RESPONSE OF WHEAT (*TRITICUM AESTIVUM)* TO *VESICULAR ARBUSCULAR MYCORRHIZA* (VAM) AND *TRICHODERMA* ON GRAIN YIELD AND UPTAKE OF PHOSPHOROUS IN ACIDIC SOILS

BY

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A dissertation submitted to the University of Zambia in partial fulfillment of the requirements for the award of the Master of Science Degree in Agronomy (Crop Science)

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DECLARATION

I Katongo Chishimba, hereby declare that all the work presented in this dissertation is my own work and has never been submitted for a degree to any University

Signed…….

Date…….

APPROVAL

This dissertation is approved as fulfilling part of the requirements for the award of the Master of Science Degree in Agronomy (Crop Science) by the University of Zambia

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ABSTRACT

Wheat is an important economic cereal crop used in the production of a wide range of foods and other products. However, due to low wheat production (< 1.5 ton/ha) in acidic soils, and the country's total demand of about 240,000 metric tones as at 2010, is unable to be met. Acid soils generally cover a large part of the Zambian soils and have problems of low soil fertility including aluminium toxicity, low pH and phosphate. This condition is detrimental to growth of wheat. Therefore, this study is important in that it may provide a cost effective remedy for growing wheat in high rainfall regions and contribute to the total country's wheat production. The research study was carried out to determine the effect of fungal treatments on selected wheat varieties with regard to uptake of phosphorous and grain yield in acidic soils. The study was conducted in Chipata District at Msekera Research Station of the Zambian Agriculture Research Institute between May and September 2010. The station is located at Latitude 13^{o} 39'N and Longitude 32^{o} 34'E. A Factorial Randomized Complete Block Design with three replications was used. The factors considered were lime at two levels (with and without lime), Variety at four levels (Sahai, Nduna, Lorrie II and UnzaWV1) and fungal treatment at four levels (Trichoderma, Versicular arbuscular mycorrhiza (VAM), VAM/Trichoderma and control). First phase of the experiment was isolation of VAM spores from the soil and the second phase was inoculation of wheat seeds with VAM and Trichoderma species at planting. Indigenous spores of VAM were extracted by the wet sieving and decanting technique of Gerdemann and Nicolson. The results of the study showed that VAM and Trichoderma spp significantly increased phosphorous uptake and grain yield for all wheat varieties when applied seperately. The grain yield

and P uptake for all four wheat varieties was still high irrespective of the liming when wheat varieties were treated with VAM and *Trichoderma* species. This is due to ability by *Trichoderma spp* and VAM to survive and increase nutrient uptake in acidic soils. Fungal treatment increased grain yield and P uptake by 200% and 400% respectively.

DEDICATION

This work is dedicated to my wife and children for their spiritual and moral support towards the completion of this dissertation.

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1.0 INTRODUCTION

Wheat is an important cereal crop which belongs to the grass family of *Poaceae* formerly *Gramineae* and makes up the genus (*Triticum spp*). It is a temperate crop that can also be grown in the tropics especially at high altitudes and in the dry cool season of the Sub-Sahara Africa. The main use of wheat is in the manufacture of flour for bread and pastries. In general, hard varieties of wheat (*T. durum*) are used for bread flour while soft varieties of wheat (*T. aestivum*) are used for pastry flour (Purseglove, 1975). Wheat is used also in the production of breakfast foods and to a limited extent in the making of beer, whiskey, and industrial alcohol. Low grades of wheat, and by-products of the flour-milling, brewing, and distilling industries, are used as feed for livestock. A minor amount of wheat is used as a coffee substitute, especially in Europe, and wheat starch is employed as a sizing for textile fabrics (Purseglove, 1975).

Soils of the high rainfall region of Zambia exhibit low soil fertility due to soil acidity and unavailability of phosphorous (P) to plants. However, aluminium (Al) toxicity is the other factor responsible for poor growth of these wheat varieties, as it causes poor root development. Under acid soil conditions, where it is difficult to separate the detrimental effects of Al from those of low P availability, the differences in Al tolerance among species or varieties seems to be positively correlated with differences in P translocation rates in the presence of Al (Foy, 1974). Wheat is the most widely grown cereal crop in temperate environments and is also cultivated in many tropical countries as is the case in Zambia. The annual harvest report of 2007 published by Famine Early Warning Systems Network (FEWS NET) of Zambia indicated that Mkushi District of Central

