seeds.

3.3 Soil sampling

Soil samples were collected from both sites of AMF and P experiment at Syferkuil at a depth of 0-30 cm before planting and after harvesting. The samples were taken to the laboratory for analysis. The sieved samples were being analysed for soil chemical and biological properties.

3.3.1 Procedures for determining soil chemical properties:

- **Soil pH (H_20)**

In all Soil samples the pH was determined using modified method of Hanlon (2015). About 10 g finely grounded soil sample were weight in a 100 ml beaker in which 25 ml of de-ionised water were poured and stirred for 5 seconds. The samples were allowed to stand for 50 minutes then stirred again. After that the samples were again allowed to stand for 10 minutes. The pH value was measured using Lasec crison GLP 21 pH meter 4510. The pH meter was calibrated an hour before the measurement.

- **Bray-1 phosphorus**

Available P was determined using Bray-1 method (Bray and Kurtz, 1945). Phosphorus in the extract was determined on a spectrophotometer by the blue ammonium molybdate method with ascorbic acid as reducing agent. The soils were weighed into extracting bottle where Bray-1 was added. The bottle was shaken by hand for 1 minute and the extract was filtered through Whatman No. 42 filter paper. Colouring reagent (ammonium molybdate) was added together with distilled water and mixed very well. The mixture was allowed to stand for 15 minutes to develop a blue colour to its maximum. The full development of the molybdenum blue colour and the absorbance subsequently read on T60 UV-visible spectrophotometer at a wavelength of 882 nm.

- **Percentage of organic carbon**

Soil organic carbon was determined using a Walkey- Black method as described by Nelson and Sommers (1982). One gram of air-dried soil was weighed into 500 ml Erlenmeyer flask and blank were also included. Potassium dichromate (0.167 mol dm^{-3}) solution was accurately added into the soil sample using pipette. Flask was swirl to disperse the soil in the solution and rapidly 20 cm^3 concentration of sulphuric acid was added. After which 150 cm^3 of de-ionised water was added and mixed well. Ortho-phosphoric acid and 1 cm^3 of diphenylamine indicator were added and titrated by adding iron (II) ammonium sulphate (0.5 mol dm^{-3}) drop by drop from a burette until the colour of solution turned sharply green at end-point from an initial dark violet brown. The volume of iron (II) ammonium sulphate solution used were recorded and percentage of organic carbon were calculated.

- **Micronutrients**

Copper and zinc were determined using di-ammonium EDTA (Beyers and Coetzer, 1971). A 5 g air-dried soil was placed into an extracting bottle where 15 cm^3 EDTA solution was added into the soil. The extracting bottle with soil was shaken horizontally for 60 minutes at 180 oscillations per minute in a reciprocating shaker at a constant temperature of 20 plus or minus 2 ˚C. After the sample was centrifuge in the same container for 5 minutes at 2 000 rpm and that sample was filtered immediately though Whatman No. 42 paper into suitable container using silicone stopper. The element

such as copper, manganese, zinc and iron in were determined in the filtrate sample using atomic absorption spectroscopy (AAS).

- **Humic and fulvic acids**

Humic and fulvic acids were extracted by treating with sodium hydroxide (NaOH) as described by (Mukherjee and Ghosh, 1984). The humic and fulvic acid fractions into the fresh soil sample were extracted using a method described by Sanchez- Monedero *et al*. (1996). The extractions were done using 0.1 m of sodium hydroxide at a ratio of 1:20. The extracts were centrifuged immediately after shaking at 8000 rpm and half of the extracts were stored for subsequent analysis of total extractable carbon fraction where the remainder acidified to pH 2 using concentrated sulphuric acid. The pH adjusted on extracts were allowed to cool over 24 hours at 4 ˚C. The fulvic acid portion in solution after centrifugation and the fraction was then analysed to extractable carbon using the dichromateoxidation method. The extractable carbon in the extracts was then calculated using Equation 3y Anderson and Ingram (1993)

3.3.2 Procedure for determining Soil biological properties

- **Microbiological analysis**

Total bacteria, fungi and phosphorus solubilising bacteria were determined using the serial dilution and standard spread plate counting method (Dick, 1996). A 1 g of fresh weight soil sample from each treatment was suspended in 10 ml of sterile distilled water and shaken for 1 minute on a rotary shaker and dilutions were prepared up to 10^{-10}. All samples were assayed by dilution with three replicates of each suspension. The different agar were used to determine microbial analysis. The Pikovskaya's agar was used for total PSB count, nutrient agar was used for total bacteria count while Rose Bengal Chloramphenicol agar was used for the total fungi count. All the plates were incubated at 37 °C for the period of 3 to 5 days and count were made to determine the number of colony forming units (CFU).

3.4 Data collection

3.4.1 Growth parameters for both pot and field experiment

At 50 % flowering data on growth parameters were measured on four selected tagged plants where plant height, stem diameter, plant vigour and dry matter were measured. The plant heights were measured using a measuring tape. Stem diameter were measured using a vernier caliper while plant vigour was determined using green seeker. The chlorophyll content were also measured using CCM-200 plus chlorophyll content meter on fully developed intact top leaf. The net photosynthesis rate and stomatal conductance was measured in each treatment using Licor system (LI-6400, 4647 superior street Lincoln, Nebraska USA). The measurements were taken between 11:00 and 13:00 during the sunny day on the two fully expanded leaves. The photosynthetic parameters were collected at the podding stage. Two fully expanded leaves were selected per plot and they were clipped to the head of the Licor 6400 to measure all required parameters. Four plants were harvested at 50 % flowering for measuring below ground parameters: the numbers of nodules were recorded. To identify the number of effective nodules the fresh nodules was dissected to see the colour inside the nodules. Nodules were oven dried at 80 °C for 24 hours, and weighed

3.4.2 Grain yield, yield related traits and total biomass data

The total numbers of plants were counted from each plot before harvesting in order to determine stand count at harvesting. In each treatment four plants were selected where the number of pods per plant, number of seeds per pod and thousand seed weight were recorded after harvesting. All the plants in the middle two rows were manually harvested and placed into a plastic bag for each plot to determine total biomass per plot. The plants from each plot were threshed and seeds were air blown to separate seeds from husk using a bucket. The grains were put into brown bags and weighed to determine yield per plot.

3.5. Data analysis

Obtained growth, nodulation, yield data, soil chemical and biological properties for the field and greenhouse experiment were subjected to Analysis of Variance (ANOVA) using Statistix 9.0 and mean separation test was done using Tukey's multiple range tests at a probability level of 5 %. The Pearson correlation analyses were carried out to observe the degree of association in regard with the growth parameters, soil chemical and biological properties. The paired T-test was used to determine the relationships between soil biological and chemical properties before planting and after harvesting. The linear regression analysis was determined on soil chemical and biological properties for both experiments, where soil biological properties are dependent and soil chemical properties are independent.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effect of arbuscular mycorrhizal fungi (AMF), bean cultivars and phosphorus (P) rate on plant growth parameters at field and greenhose experiment

Under field experiment red speckled bean had significantly taller plants and larger leaf area than the small white haricot bean (P≤0.05) and under greenhouse are not significant. The mean plant height and leaf area for red speckled bean under field experiment was 101.77 cm and 26.49 cm^2 respectively (Table1). Under field condition small white haricot bean had bigger stem diameter (28.14 cm) compared to red speckled bean (23.24 cm). However, under greenhouse condition the root fresh weight, leaf fresh weight and number of nodules per plant were not significantly different in both cultivars (P>0.05) (Table 4). The variations could be due to the morphological differences in the two cultivars. The red speckled bean has an indeterminate growth habit where vegetative growth continues after flowering or pod filling resulting in higher plant height as compared to the determinate white haricot whose vegetative growth stop once maturity has been reached. The results under greenhouse condition showed that the growth parameters of red speckled bean are greater than small white haricot bean and this might be due to indeterminate growth of red speckled bean. The indeterminate cultivar resulted in a higher growth parameters and yield (Liebenberg, 2002).

In field experiments addition of P fertiliser did however; affect growth parameters of both cultivars (P≤0.05) except the plant height. The application of P at the rate of 20 kg/ha resulted in bigger stem diameter, leaf fresh weight and leaf area than other P rates for red speckled bean. The mean values were 26.97 cm, 535.18 g and 248.45 cm^2 respectively. However, in the greenhouse experiment all P rates showed positive responses on chlorophyll, leaf fresh weight and dry weight (P≤0.05) except plant vigour, number of nodules per plant and root dry weight (Table 5).The results are in line with those of Turuko and Mohammed (2014) who indicated that, application of P fertiliser had no effect on plant height in common bean. Similarly, Murut *et al.* (2014) reported that phosphorus application did not significantly affect various parameters examined and its effect was sometimes erratic and inconsistent. Mean values of the

data indicated an increase on chlorophyll content (ccl), leaf fresh and dry weight of dry bean was recorded in plots with P applied at the rate of 40 kg P/ha followed by P applied at the rate of 0 kg P/ ha on both cultivars. Similarly, Pharudi (2010) reported that, the application of P level at 30 kg P/ha produce significantly higher plant growth parameters (fresh and dry mass per plant).