Province of Zambia continued to be the major producer of wheat contributing 60% to the total production, followed by Lusaka and Copperbelt at 20% and 12% respectively. Contribution by Southern Province lagged behind at 8% of the national production while little or no wheat was produced in the remaining five provinces. Although impressive yields of above 7 ton/ha are obtained under irrigation in regions with less acid soils, poor yields of less than 1.5 ton/ha are obtained in regions with high rainfall such as the Northern, North Western, Copperbelt and Luapula provinces of Zambia (FEWS NET, 2007). This situation has resulted in wheat being grown only in a few places where climatic conditions and physical and chemical properties of soil are favorable. In 2007 Zambia's consumption of wheat was over 200,000 metric tons, but production stood at only 40% of the total demand (University of Zambia Conference Papers, 2007). Therefore, in order to overcome food deficits, technologies that enhance growth of wheat in high rainfall regions without the use of excessive amounts of fertilisers and lime to amend acidic soils should be applied. An example of such a technology is the use of Versicular arbuscular mycorrhiza and Trichoderma fungus.

1.1. Objectives of the Study

i. To determine the single and combined effect VAM and Trichoderma fungus on the uptake of phosphorous and grain yield of wheat grown in acidic soil.

ii. To determine the relationship between phosphorous uptake and grain yield of wheat grown on acidic soils

1.2. Research Hypothesis

i. Inoculating wheat grown on acidic soil with VAM and/or Trichoderma fungus significantly increases phosphorous uptake and grain yield.

ii. Increased uptake of phosphorous significantly increases grain yield of wheat grown in acidic soils.

2.0 LITERATURE REVIEW

2.1 Vesicular Arbuscular Mycorrhiza

Mycorrhiza has been defined as the mutualistic symbiosis or non-pathogenic association between soil-borne fungi with the roots of higher plants (Sieverding, 1991). Two types of mycorrhiza are known to occur (Quilambo, 2000). These are ecto- and endomycorrhiza. The ectomycorrhizae are characterized by fungal growth outside the root cells. They are quite common in temperate and boreal forest trees and number over 5000 species mainly within the Basidiomycetes (Sieverding, 1991). Some tropical trees such as pine and eucalyptus have also been found to form ectomycorrhizae associations.

The endomycorrhizae on the other hand are characterized by inter-and intracellular fungal growth in cells of root cortex, forming specific fungal structures, referred to as vesicles and arbuscules. This characteristic growth gives the endomycorrhiza the alternate name, vesicular arbuscular mycorrhiza (VAM). It is the most widely distributed association in plants. About 80% of all terrestrial plant species form this type of symbiosis (Smith and Read, 1997) and 95% of the world's present species of vascular plants belong to families that are characteristically mycorrhizal (Quilambo, 2000).

The vesicular arbuscular mycorrhizae belong to taxonomic order called Glomales, which currently comprises 6 genera. They are found in a wide range of habitats usually in the roots of angiosperms, gymnosperms and pterydophytes. They also occur in the gametophytes of some mosses, lycopods and Psilophytes which are all rootless (Mosse

et al., 1981; Pocock and Duckett, 1985). They also occur in aquatic plants (Beck-Nielsen and Madsen, 2001). There are plants, however, that have been shown to be mycorrhiza free, such as Proteacea, Cruciferae and Zygophyllaceae (Nicholson, 1967; Brundrett *et al*., 1996),

The reason why some plants do not form mycorrhizae is not fully known, but it may be related to the presence of compounds toxic to fungus in root cortical tissue or in root exudates. It may also be due to unfavourable interactions between the fungus and the plant at the cell wall and or middle lamella level (Tester *et al*., 1987). High concentrations of salycilic acid have been found to reduce mycorrhization (Medina *et al*., 2003), meaning that plants with a genetic basis for high salycilic acid content have evolved to be mycorrhiza free.

However, about 80% of plant species, including many important crops such as grapes and vines do form VAM. In the case of grapes the concentration may peak after about 15 years and it is considered that VAM contribute to improved wine quality. Some important mycorrhiza free plants are canola and other members of the cabbage family, lupins and beets (Nicholson, 1967). Other families of crop plants do host the fungi, but the degree to which they respond to the symbiosis is variable. This often relates to the speed of root growth and development of root hairs by the plant and to soil conditions, particularly nutrient levels. Knowledge of crops that form mycorrhiza association and are highly responsive would help improve crop productivity, especially in soils with low nutrient availability.