The application of AM fungi did not significantly influence any plant growth parameters ($P \geq 0.05$) for both cultivars. Pharudi (2010), reported that inoculation with AM fungi did not have any beneficial effect on fresh and dry biomass of maize plant at P application levels of 0, 10 and 20 kg P/ha. According to Dash and Gupta (2011) the AM fungi are important rhizospheric microorganisms and they increase the plant uptake of nutrients especially relatively immobile elements such as phosphorus and zinc. In this study no improvement of soil nutrients was observed, hence no significant increase in growth parameters. There could be a time needed to establish indigenous isolates obtained to adapt on dry and semi-arid conditions. The fact that the taller plants and bigger stems were observed, though not statistical significant means that given time for AM fungi to adapt to environmental conditions in Limpopo province, the Mycoroot could produce different results. There is need to repeat the experiment over 3-4 seasons to allow the Mycoroot to adapt to the local conditions.

4.2 Interactive effect of phosphorus fertiliser rates and bean cultivars on plant growth parameters

Stem diameter, leaf fresh weight and number of nodules/plant responded significantly to interactive effects between phosphorus application rates and bean cultivars ($P \leq 0.05$) However, no interactive effects were found for plant height, root fresh weight and leaf area ($P > 0.05$). The significant P rates × cultivars interaction clearly indicate that, cultivars produced different stem diameter, leaf fresh weight and number of nodules/plant at five soil P levels.

The best responses for small white haricot bean was recorded at 20 kg P/ha for leaf fresh weight (432.97 g) and number of nodules/plant (24) while a thicker stem diameter was recorded at 0 kg P/ha (Table 2). The results are contradicted with the findings of El-Gizawy (2009) who reported that stem diameter increases with an increase of P rates.

The increase in stem diameter under control treatment might be due to the high immobility of P in the soil. The red speckled bean showed the best response at 40 kg/ha for stem diameter and number of nodules/plant while leaf fresh weight was highest at a rate of 20 kg P/ha. Identification of dry bean cultivar efficient in P utilization may be useful in developing a breeding program to produce superior P use efficient cultivars for low P soil.

4.3 Relationship between application of phosphorus fertiliser rate and plant growth parameters

Regression equations showed a quadratic increase in stem diameter, leaf fresh weight and number of nodules/plant as P fertiliser rates were increased (P≤0.05) for both cultivars. Maximum stem diameter, leaf fresh weight and number of nodules/plant of red speckled bean was obtained at 40 kg P/ha (Figures 1 a, b and c) while for small white haricot bean the maximum rate was rate of 20 kg P/ha. The small white haricot bean showed an inverse quadratic decrease of stem diameter as P rate increased while the leaf fresh weight and number of nodules/plant increased as P rate increased (Figure 2. a, b and c). Pharudi (2010), El-Gizawy (2009) and Masood *et al.* (2011) reported that generally growth parameters showed an increased with an increase in P application level and tended to reach maximum value at 30 kg P/ha and 40 kg/ ha respectively. This is in agreement with the study conducted on soybean which indicated that increasing the phosphorus fertiliser rates concentration in the soil increased the whole plant dry matter accumulation and total leaf area (Turuko and Mohammed, 2014).

4.4 Yield components and yield associated plant parameters

As expected Red speckled bean had significantly higher grain yield as compared to small white haricot bean (P≤0.05). The mean value for grain yield was 743.47 kg/ha (Table 3). However, numbers of pod/plant and 100-seeds weight was not significantly different in both cultivars. The difference is probably due to the genetic differences between the two cultivars. The red speckled bean as expected had higher grain yield because it had a large seeded cranberry type whilst small white haricot had a small white seeded type (Ndlovu, 2015). Tagore *et al.* (2013) also reported that the difference in grain yield of chickpea among genotype was due to the differences in

seed size of the individual genotype. Similarly, Zafar *et al*. (2011) reported that cultivar significantly influence grain yield.

The application of AMF did not affect growth parameters of both bean cultivar (P≥0.05). However, the inoculated treatment visually showed higher number of pods per plants, 100-seed weight and grain yield. The most publicised benefit of mycorrhiza is the improved growth rate, often shown in experimental comparisons of mycorrhizal and non-mycorrhizal plants through its inoculation (Rai *et al*., 2013). Some studies observed an increase in plant height, pods per plant and seeds per pod, 100-seed weight and grain yield of soya bean seed inoculation with AMF and Rhizobum (Malik *et al.,* 2006; Murtaza *et al*., 2014 and Nursu'aidah, 2011).

The application of P rates showed significant effect on number of pod/plant and grain yield except 100-seeds weight. The maximum grain yield was recorded on the treatment of 40 kg/P ha (808.25 kg/ha) followed by the 20 kg P/ ha (691.64 kg/ha) (Table 5). The findings are line with those of Zafar *et al*. (2011) who reported that the application of P at the rate of 20 kg/ha significantly increased yield of dry bean. The improvement in the number of pods due to phosphorus could have resulted from the availability of plant nutrient which stimulated the plants to produce more pods (Dash and Gupta, 2011; Zafar *et al*., 2011).

Table 1: Effect of AM fungi, bean cultivars and P fertiliser on growth parameters of dry bean under field conditions

Treatments		Plant height (cm)	Stem diameter (cm)	RFW (g)	LFW (g)	NNPP	LA (cm^2)
Cultivars	Red speckled bean	101.77^{b}	23.24^{a}	11.98a	545.51a	47	26.49^{a}
	Small white haricot bean	82.41^{a}	28.14^{b}	8.89a	295.16	12	15.78^{b}
	P-value	0.01	0.04	0.08	0.06	0.06	0.00
	Significant difference	**	*	ns	ns	ns	***
	Tukey's HSD	11.27	4.67	4.26	282.23	40	46.19
	CV%	8.41	14.61	32.82	53.97	109	18.01
Fertiliser application rate	0 kg/ha	91.42	24.81ab	8.47	285.78^{c}	9^{b}	18.94^{b}
	20 kg/ha	92.55	26.97^{a}	11.69^{ab}	535.18^{a}	32^{ab}	24.45^{a}
	40 kg/ha	99.2	26.96^{ab}	12.94^{a}	483.18^{ab}	45^{a}	21.05^{ab}
	60 kg/ha	85.18	24.04^{b}	8.68^{b}	377.19^{bc}	33^{ab}	17.08^{b}
	P value	0.87	0.01	0.00	0.00	0.00	0.00
	Significant difference	ns	**	***	***	***	***
	Tukey's HSD	45.73	2.92	3.78	121.14	26	56.55
Inoculation	Inoculated	93.86	25.37	11.46	448.95	29	21.29
	Uninoculated	90.32	26.01	9.42	391.72	30	20.97
	P-value	0.7	0.63	0.22	0.26	0.62	0.85
	Significant difference	ns	ns	ns	ns	ns	ns
	Tukey's HSD	24.05	3.42	3.93	121.34	20.55	61.45

NB: Means followed by the same letter in a column are not significantly different at P≤ 0.05 *= P≤ 0.05, ** =P≤ 0.01*** =P≤ 0.0001, ns=non-significant. RFW= Roots fresh weight, LFW= Leaf fresh weight, LA=Leaf area, NNPP= Number of nodules/plant AMF= Arbuscular mycorrhizal fungi

Table 2: Interactive effects of bean varieties and P fertiliser on growth parameters of dry bean

Treatments		PH (cm)	SD (cm)	RFW (g)	LFW (g)	NNPP	Leaf area (cm^2)
Cultivars	Fertiliser (kg P/ha)						
Red speckled bean	0	74.42	24.52	9.94	339.50	12	24.6^{ab}
Red speckled bean	20	76.1	29.23	11.35	646.40	40	29.79^{a}
Red speckled bean	40	96.38	31.11	14.95	640.65	75	27.14^{a}
Red speckled bean	0	82.75	27.71	11.69	555.50	64	22.41^{abc}
small white haricot bean	0	108.43	25.09	7.00	232.07	8	13.29^{bc}
small white haricot bean	20	109.01	24.72	12.02	423.97	24	19.12^{abc}
small white haricot bean	40	102.04	22.81	10.88	325.72	15	15.95^{bc}
small white haricot bean	60	87.63	20.37	5.68	198.88	3	11.74^{c}
P value		0.69	0.00	0.12	0.04	0.00	0.90
Significant difference		ns	***	ns	*	***	ns
Tukey's HSD		77.63	4.97	6.42	205.63	44.46	96.00

NB: Means followed by the same letter in a column are not significantly different at P≤ 0.05 *= P≤ 0.05, **=P≤ 0.01***=P≤ 0.0001, ns = non-significant. RFW= Root fresh weight, LFW= Leaf fresh weight, NNPP= Number of nodules/plant.

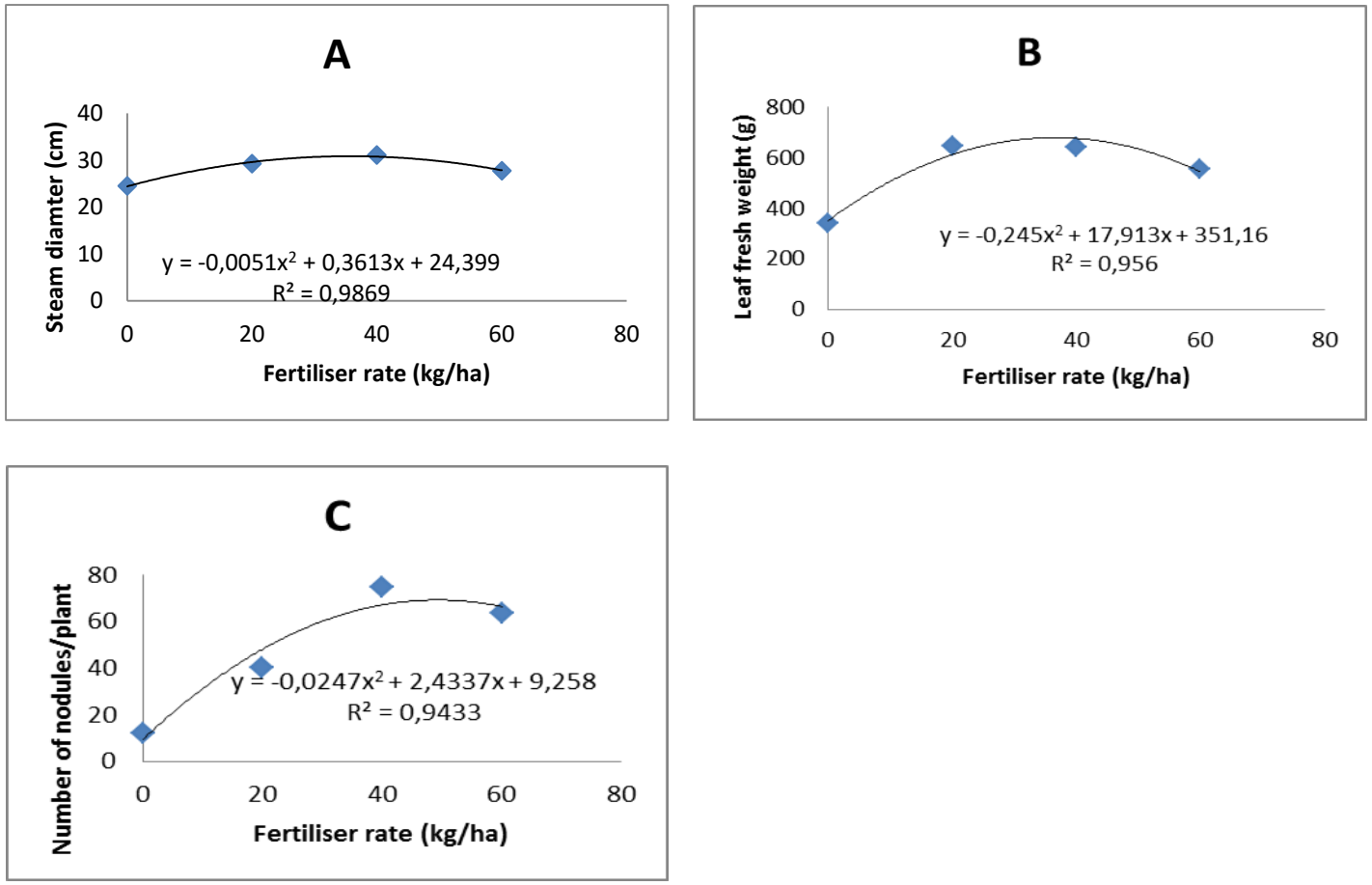

Figure 1: Stem diameter (A), leaf fresh weight (B) and number of nodules/plant as (C) influenced by P rates on red speckled bean

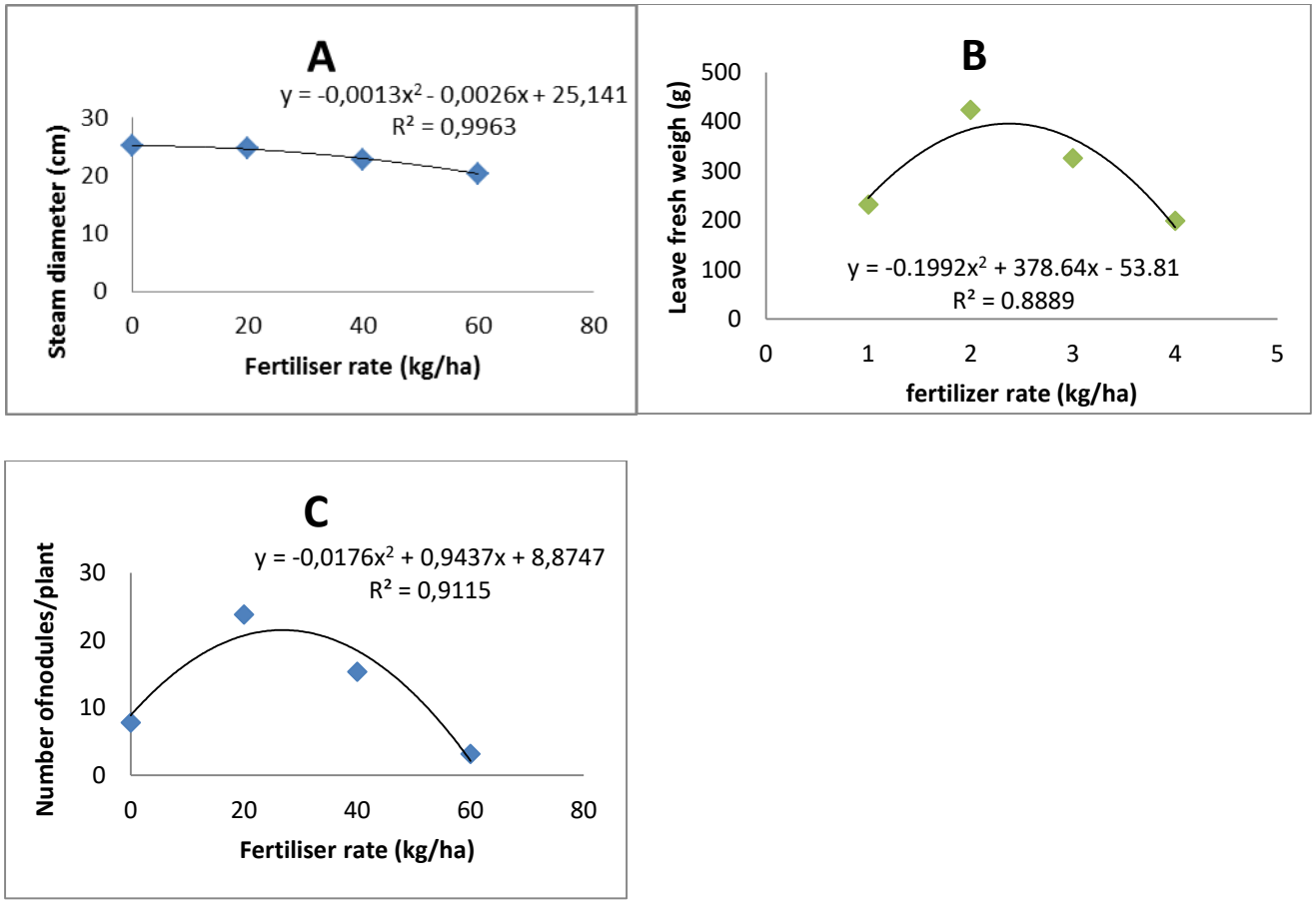

Figure 2: Stem diameter (A), leaf fresh weight (B) and number of nodules/plant as (C) influenced by P rates on small white haricot bean

Table 3: Effects of bean cultivars, AM fungi and P application rate on yield components and grain yield for field experiment

Treatments		NPPP	100seeds (g)	Grain (kg/ha)
cultivars	Red speckled bean	74a	60.10	743.47[a]
	Small white haricot bean	71a	57.59	572.27[b]
	P-value	0.74	0.92	0.045
	Significant difference	ns	ns	*
	Tukey's HSD	34.08	89.63	165.72
	CV%	37.64	122.43	20.25
Fertiliser application rate	0 kg/ha	55[b]	97.66	458.16[b]
	20 kg/ha	76[b]	47.23	691.64[a]
	40 kg/ha	79[a]	47.30	808.25[a]
	60 kg/ha	80[a]	43.20	673.14[a]
	P value	0.00	0.22	0.00
	Significant difference	***	ns	***
	Tukey's HSD	21	80.02	194.44
Inoculation	Inoculated	78	48.76	694.52a
	Uninoculated	67	68.94	621.23a
	P value	0.32	0.38	0.34
	Significant difference	ns	ns	ns
	Tukey's HSD	26.21	54.31	190.05