2.2. Vesicular Arbuscular Mycorrhiza and Soil Fertility

Three main components involved in VAM association are soil, fungus and the plant (Brundrett *et al*., 1996). The fungal component involves the fungal structure within the cortical cells of the root and the extra radical mycelium in the soil. The last may be quite extensive under some conditions, but does not form any vegetative structures (Smith and Read, 1997). Its primary function is the absorption of resources from the soil. The increased efficiency of mycorrhiza infested roots versus non-mycorrhiza roots is caused by the active uptake and transport of essential nutrients by mycorrhizae.

Mycorrhizae are described as improving the absorption of several nutrients. Inoculation with *Glomus mosseae* not only affected plant growth and nutrition in *Medicago sativa*, but also enhanced the activity of *Rhizobium meliloti* when it was applied as an inoculant (Azcón-Aguilar *et al*., 1979). Versicular arbuscular mycorrhiza has been shown to improve productivity in soils of low fertility (Jeffries, 1987). They are particularly important for increasing the uptake of slowly diffusing ions such as PO_4^{3-} (Jacobsen *et al*., 1992), immobile nutrients such as P, Zn and Cu (Liu *et al*., 2002) and other nutrients e.g. Cadmium. Under drought conditions the uptake of highly mobile nutrients such as NO_{3-} can also be enhanced by mycorrhiza associations (Subramanian and Charest, 1999). In legume plants the importance of VAM symbiosis has been attributed to high P requirements on the nodulation and N_2 fixation process which requires enhanced P uptake (Barea and Ázcón-Aguilar, 1983). Improved P nutrition has been shown to increase in infertile and P fixing soils of the tropics (Dodd, 2000).

Mycorrhiza fungi can also improve absorption of N from NH_{4+} -N mineral fertilizers, transporting it to the host plant (Johansen *et al*., 1993). Its transport and absorption can also increase biomass production in soils with low Potassium, Calcium and Magnesium (Liu et al., 2002).

2.3 Uptake of Phosphorous (P) by Versicular Arbuscular Mycorrhiza

The type of mycorrhiza that improves P uptake by plants is VAM. Versicular Arbuscular fungi infect the cells of the root cortex and form both an internal network of hyphae and an external growth of hyphae. They possess special structures known as vesicles and arbuscules. The highly branched arbuscules help in the transfer of nutrients from the fungus to the plant-root cells, and the vesicles are sac-like structures, which store P as phospholipids. Versicular arbuscular mycorrhizae are geographically ubiquitous and occur over a wide ecological range from aquatic to desert environments (Mosse *et al.*, 1981). VAM fungi colonize roots of many plants.

Troeh and Loynachan (2003) have reviewed the integration of VAM into cropping systems to maintain high yields and to reduce P inputs. The mode of action that enhanced P uptake in VAM-infected plants is facilitated by:

i. The fungal Hyphae exploring a greater volume of soil for P and also intercepting a greater number of point sources of P,
ii. The fungi dissolving sparingly soluble P minerals such as phosphate rock and

iii. The infected roots increasing the rate of P uptake, by increasing the diffusion gradient by depleting P to lower P concentrations than can non-mycorrhizal roots and by enhancing the transfer of P between living roots and from dying roots to living roots (Angus, 2002).

The P inflow rates of mycorrhizal roots are calculated to be 2–6 times those of non-mycorrhizal roots (Sengupta and Chaudhuri, 2002).

Phosphatase taken up by VAM fungi play an important role in translating fixed or insoluble P into soluble P, which can be used by plant freely. At the same time, hyphae are also important ways of transporting P in the soil. However, other elements such as Zinc (Zn) and Copper (Cu) are not readily mobile in soil. Bryla and Duniway (1997) measured contents of Cu and Zn in clover planted in five compartments with an air gap and found more than half of the total of each nutrient were absorbed by extension hyphae (Gemma *et al*., 1997). The absorption of calcium (Ca), silicon (Si), Nickel (Ni), and cobalt (Co) was also reported to have been increased by VAM symbiosis (Goicoechea *et al*., 1997).

It is still accepted that VAM enhance resistance to high stress of host plants by improving their nutritional status. Drought stress is a major agricultural constraint in the semi-arid tropics. It is known to have a considerable negative impact on nodule function (Sprent, 1971). Drought inhibits photosynthesis and disturbs the delicate mechanism of oxygen control in nodules. The latter is essential for active nitrogen fixation (Goicoechea *et al*., 1997). Versicular arbuscular mycorrhiza symbiosis can protect host

plants against detrimental effects of drought stress (Ruiz-Lozano *et al*., 1999). Quilambo (2000) reported that inoculation with an indigenous inoculant resulted in increased leaf and root growth and prevented the expected increase in root to shoot ratio and root-weight ratio that are normally observed under phosphorus deficient and drought stress conditions in peanut. In watermelon (*Citrullus lunatus Thunb*) mycorrhiza colonization was found to improve not only the plant yield and water use efficiency, but also the quality of the fruit (Kaya *et al*., 2003).

Versicular arbuscular mycorrhiza extends the plant root system and the whole mycorrhiza or fungus plant root system can exploit the soil nutrients much more effectively than the plant alone. Some plant nutrients, such as phosphorus and zinc, move very slowly in the soil solution. Therefore, when a plant removes these nutrients from the soil near the root, there can be a delay before they are replaced at the root surface. A zone of nutrient depletion may occur near the root and slow down plant nutrient uptake. The fungi grow out into the soil, sometimes several centimeters from the root and pick up nutrients at a distance where they are still readily available. The fungal hyphae then transport the nutrients quickly back to the plant – a kind of rapid transit system - overcoming the slow movement in the soil (Gemma *et al*., 1997). Tolerance to drought can be increased as the rapid transit system overcomes slow movement of nutrients in dry soil. However Sengupta and Chaudhuri (2002) reported that there was insufficient evidence that the fungi actually transport water.

Additionally, the hyphae are very narrow, only about 10µm diameter or less (Siddiqui and Mohmood**,** 1996). This means that they have a huge surface area for nutrient

absorption and can squeeze into soil pores that are not accessible to roots that will be 10 times, or more, the width of a VAM fungal hyphal. VAM hyphae growing out of the roots bind soil particles together, like a 'sticky string bag'. This improves soil stability and can help to prevent erosion. The benefits do not come absolutely free, because the fungus needs sugars provided by the plant. Under most conditions, the plant produces sugars to spare, so the 'cost' of supporting the fungi is well invested. This results in enhanced nutrient uptake and more effective use of fertilizers (Siddiqui and Mohmood**,** 1996).

2.4 Trichoderma Fungi

Trichoderma species are fungi that are present in nearly all soils and other diverse habitats. *Trichoderma* species include *T. harzianum, T. viride, T. koningii, T. hamatum* and other species (McAllister *et al*., 1994). In soil, they are frequently the most prevalent culturable fungi. Some strains are highly rhizosphere competent, i.e., able to colonize and grow on roots as they develop. The most strongly rhizosphere competent strains can be added to soil or seeds by any method. Once they come into contact with roots, they colonize the root surface (Ghahfarokhy *et al.,* 2011*)* or cortex. If added as a seed treatment, the best strains will colonize root surfaces even when roots are a meter or more below the soil surface and they can persist at useful numbers up to 18 months after application. However, ordinary strains lack this ability (Harman and Taylor, 1988).

In addition to colonizing roots, *Trichoderma* species attack, parasitize and otherwise gain nutrition from other fungi. Since *Trichoderma* species grow and proliferate best

when there are abundant healthy roots, they have evolved numerous mechanisms for both attack of other fungi and for enhancing plant and root growth (Elad and Kapat, 1999). Several new general methods for both biocontrol and for causing enhancement of plant growth have recently been demonstrated and it is now clear that there must be hundreds of separate genes and gene products involved in these processes. A recent list of mechanisms is as follows.

i. Mycoparasitism
ii. Antibiosis
iii. Tolerance to stress through enhanced root and plant development
iv. Solubilization and sequestration of inorganic nutrients
v. Induced resistance
vi. Inactivation of the pathogen's enzymes

2.4.2 Pesticide susceptibility

Trichoderma species possess innate resistance to most agricultural chemicals, including fungicides, although individual strains differ in their resistance (Bolar *et al*., 2000). Some lines have been selected or modified to be resistant to specific agricultural chemicals (Lumsden and Vaughn. 1993). *Trichoderma* fungi are also resistant to low pH and able to survive and multiply in acidic soils, hence making them suitable in high rainfall areas to help plants resist against fungal attack and improve uptake of mineral nutrients.