NB: Means followed by the same letter in a column are not significantly different at P≤ 0.05 *= P≤ 0.05, ** =P≤ 0.01*** =P≤ 0.0001, ns=non-significant. NPPP=number of pod/ plant AMF= Arbuscular mycorrhizal fungi

Table 4: Effect of AM fungi, bean cultivars and P fertiliser rate on plant growth parameters for greenhouse

Treatment							
cultivars		**Chlorophyll (CCI)**	**Plant vigour (NDVI)**	**NNPP %**	**RDW (g)**	**LFW (g)**	**LDW (g)**
	Red speckled bean	39.73	0.62[a]	97.22[a]	1.65[a]	60.92	8.39
	Small white haricot bean	35.63	0.53[b]	42.44[b]	1.09[b]	57.81	8.20
	P value	0.24	0.01	0.03	0.009	0.40	0.37
	Significant difference	ns	*	*	***	ns	ns
	Tukey's HSD	9.045	0.05	45.72	0.29	10.19	0.59
	CV%	30.17	11.49	82.29	26.45	21.57	9.02
Fertiliser application rate	0 kg/ha	33.51[b]	0.56	61.69	1.24	48.83[b]	6.21[b]
	20 kg/ha	43.04[a]	0.57	62.19	1.37	60.63[a]	8.58[a]
	40 kg/ha	42.27[ab]	0.60	84.06	1.46	65.44[a]	9.59[a]
	60 kg/ha	43.62[a]	0.58	71.38	1.46	62.56[a]	8.82[a]
	P value	0.00	0.48	0.06	0.53	0.00	0
	Significant difference	***	ns	ns	ns	***	***
Inoculation(AMF)	Tukey's HSD	40.529[a]	0.0727	24.825	0.41	9.86	1.36
	Inoculated		0.6a	76.094[a]	1.34[a]	59.46[a]	8.00[a]
	Uninoculated	34.83[b]	0.56[a]	63.56[a]	1.40[a]	59.26[a]	8.59[a]
	P value	0.01	0.39	0.15	0.62	0.94	0.17
	Significant difference	*	ns	ns	ns	ns	ns
	Tukey's HSD	4.09	0.10	18.87	0.29	6.36	0.94

NB: Means followed by the same letter in a column are not significantly different at P≤ 0.05 *, ns=non-significant (P≥ 0.05). NNPP= number of nodules/plant, RDW =Root dry weight, LFW=leaf fresh weight and LDW= leaf dry weight, AMF=arbuscular mycorrhizal fungi.

4.5 Effect of bean cultivars, AM fungi and P fertiliser on Soil chemical properties for both greenhouse and field experiments

4.5.1. Soil pH and P

Under both experiments the results showed that, available soil phosphorus and soil pH were not significantly influenced by the cultivars (P≥0.05). The soil pH ranged from 6.8-7.5 (Table 5). Paired T-tests showed a significant decrease between the soil pH before and after planting whilst the phosphorus was non-significant (Table 6). Availability of P showed significant positive correlations with the soil pH in the field experiment (r=0.36, P = 0.01) but a negative correlation in greenhouse experiment (r = -0.05, P = 0.70). Soil available P differences can be attributed to the soil pH. Similarly Jensen (2010) reported that P is the most soil nutrient that is mostly affected by soil pH. Moradi *et al*. (2015) also reported negative correlation between soil available P and soil pH. Zhong and Cai (2007) reported that available P increases with decreasing pH. It should be noted that in alkaline soil with pH above seven, P is retained by Calcium ions and can be quickly converted to insoluble compounds. The macronutrients such as phosphorus are limited in soil with pH above seven due to its precipitation in the soil solution (Yousefi *et al*., 2011). In general some of nutrients cannot be efficiently absorbed by plants roots if soil pH is too high (above 7).

The AMF application did not showed any effect on soil pH and available P (P≥0.05). Visually the treatment that are inoculated (AMF) showed higher soil pH and P than uninoculated treatment. According to Yadav and Aggarwal (2014) inoculation of soil with AM fungi and different levels of superphosphate markedly improved P contents in soybean in comparison to control. Some studies revealed that AM fungal colonisation pattern was related to soil pH and available phosphorus in the soil (Pharudi, 2010 and Moradi *et al*., 2015).

4.5.2 Copper and zinc for both trials

Under field condition there were significant differences in zinc mobilisation in both cultivars except in greenhouse. However, in the greenhouse experiment zinc responded to cultivar differences (P≤0.05). Cultivars showed significant effect on copper in field but not in in greenhouse experiment (Table 5 and 7). This variation was due to the higher soil pH, the availability of many micronutrients such as zinc and

copper reduced at higher pH values. In this study, the soil type was sandy loam and according to Schulte (2004) sandy soil contains low total zinc levels. Since in greenhouse plants are planted into pots this might cause the soil to be compacted and could lead to low availability of zinc to plant uptake (Schulte, 2004). Soil pH influences the availability of micronutrients especially zinc more than any other factor. The correlation effects between the soil pH, phosphorus on zinc availability were studied (Table 10). In this study Zinc is positively correlated with soil pH together in greenhouse ($r = 0.13$, $P = 0.27$) and field experiment ($r = 0.31$, $P = 0.03$).

This study showed that the application of P at 20 kg/kg increased copper in greenhouse whilst in field experiment did not showed any increase on copper and zinc. The higher levels of P may induce zinc deficiency in some cases. When phosphorus rate increases it also decrease the availability of zinc into the soil (Table 10). In this study the zinc shown a non-significant negative correlation with phosphorus in greenhouse experiment ($r = - 0.14$, $P = 0.26$) but positively correlated in the field experiment ($r = 0.41$, $P = 0.00$). It indicates that soils with high phosphates are low in available zinc. Similarly, relationship of available phosphorus and zinc observed in coastal soil by was observed between zinc with phosphorus by Ali *et al.* (2002).

Arbscular Mycorrhizal fungi had an effect on copper and zinc availability on both experiment ($P<0.05$). Paired T-test showed a significant increase in zinc and copper (Table 6). Similarly, Mokoi and Ndakidemi (2009) reported that Zn availability was influenced by AM fungi applications. The AM fungi are important rhizospheric microorganism that can increase mobilisation of relatively immobile elements such as phosphorus, zinc and copper (Dash and Gupta, 2011).

4.5.3 Humic substances and organic carbon

Humic substances are considered as the most important constituents of soil and play a dominant role in improving soil productivity. In this study all the treatments (AMF and P rate) under both experiments did not significantly influence humic, fulvic acid and organic carbon in both experiments ($P\geq0.05$).

However, the paired T-tests showed significant effect on organic carbon and humic acid except fulvic acid (Table 6). The humic acid was higher at harvesting stage compared to before planting while the fulvic acid showed no differences between the

two stages. The increase in humic acid might be due to the decomposition of leaves of dry bean above ground (Tables 5 and 7). In the decomposition process different products are released such as carbon dioxide and resynthesised organic matter called humus (FAO, 2005). Most of the soil organisms are concentrated around roots and in the litter on humus that why in this study the humic acids were higher during harvesting stage. The humic acids has the ability to interact with organic compound and through the formation of these complexes humic substance can dissolve, mobilize and transport organics in soil or accumulate in certain horizon.

The humic substances, such as fulvic acids that result from the decomposition of organic fertilisers, adsorb free cations from the soil solution and may favour the physiological functions of the fungal mycelia (absorption and transport) (Gryndler *et al*., 2005). Fulvic acid in this study showed no differences in the two stages (after planting and harvesting stages) because fulvic acid is produced in the earlier stages of humus formation. The relative amounts of fulvic acids in soils vary with soil type and management practices.

4.6 Soil biological properties for both trails

4.6.1 Total fungi

The total fungus was not significantly different between the cultivars for both trials. The total fungi did not significantly influenced by both cultivars on both experiments. For the greenhouse trial red speckled bean had higher population of fungi than small white haricot bean. In field trial small white haricot bean had higher population of fungi 4.94 $\times 10^{10}$ CFU/g than the red speckled bean 5.23×10^{10} CFU/g. The Application of AMF and Phosphorus fertiliser rate did not significantly influence total fungi in both trials. Application of P at a rate of 60 kg/ha numerically resulted in a higher count of total fungi in greenhouse whilst on field 20 kg/ha produced higher fungi population. Similarly Nashwa *et al*. (2015) reported that the treatment with higher inorganic fertiliser results in higher total yeast population 0.44 $\times 10^{5}$ CFU/g. Fungi has a fine root-like structure (mycelium) that actually cells linked in long strands and makes it well adapted for acquiring phosphorus. The fungi can then use the phosphorus to help its own growth.