2.4.3 Uses of Trichoderma

These versatile fungi are used commercially in a variety of ways, including the following:

2.4.3.1 Plant growth promotion

For many years, the ability of these fungi to increase the rate of plant growth and development, including, especially, their ability to cause the production of more robust roots has been known (Chet, 1987). Some of these abilities are likely to be quite profound. Recently, it has been found that one strain increases the numbers of deep roots at as much as a meter below the soil surface (Kubicek and Harman, 1998). These deep roots cause crops, such as corn, and ornamental plants, such as turf grass, to become more resistant to drought. Perhaps even more importantly, recent research indicates that corn plants whose roots are colonized by *Trichoderma* strain T-22 require about 40% less nitrogen fertilizer than corn plants whose roots lack the fungus. Therefore, the use of nitrogen fertilizer may be curtailed to minimize damage to estuaries and oceanic environment (Herman, 2000).

2.4.3.2 Source of Transgenes

Biocontrol microbes, by definition, must contain a large number of genes that encode products that permit biocontrol to occur. Several genes have been cloned from *Trichoderma* spp. that offer great promise as transgenes to produce crops that are resistant to plant diseases. No such genes are yet commercially available, but a number are in the process of development. These genes, which are contained in *Trichoderma*

spp. and many other beneficial microbes, are the basis for much of "natural" organic crop protection and production (Harman and Kubicek, 1998).

2.5 Status of Wheat Production in Zambia

According to a report released by Meas Consultancy and Services Ltd (2011), major wheat producing areas in Zambia as at 2010 were Central Province with a share of 60%, Copperbelt with a share of 19%, Southern Province 12%, and Lusaka 9%. Out of the total national production for 2010, Mkushi district contributed 30%. The number of wheat farmers was 168 as of 2009. These farmers cultivate, on average, between 30 and 2000 hectares of wheat crop per year. The largest number of farmers was in Central province with 103, Southern province with 26, Lusaka with 33 and Copperbelt province with 6. Wheat production in Zambia over the last 10 years has increased from just below 100,000 MT in the year 2000 to about 254,000 MT for 2012. Wheat yields in Zambia range from about 5 MT to over 8 MT per hectare while national average wheat yield is estimated at about 6.5 MT per hectare (Meas Consultancy and Services Ltd, 2011).

There has not been enough work in the wheat value chain to determine reliable data on wheat consumption levels and trends in Zambia. However, wheat consumption was estimated at between 200,000 MT and 240,000 MT in 2010. Almost all the wheat produced in the country is taken up by the milling industry for processing into bread and cake flour. Major challenges influencing wheat production in Zambia include among others; issues of asymmetric information, high seed prices, limited number of seed suppliers, high energy costs coupled with erratic supply in irrigated farms,

inadequate research and extension services, lack of adoption of appropriate precision technologies as well as illegal wheat imports (Meas Consultancy and Services Ltd, 2011).

3.0 MATERIALS AND METHODS

3.1 Site Description

The experiment was conducted at Zambia Agriculture Research Institute, Msekera Research Station in Chipata district shown in figure 3.1. The Station is located about 12 km due West of Chipata Township, in between the Great East Road and the Msoro Road. Its approximate co-ordinates are Latitude 13^{o} 39'N and Longitude 32^{o} 34' E and covers an area of about 406 Ha at an altitude of 1016 meters. The soils at the experimental site in 0-20 cm soil layer are composed of 1.2% carbon content, pH ($CaCl_2$) of 4.0, 25% clay, 67% sand. In general, the surface texture for the experimental site is sandy clay loam with reddish brown top and sub soils, classified as Typic kandiustalf (USDA, 1975) or Haplic luvisols (FAO, 1988).