The correlation effects between the soil parameters on bacterial count were also studied and results are presented in Table 8 and 9. Total fungal count was positively

correlated with P (r = 0.09, P = 0.05) in greenhouse experiment, and negatively correlated with P (r = -0.25, P = 0.05) in field experiment. The previous study showed that total yeast count increased with increase of P fertiliser (Nashwa *et al*., 2015). Phosphorus is an important element which is actively supplied to host plant by AMF and fungi are highly depended on the host plant for carbon nutrition because plants are good in acquiring carbon which is other essential element that fungi need (Qin *et al*., 2015). Fungi often invade the cells of plants roots; trade their excess phosphorus for the plants excess carbon

4.6.2 Total bacteria

Under both experiments all treatments did not show any statistical significant influence on total bacteria count. However, the red speckled bean showed higher bacterial population compared to small white haricot bean in both trials (Table 5 and 7). The application of P at a rate of 60 kg/ha resulted in higher population of bacteria compared to other treatments. The uninoculated treatment results in both experiment resulted at higher bacterial population in the soil than inoculated treatment. In this study the bacteria isolation was not done so it was not clear which bacteria dominated. However, in some studies higher silicate bacteria counts were observed in treatment with inorganic fertilizer which is P (Nashwa *et al*., 2015). This observation could be due to the habit of these bacteria to attach soil particles and colonize the rhizospheric area (Nashwa *et al*., 2015). Since no isolation of bacterium was done it is suspected that those silicate bacteria might have been present in the current study.

The correlation effects between the soil biological properties and pH on bacterial count were studied on both experiments (Table 8 and 9). Bacterial count was positively correlated with soil pH in greenhouse (r = 0.50, P = 0.69). Similarly, Rousk *et al.* (2010) reported that there was a strong influence of soil pH on the composition of the bacterial communities across the gradient. The regression model for relationship between soil pH and bacterial count for field experiment are shown in Figure 9. The results showed that bacterial count was negatively correlated with soil pH in field (r = - 0.04, P = 0.77). Similarly, Hassan and El-Kamal (2015) reported that the bacterial count has a negative correlation on P and pH. The microbial community especially bacterial population is influenced by the soil pH. Some bacteria can only live and reproduce within a certain range of environmental conditions. The factors that can influence the growth of

bacteria are water availability, temperature and soil pH. Bacteria are sensitive to the hydrogen ion concentration they find in environment especially in low pH bacterial population decreases.

4.6.3 Phosphorus solubilizing bacteria (PSB)

Small white haricot bean showed higher population of phosphorus solubilizing bacteria compared to red speckled bean in field (Table 5). For greenhouse experiment red speckled bean produce more PSB than small white haricot bean (Table 7). However, the paired T-test did not show any significant difference between the mean of before planting and after harvesting on PSB (Table 6). The application of P fertiliser rates did not significantly influence PSB on both trials. Yousefi *et al*. (2011) reported that the application of higher phosphorus fertiliser reduce the colonisation rate of plant roots. Small amount of phosphorus at the beginning of crop is essential to create symbiosis between plant roots and fungi. However, numerically the application of P at the rate of 20 kg P/ha showed high population of PSB as compared to control. Similarly, Nashwa *et al*. (2015) reported that 20 kg P/ha increases population of PSB. Most of soil P is usually presented as insoluble metal chelates, moreover substantial amounts of applied chemical phosphate fertiliser are rapidly converted into insoluble phosphate source. Several bacteria with varied potential to solubilise inorganic phosphate, known as phosphate solubilising microorganisms, have been found in the rhizosphere of plants (Charana Walpola and Yoon, 2013).

Under greenhouse and field experiments inoculation with AM fungi did not significantly show any effect on phosphorus solubilizing bacteria. Such results are in line with findings of Yousefi *et al.* (2011), which reported that, AM fungi inoculation have no effect of P-Olsen% and PSB numbers. The AM fungal colonisation pattern was related to soil pH and availability of P into the soil (Kumar *et al*., 2008). According to Yadav and Aggarwal (2014) the increased phosphorus concentration in soil solution decreased mycorrhizal association and formation of secondary external hyphae. However, in the current study, the treatments that were inoculated with AM fungi numerically showed higher population of PSB as compared to uninoculated treatment.

Total phosphorus solubilizing bacteria count was positively correlated with pH (r = 0.14 and P = 035) in greenhouse and negatively correlated with P (r = -0.06, P = 0.66) (Table 8 and 9) in field experiment. Available soil phosphorus increases with an increase of PSB population in the field whilst in greenhouse there is no relationship between the two (Figure 8 and 9). The negative correlation between PSB and available soil P content, as well as the positive correlation between pH suggested that acidification of the medium can facilitate phosphate solubilisation. Adding an insoluble phosphate source significantly increased total PSB population in soil. The phosphorus solubilising potentials has been attributed to the strains ability to reduces pH of the surrounding either by realising protons (Charana Walpola and Yoon, 2013). A similar increase in the PSB population and available P content was observed by Qin *et al*. (2015). They also observed a positive correlation between available soil P content and PSB population.

Table 5: Effect of bean cultivars, AM fungi and P fertiliser on Soil chemical and biological properties for field experiment

Treatments		pH	P mg/kg	Zn Mg/kg	Cu Mg/kg	HA %	FA %	Total Fungi 10^{10} CFU/g	Total PSB 10^{10} CFU/g	Total Bacteria 10^{10} CFU/g	OC %
cultivars	Red speckled bean	7.71	5.39	1.09	6.52[a]	0.17	0.09	4.94	5.5	4.28	2.08
	Small white haricot bean	7.55	4.97	1.00	5.54[b]	0.20	0.05	5.23	5.26	4.91	2.13
	P-value	0.32	0.37	0.71	0.00	0.37	0.31	0.21	0.66	0.51	0.81
	Significant difference	ns	ns	ns	***	ns	ns	ns	ns	ns	ns
	Tukey's HSD	0.52	2.05	0.87	1.27	0.11	0.12	0.69	2.02	0.76	0.76
	CV%	5.55	5.18	67.37	5.52	51.80	134.84	11.06	30.18	60.60	29.14
Fertiliser application rate	0 kg/ha	7.69	4.51	0.62	5.80	0.16	0.07	4.18	5.16	4.86	2.11
	20 kg/ha	7.65	5.08	1.29	5.47	0.20	0.05	5.73	4.91	3.44	2.38
	40 kg/ha	7.60	5.28	1.12	6.21	0.20	0.09	5.46	5.67	4.90	2.14
	60 kg/ha	7.58	5.87	1.14	6.65	0.16	0.07	4.96	5.76	5.19	1.78
	P-value	0.79	0.58	0.16	0.55	0.58	0.83	0.14	0.62	0.16	0.10
	Significant difference	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Tukey's HSD	0.33	4.22	1.17	2.36	0.12	0.12	1.88	2.05	2.26	3.46
Inoculation	Inoculated	7.67	5.74	1.27	5.79	0.18	0.08	4.77	5.29	5.06	2.17
	uninoculated	7.59	4.63	0.81	6.27	0.18	0.06	5.40	1.74	4.13	2.04
	P-value	0.56	0.42	0.44	0.35	0.87	0.67	0.36	0.79	0.19	0.65
	Significant difference	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Tukey's HSD	0.34	3.47	0.7501	1.27	0.11	0.07	1.72	1.74	1.64	0.64

NB: Means followed by the same letter in a column are not significantly different at P≤ 0.05 *= P≤ 0.05, ns=non-significant. Zn= zinc, Cu= copper, HA= humic acid, FA= fulvic acid, PSB= phosphorus solubilizing bacteria, OC= organic carbon, CFU=colony forming units and AMF= Arbuscular mycorrhizal fungi

Table 6: Paired T-test for soil parameters before and after harvesting for field experiment

Treatments	P Mg/kg	pH	Total PSB CFU	HA %	FA %	Zn Mg/kg	Cu Mg/kg	OC %	Total Fungi CFU	Total Bacteria CFU
Mean	-0.74	-0.33	0.19	0.06	0.01	0.93	5.07	0.52	0.67	0.51
Std Error	0.61	0.06	0.3949	0.01	0.01	0.17	0.41	0.09	0.18	0.13
Lower 95% CI	-1.98	-0.45	-0.5986	0.03	-0.02	0.58	4.26	0.32	0.29	0.24
Mean - H0	-0.74	-0.33	0.1958	0.06	0.02	0.93	5.07	0.52	0.67	0.51
Upper 95% CI	0.50	-0.20	0.9902	0.09	0.05	1.27	5.89	0.72	1.05	0.77
T	-1.2	-5.44	0.5	4.98	1.01	5.4	12.37	5.29	3.59	3.82
DF	47	47	47	47	47	47	47	47	47	47
P-value	0.23	0	0.62	0	0.32	0	0	0	0.00	0.00
significant	ns	***	ns	***	ns	***	***	***	***	***

NB: Means followed by the same letter in a column are not significantly different at P≤ 0.05 *= P≤ 0.05, ns=non-significant. Zn= zinc, Cu= copper, HA= humic acid, FA= fulvic acid, PSB= phosphorus solubilizing bacteria and OC= organic carbon,T=test statistis, DF=decrease of freedom, Cl=confidence of interval, Std= standard, H0=hypothesis

Table 7: Effect of AMF, bean type and phosphorus fertiliser rate on soil chemical and biological properties under greenhouse experiment

Treatment											
Cultivars		**pH**	**Zn Mg/kg**	**P Mg/kg**	**HA %**	**FA %**	**Cu Mg/kg**	**OC %**	**Total Bacteria 10^{10}CFU/g**	**Total Fungi 10^{10}CFU/g**	**Total PSB 10^{10}CFU/g**
	Red speckled bean	7.15	5.64[b]	2.91	0.10	0.13	4.48	1.60	3.98	3.93	5.28
	Small white haricot bean	7.29	6.35[a]	4.22	0.16	0.13	5.22	1.39	6.85	3.37	5.6938
	P-value	0.46	0.02	0.09	0.08	0.64	0.15	0.38	0.12	0.30	0.47
	Significant difference	ns	*	ns	ns	ns	ns	ns	ns	ns	ns
	Tukey's HSD	0.53	0.56	1.74	0.08	0.01	1.24	0.65	4.32	1.45	1.60
	CV%	9.37	11.76	61.24	76.48	33.20	32.15	54.92	100.35	50.09	36.65
Fertiliser application rate	0 kg P/ha	7.52	6.50	2.78	0.12	0.14	5.09	1.40	4.00	3.31	4.81
	20 kg P/ha	7.18[a]	5.60	3.11	0.10	0.10	5.20	1.70	4.20	3.37	6.08
	40 kg P/ha	7.18[ab]	5.91	4.56	0.15	0.16	4.73	1.46	6.56	3.31	5.19
	60 kg P/ha	7.02[b]	5.97	3.81	0.15	0.13	4.38	1.42	6.9	4.62	5.85
	P value	0.02[ab]		0.17	0.49	0.16	0.77	0.1444	0.49	0.20	0.15

	Significant difference	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Turkey HSD	0.43	1.15	2.29	0.09	0.06	2.35	0.38	6.41	1.94	1.63
Inoculation (AMF)	inoculated	7.22	6.02	4.05	0.13	0.13	4.65	1.52	5.26	3.71	5.58
	uninoculated	7.23	5.97	3.08	0.13	0.13	5.05	1.47	5.56	3.59	5.39
	P-value	0.96	0.23	0.07	0.96	0.82	0.56	0.77	0.88	0.77	0.68
	Significant difference	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Tukey's HSD	0.37	1.23	1.10	0.05	0.05	1.64	0.33	4.86	1.03	1.09

NB: Means followed by the same letter in a column are not significantly different at P≤ 0.05 *= P≤ 0.05, ns=non-significant. Zn= zinc, Cu= copper, HA= humic acid, FA= fulvic acid, PSB= phosphorus solubilizing bacteria, OC= organic carbon, CFU=colony forming units and AMF= Arbuscular mycorrhizal fungi

Table 8: Correlation relationship between soil biological properties and Phosphorus for field experiment

	Fungi	Phosphorus	pH	PSB
Phosphorus	0.09			
P-value	0.54			
pH	-0.04	0.36		
	0.79	0.01		
PSB	0.18	-0.06	-0.19	
	0.22	0.66	0.19	
Bacteria	0.06	-0.04	-0.04	0.26
	0.68	0.80	0.77	0.07

NB: PSB = phosphorus solubilizing bacteria

Table 9: Correlation relationship between soil biological properties and Phosphorus for greenhouse experiment

	bacteria	Fungi	Phosphorus	PSB
Fungi	0.12			
P-value	0.33			
Phosphorus	0.07	-0.25		
	0.57	0.05		
PSB	0.08	-0.05	-0.00	
	0.51	0.68	0.96	
pH	0.05	0.09	-0.05	-0.12
	0.69	0.49	0.70	0.35

NB: PSB = phosphorus solubilizing bacteria

Table 10: Correlation relationship between soil pH, Zn and Phosphorus for both experiments

Greenhouse experiment			Field experiment	
	phosphorus	**pH**	**phosphorus**	**pH**
Zinc	-0.14	0.14	0.41	0.31
P-value	0.26	0.27	0.00	0.03

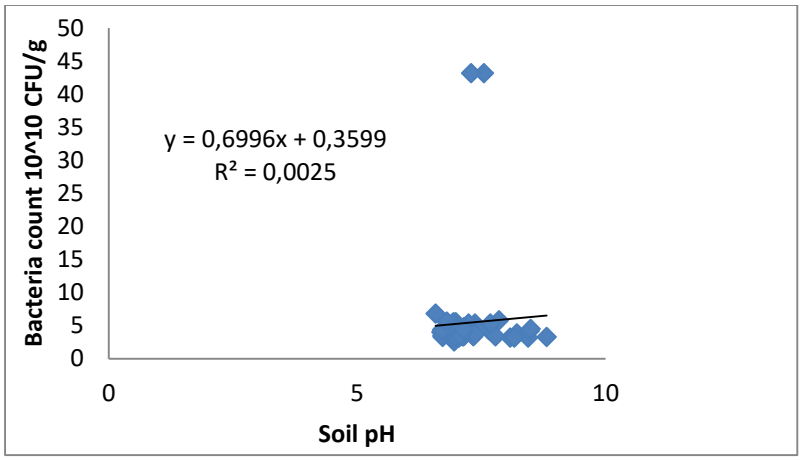

Figure 3: Relationship between soil pH and bacterial count in greenhouse experiment

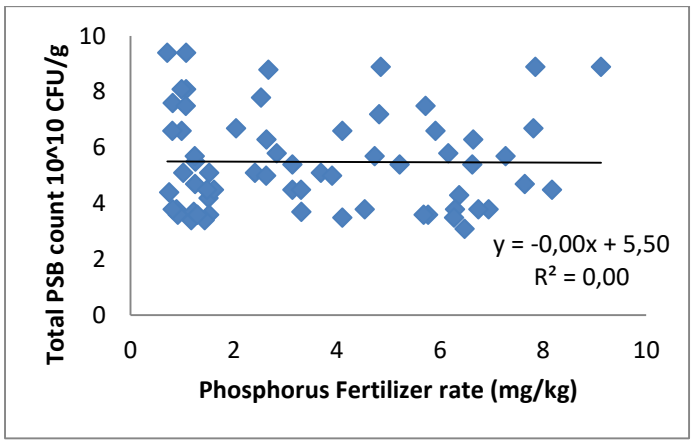

Figure 4: Relationship between available soil P and total PSB count in greenhouse experiment

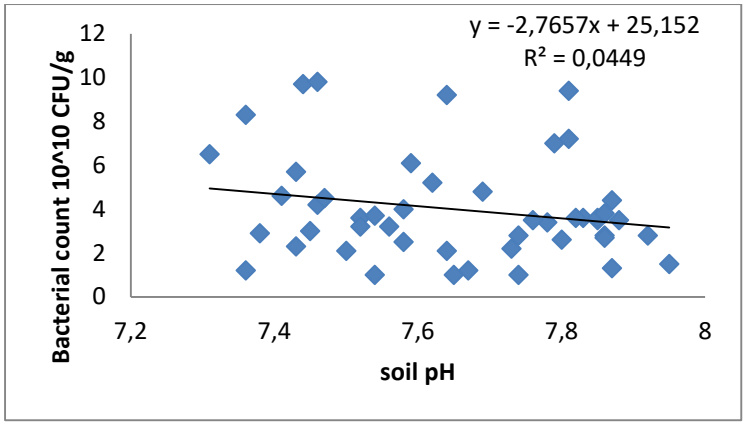

Figure 5: Relationship between available soil pH and total bacterial count in field experiment

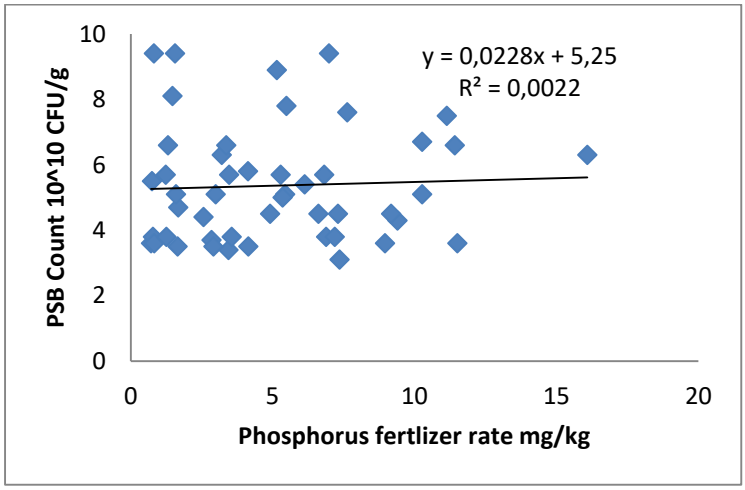

Figure 6: Relationship between available soil P and total PSB count in field experiment

CHAPTER 5

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Soil infertility is one of the major problems that cause lower production in South Africa. South African soils are generally deficient in phosphorus which is one of the most essential macro-nutrients for growth and root development of legumes. The amount of available phosphorus in soils is largely insufficient to meet the demand of legumes and thus phosphorus deficiency is widespread in such crops. In addition, the cost of production of high P demanding crops such as dry bean is high due to the high cost of artificial fertilisers. Most SH farmers in South Africa cannot afford to buy phosphorus fertiliser input due to financial problems as the majority of them are resource poor.