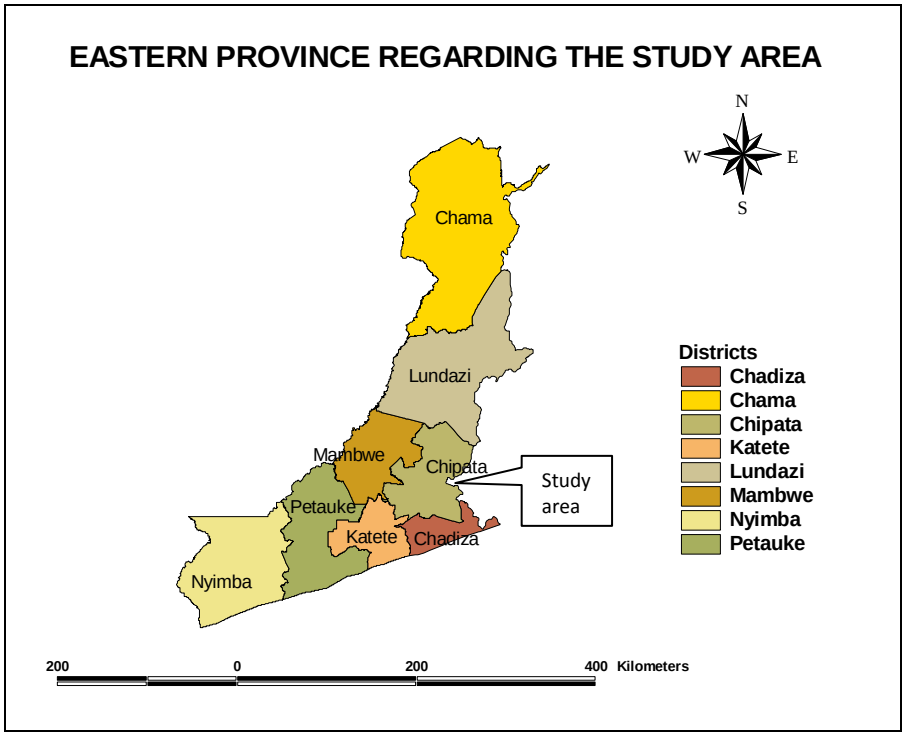

Figure 3.1 Eastern Province regarding the study area

In this region, the rainy season extends from November to April with an average seasonal rainfall of 1092 mm per annum. The rain season is followed by dry cool season from May to August and a hot dry season with low humidity and high sunshine hours from September to October. The climate in this region is thus Tropical Continental.

The study was conducted between the month of May and September 2010. The first phase of the experiment involved the isolation of VAM spores from the soil while the second phase involved the inoculation of wheat seeds with VAM spores and Trichoderma at planting, and the raising of the crop till maturity and harvest.

3.2 Phase 1: Isolation of the Vesicular Arbuscular Mycorrhiza Spores

Numerous techniques are used to recover VAM propagules from soil. The most basic of these is wet sieving and decanting to remove the clay and sand fractions of the soil while retaining spores and other similar sized soil and organic matter particles on sieves of various sizes. This technique is relatively fast. Therefore, a blend of several indigenous spores was extracted by the wet sieving and decanting technique of Gerdemann and Nicolson (1963). The material collected on the 50 µm sieve is what comprised the VAM spores.

3.3 Phase II: Planting and Inoculation

Drops of VAM spores were uniformly blended with wheat seeds before planting. The collected acidic soil was mixed with sand to improve aeration and development of VAM. Trichoderma fungi have been blended into a number of products such as Mycomineral and Eco-T. These products are available on the market, but in Zambia

they are promoted by the Participatory Ecological Land Use Management (PELUM) and the Organic Producers and Processors Association of Zambia (OPPAZ). These organizations promote organic farming in Zambia. In this research Eco-T was used as a source of Trichoderma fungi. Seed treatment is the most economic method for the treatment of extensive crops such as maize (corn) and wheat. As such wheat seeds were mixed with sticker (1% CMC (carboxyl methyl cellulose)) until all seeds were damp. Enough sticker should be used to wet the seed surface with no excess. Eco-T powder was then added to the wet seeds and thoroughly mixed at a rate of 2g/kg seed. Finally the seeds were dried and planted.

3.4 Source of Research Materials for the Study

Pot Experiment was used and the size used was 10kg soil. Soils were collected from Luangeni Constituency where soils are acidic with a pH of 4.0. Vesicular Arbuscular Mycorrhiza was extracted as described in phase 1 of the experiment, where as Eco-T and lime were obtained from the commercial existing markets. Variety UNZA WV1 (University of Zambia wheat variety 1) which is heat tolerant was collected from University of Zambia School of Agriculture, Lorrie II was obtained from Zambia Agriculture Research Institute at Mount Makulu. Sahai and Nduna were obtained from SEEDCO Company Limited. These four wheat varieties where used as test crops because they are acid sensitive varieties. They were ideal for this experiment because the soils used were acidic.