To address such problem this research was conducted to determine optimum rate of phosphorus nutrient for legume production in combination with AM fungi. The use of AM fungi has a potential to improve crop growth and development in dry bean and increase availability of immobile nutrients such as phosphorus. In this study, the use of this technology (AM fungi) will reduce the application of artificial phosphate fertilizers such as single super phosphate and the dangers that such fertilisers pose to the environment. The proposed low cost production initiatives could trigger an increase in area planted to dry bean, as well as its productivity levels. Thus, this study was initiated with the following objectives: (i). To evaluate the effect of AM fungi on growth responses (grain yield, number of nodules/ plant and number of pods/plant) of the small white haricot and red speckled bean cultivars, (ii) to evaluate the effect of P rates on growth responses (grain yield, number of nodules/ plant and number of pods/plant) of the small white haricot and red speckled bean cultivars, (iii). to evaluate the interaction of AMF and P rates has effect on growth parameters of dry bean and (iv) to evaluate the effect of AM fungi on soil chemical, soil biological properties and phosphorus mobilisation.

In green house experiment, the growth parameters such as number of nodules per plant of red speckled bean cultivar showed negative responses after application of P. However, the field experience showed a positive association between a number of nodules per plants, grain yield and P.

For the SH farmer to increase nodulation of red speckled bean have to apply P at lower rate 20 kg P/ha which is far lower than the currently recommended rates of 60 kg P/ha. Improved nodulation also translates to better yields. Obtaining higher yield with lower P fertiliser application rate could improve food security and reduce pollution such as eutrophication of rivers and soil degradation through acidification by artificial fertilisers.

In both the field and greenhouse experiments, small white haricot bean responded better to P application rate in a number of growth indicators such as chlorophyll content, leaf fresh weight and dry weight except the plant height and grain yield. The increase in leaf dry weight is very much useful as it indicates an increase in biomass for green manuring, mulch or even fodder. The increase in leaf dry weight practically can be used by SH farmers for mulching protecting soil erosion. The most important chemical reaction in nature is photosynthesis, so the increase in chlorophyll content through the application of P at 40 kg P/ ha will enhance the photosynthesis process.

The application of AM fungi did not show any statistically significant effect on growth parameters of small white haricot bean in both experiments. However, visually there were increases in growth parameters. The AMF need time to adapt under soil conditions prevailing in Limpopo Province.

From this study, the application of P at 20 kg/ha increased copper in greenhouse whilst in field experiment did not show any increase on copper and zinc. The higher levels of P may induce zinc deficiency in some cases. When phosphorus rates increases it also decrease the availability of zinc in soil. In both trials application of 80 kg P/ha had a negative impact on soil available P, fulvic, humic and organic carbon. Arbuscular Mycorrhizal fungi had an effect on copper and zinc availability on both experiment. The AM fungi are important rhizospheric microorganism that can increase mobilisation of relatively immobile elements such as phosphorus, zinc and copper. From these results, it is recommended that attention should be given to applying lower P (20 kg P/ha) if the increase in soil chemical properties are needed to be achieved.

In field and greenhouse experiments soil biological properties showed a negative response to the AM fungi and P rates such as total bacterial count, total fungus count and population of phosphorus solubilizing bacterial. No positive responses were observed on both experiments. However, the recommended P rates from these study suggest that is 20 kg P/ha.

The results of this study also revealed that the combination of application of P and AM fungi did not significantly affect the soil microbial and chemical properties on both green house and field experiment. Further investigations over longer period need to be carried out to explain this finding.

The following recommendations can be made:

- There is need to repeat the experiment over 3-4 seasons to allow the mycoroot to adapt to the local conditions.
- These study showed that there is need to test AM fungi inoculation in different soils and climatic locations in order to make sure that they can mobilise P into the soil before introducing this technology (AMF inoculation) for SH farmers.
- The P rate should be applied at the rate of 40 kg P/ha to improve dry bean production and 20 kg/ha for soil chemical properties and biological properties

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APPENDICES

Analysis of Variance (ANOVA) tables for field study

Appendix 1: Analysis of variance for plant height

Source	DF	SS	MS	F	P
Replication (A)	2	613.4	306.71		
Cultivars (B)	1	4499.8	4499.85	54.79	0.0178
Error A*B	2	164.3	82.13		
Inoculation (C)	1	150.2	150.17	0.17	0.7037
B*C	1	4433.3	4433.29	4.93	0.0906
Error A*B*C	4	3596.3	899.07		
Fertiliser (D)	3	1188.1	396.04	0.24	0.8674
B* D	3	2385.7	795.22	0.48	0.6976
C*D	3	3594.5	1198.17	0.73	0.5460
B*C*D	3	417.9	139.29	0.08	0.9678
Error A*B*C*D	24	39561.7	1648.40		
Total	47	60605.1			

Appendix 2: Analysis of variance for stem diameter

Source	DF	SS	MS	F	P
Replication (A)	2	298.99	149.493		
Cultivars (B)	1	287.62	287.618	20.40	0.0457
Error A*B	2	28.20	14.099		
Inoculation (C)	1	4.81	4.805	0.26	0.6347
B*C	1	57.22	57.220	3.14	0.1512
Error A*B*C	4	72.92	18.231		
Fertiliser (D)	3	81.22	27.074	4.01	0.0190
B*D	3	142.85	47.618	7.06	0.0015
C*D	3	7.65	2.550	0.38	0.7696
B*C*D	3	11.76	3.918	0.58	0.6331
Error A*B*C*D	24	161.85	6.744		
Total	47	1155.08			

Appendix 3: Analysis of variance for root fresh weight

Source	DF	SS	MS	F	P
Replication (A)	2	32.169	16.084		
Cultivars (B)	1	114.701	114.701	9.77	0.0889
Error A*B	2	23.479	11.740		
Inoculation (C)	1	50.062	50.062	2.09	0.2220
B*C	1	4.941	4.941	0.21	0.6734
Error A*B*C	4	95.917	23.979		
Fertiliser (D)	3	175.525	58.508	5.19	0.0066
B*D	3	71.075	23.692	2.10	0.1268
C*D	3	5.759	1.920	0.17	0.9155
B*C*D	3	48.256	16.085	1.43	0.2598
Error A*B*C*D	24	270.777	11.282		
Total	47	892.661			

Appendix 4: Analysis of variance for leaf fresh weight

Source	DF	SS	MS	F	P
Replication (A)	2	635004	317502		
Cultivars (B)	1	752127	752127	14.61	0.0621
Error A*B	2	102936	51468		
Inoculation (C)	1	39314	39314	1.72	0.2601
B*C	1	11992	11992	0.52	0.5091
Error A*B*C	4	91498	22874		
Fertiliser (D)	3	445267	148422	12.83	0.0000
B*D	3	110004	36668	3.17	0.0426
C*D	3	125566	41855	3.62	0.0276
B*C*D	3	80844	26948	2.33	0.0997
Error A*B*C*D	24	277586	11566		
Total	47	2672137			

Appendix 5: Analysis of variance for number of nodules per plant

Replication (A)	2	8365.6	4182.8		
Cultivars (B)	1	14840.3	14840.3	13.76	0.0656
Error A*B	2	2156.3	1078.1		
Inoculation (C)	1	1.3	1.3	0.00	0.9662
B*C	1	2.1	2.1	0.00	0.9578
Error A*B*C	4	2624.6	656.1		
Fertiliser (D)	3	7808.8	2602.9	4.81	0.0092
B*D	3	7686.5	2562.2	4.74	0.0098
C*D	3	2704.8	901.6	1.67	0.2005
B*C*D	3	373.4	124.5	0.23	0.8744
Error A*B*C*D	24	12975.5	540.6		
Total	47	59539.2			

Appendix 6: Analysis of variance for leaf area

Source	DF	SS	MS	F	P
Replication (A)	2	19396	9698		
Cultivars (B)	1	141812	141812	102.84	0.0096
Error A*B	2	2758	1379		
Inoculation (C)	1	225	225	0.04	0.8544
B*C	1	6131	6131	1.05	0.3644
Error A*B*C	4	23466	5867		
Fertilize (D)	3	41416	13805	5.48	0.0052
B*D	3	1413	471	0.19	0.9043
C*D	3	11182	3727	1.48	0.2455
B*C*D	3	17877	5959	2.36	0.0963
Error A*B*C*D	24	60507	2521		
Total	47	326184			

Appendix 7: Analysis of variance for grain yield

Source	DF	SS	MS	F	P
Replication (A)	2	334642	167321		
Cultivars (B)	1	351689	351689	19.82	0.0469
Error A*B	2	35491	17746		
Inoculation (C)	1	64456	64456	1.15	0.3442
B*C	1	88558	88558	1.58	0.2774
Error A*B*C	4	224467	56117		
Fertiliser (D)	3	766715	255572	8.58	0.0005
B*D	3	41213	13738	0.46	0.7121
C*D	3	205420	68473	2.30	0.1031
B*C*D	3	134971	44990	1.51	0.2374
Error A*B*C*D	24	715116	29797		
Total	47	2962739			

Appendix 8: Analysis of variance for number pods per plant

Source	DF	SS	MS	F	P
Replication (A)	2	4005.3	2002.65		
Cultivars (B)	1	108.0	108.00	0.14	0.7409
Error A*B	2	1501.1	750.56		
Inoculation (C)	1	1386.8	1386.75	1.30	0.3179
B*C	1	1875.0	1875.00	1.76	0.2556
Error A*B*C	4	4267.7	1066.94		
Fertiliser (D)	3	5062.9	1687.64	4.76	0.0097
B*D	3	354.0	118.00	0.33	0.8019
C*D	3	1046.9	348.97	0.98	0.4171
B*C*D	3	337.0	112.33	0.32	0.8132
Error A*B*C*D	24	8515.2	354.80		
Total	47	28459.9			

Appendix 9: Analysis of variance for copper (Cu)

Source	DF	SS	MS	F	P
Replication (A)	2	52.174	26.0868		
Cultivars (B)	1	11.662	11.6624	104.92	0.0094
Error A*B	2	0.222	0.1112		
Inoculation (C)	1	2.736	2.7361	1.08	0.3577
B*C	1	1.591	1.5914	0.63	0.4727
Error A*B*C	4	10.149	2.5372		
Fertiliser (D)	3	9.342	3.1140	0.70	0.5588
B*D	3	11.133	3.7111	0.84	0.4857
C*D	3	23.729	7.9096	1.79	0.1762
B*C*D	3	12.779	4.2596	0.96	0.4261
Error A*B*C*D	24	106.115	4.4214		
Total	47	241.633			

Analysis of Variance (ANOVA) tables for greenhouse study

Appendix 10: Analysis of variance for chlorophyll content

Source	DF	SS	MS	F	P
Replication (A)	3	372.51	124.170		
Cultivars (B)	1	269.06	269.063	2.08	0.2448
Error A*B	3	387.76	129.254		
Inoculation (C)	1	519.70	519.698	11.61	0.0144
B*C	1	38.87	38.867	0.87	0.3874
Error A*B*C	6	268.63	44.772		
Fertiliser (D)	3	1040.05	346.683	6.23	0.0016
B*D	3	28.28	9.427	0.17	0.9163
C*D	3	96.41	32.136	0.58	0.6334
B*C*D	3	76.11	25.371	0.46	0.7146
Error A*B*C*D	36	2002.50	55.625		
Total	63	5099.88			

Appendix 11: Analysis of variance for plant vigour

Source	DF	SS	MS	F	P
Replication (A)	3	0.10346	0.03449		
Cultivars (A)	1	0.14440	0.14440	32.36	0.0108
Error A*B	3	0.01339	0.00446		
Inoculation (C)	1	0.02250	0.02250	0.83	0.3964
B*C	1	0.01690	0.01690	0.63	0.4589
Error A*B*C	6	0.16195	0.02699		
Fertiliser (D)	3	0.01456	0.00485	0.83	0.4842
B*D	3	0.00661	0.00220	0.38	0.7690
C*D	3	0.00934	0.00311	0.53	0.6615
B*C*D	3	0.02859	0.00953	1.64	0.1980
Error A*B*C*D	36	0.20960	0.00582		
Total	63	0.73130			

Appendix 12: Analysis of variance for number of nodules per plant

Source	DF	SS	MS	F	P
Replication (A)	3	17347	5782.3		
Cultivars (B)	1	48016	48015.8	14.54	0.0317
Error A*B	3	9905	3301.6		
Inoculation (C)	1	2513	2512.5	2.64	0.1554
B*C	1	1454	1453.5	1.53	0.2628
Error A*B*C	6	5713	952.2		
Fertiliser (D)	3	5275	1758.2	2.59	0.0680
B*D	3	2381	793.7	1.17	0.3353
C*D	3	1074	358.1	0.53	0.6665
B*C*D	3	549	183.1	0.27	0.8469
Error A*B*C*D	36	24459	679.4		
Total	63	118685			

Appendix 13: Analysis of variance for root dry weight

Source	DF	SS	MS	F	P
Replication (A)	3	0.8275	0.27583		
Cultivars (B)	1	4.8290	4.82901	36.68	0.0090
Error A*B	3	0.3950	0.13167		
Inoculation (C)	1	0.0613	0.06126	0.26	0.6274
B*C	1	0.0016	0.00160	0.01	0.9368
Error A*B*C	6	1.4059	0.23431		
Fertiliser (D)	3	0.4175	0.13918	0.75	0.5301
B*D	3	0.0760	0.02534	0.14	0.9377
C*D	3	0.6193	0.20642	1.11	0.3574
B*C*D	3	0.0122	0.00405	0.02	0.9955
Error A*B*C*D	36	6.6890	0.18581		
Total	63	15.3342			

Appendix 14: Analysis of variance for leaf fresh weight

Source	DF	SS	MS	F	P
Replication (a)	3	260.78	86.927		
Cultivars (B)	1	154.57	154.567	0.94	0.4032
Error A*B	3	492.00	163.999		
Inoculation (C)	1	0.66	0.656	0.01	0.9405
B*C	1	87.38	87.376	0.81	0.4037
Error A*B*C	6	649.91	108.318		
Fertiliser (D)	3	2556.41	852.137	7.94	0.0003
B*C	3	524.76	174.921	1.63	0.1994
C*D	3	9.93	3.309	0.03	0.9926
B*C*D	3	110.65	36.882	0.34	0.7938
Error A*B*C*D	36	3862.04	107.279		
Total	63	8709.07			

Appendix 15: Analysis of variance for leaf dry weight

Source	DF	SS	MS	F	P
Replication (A)	3	30.176	10.0587		
Cultivars (B)	1	0.608	0.6084	1.08	0.3743
Error A*B	3	1.684	0.5613		
Inoculation (C)	1	5.676	5.6763	2.40	0.1720
B*C	1	6.338	6.3378	2.68	0.1524
Error A*B*C	6	14.166	2.3610		
Fertiliser (D)	3	102.579	34.1932	16.65	0.0000
B*D	3	21.433	7.1445	3.48	0.0257
C*D	3	6.606	2.2021	1.07	0.3729
B*C*D	3	0.980	0.3268	0.16	0.9231
Error A*B*C*D	36	73.909	2.0530		
Total	63	264.157			

Appendix 16: Analysis of variance for soil pH

Source	DF	SS	MS	F	P
Replication (A)	3	0.1533	0.05109		
Cultivars (B)	1	0.3249	0.32490	0.71	0.4619
Error A*B	3	1.3768	0.45893		
Inoculation (C)	1	0.0008	0.00076	0.00	0.9661
B*C	1	0.0144	0.01440	0.04	0.8529
Error A*B*C	6	2.3069	0.38449		
Fertiliser (D)	3	2.0929	0.69762	3.41	0.0276
B*D	3	0.2264	0.07547	0.37	0.7757
C*D	3	0.5147	0.17157	0.84	0.4813
B*C*D	3	1.0214	0.34047	1.67	0.1917
Error A*B*C*D	36	7.3594	0.20443		
Total	63	15.3918			

Appendix 17: Analysis of variance for zinc (Zn)

Source	DF	SS	MS	F	P
Replication (A)	3	22.341	7.44695		
Bean (B)	1	7.858	7.85835	15.78	0.0285
Error A*B	3	1.494	0.49803		
Inoculation (C)	1	0.055	0.05492	0.01	0.9117
B*C	1	0.648	0.64827	0.16	0.7047
Error A*B*C	6	24.613	4.10223		
Fertiliser (D)	3	6.600	2.20000	1.50	0.2317
B*D	3	9.521	3.17374	2.16	0.1097
C*D	3	3.885	1.29500	0.88	0.4598
B*C*D	3	3.543	1.18084	0.80	0.5000
Error A*B*C*D	36	52.884	1.46899		
Total	63	133.442			