3.5 Experimental Design and Treatments

This research was a factorial experiment with three factors and three replications in Randomized Complete Block Design (RCBD). The following were the three factors considered in the experiment:

(i) Liming: At two levels (L_1 and L_0). L_1- lime applied at 2.5 ton/ha and L_0 -no lime applied.

(ii) Variety: Four varieties (Sahai, Nduna, Lorrie II and UNZA WV1).

(iii) Soil Treatments: At four levels (Vesicular Arbuscular Mycorrhiza, Trichoderma Fungus, Vesicular Arbuscular Mycorrhiza/Trichoderma Fungus and control).

The experiment had 32 treatment combinations resulting in a total number of ninety six (96) treatments. All the soils received applications of N P K at rates equivalent to 20 kg N/ha, 40 kg P_2O_5/ha and 20 kg K_2O/ha. Top dressing (urea) was applied at the rate of 300kg/ha. The following parameters were measured: Levels of soil and plant phosphorus, tillering (number of tillers), grain yield, height, aluminium, calcium and magnesium and soil pH. Part of the lay out of the experiment in the field is shown in Figure 3.2 below

Figure 3.2 General View of Experiment in the Field

3.6 Laboratory Extraction and Analysis Methods

The quantity of phosphorus in plant and soil material was determined by the Colorimetric method as described by Murphy and Riley (1962) and Watanabe and Olsen (1965). The concentration of Phosphorus was read at 882 nm, 15 minutes after addition of 8 ml of a molybdate reagent for colour development. The procedure was repeated for all samples where P was to be determined and results recorded accordingly. The analysis of Nitrogen in soil samples was done by use micro-kjeldahl digestion followed by distillation and titration (Anderson and Ingram, 1993).

Base cations (Ca, Mg, Na and K) in the soil samples were extracted with 1 *M* NH_4OAc (pH 7) followed by centrifugation (Page, 1982) and determined by Atomic Absorption Spectrometry (Dahlquist and Knoll, 1978). Zinc and Manganese were extracted with 0.005*M*DTPA (diethylenetriaminepentaacetic acid) and determined with Atomic absorption Spectrometry.

For the analysis of aluminium, extraction was done with 1 *M*KCl. The extract was determined by atomic absorption spectrometry. The pH was determined on a 1:1 soil/water mixture and measured on a Beckman pH meter with glass and calomel reference electrodes calibrated to buffers pH 4 and 7. Finally, Organic carbon was determined by Walkley and Black method.

3.7 Data Analysis

The data were subjected to Analysis of Variance using GenStat edition 14. For mean comparisons, significance was tested at $P \leq 0.05$ and standard error was used for mean separation (Gomez and Gomez, 1984).

4.0 RESULTS

4.1 Soil Chemical Properties

Selected chemical properties of the soil used in the study are presented in Table 4.1. The soil used was acid sandy clay loam. It had very low levels P, N and K for requirements of most important field and horticultural crops.

Table 4.1 Selected chemical properties of the soil used in the study.

pH ($CaCl_2$)	**Avail P mg/kg**	**Total N (%)**	**Org C (%)**	**Na**	**Ca**	**Mg**	**K**	**Zn**	**Mn**
				meq/100g				mg/kg soil	
4.0	2.96	0.003	0.20	0.31	0.19	0.92	0.39	3.49	14.58

4.2 The Effect of Root Growth to Treatment of Trichoderma (TF) and Vesicular Arbuscular Mycorrhiza

After thinning of plants, some roots of wheat varieties were examined. The results of root volume calculation shown in figure 4.1 (b) derived from figure 4.1 (a) showed that treatment with VAM increased root volume by 200%, where as treatment with VAM/TF increased by 216% and treatment with TF increased root volume by 400% as compared to the control experiment. Therefore, treatment with TF gave the best root development after one month of growth.

Figure 4.1(a) Response of root growth to *Trichoderma* and mycorrhiza

Figure 4.1 (b) Root volume determination

4.3 The Response of P uptake to Treatment with Trichoderma (TF) and Vesicular Arbuscular Mycorrhiza (VAM)

Trichoderma (TF) and Mycorrhiza (VAM) significantly increased the uptake of P at 5% level of significance. The effect of TF and VAM on the uptake of P was almost equal with treatment TF giving a mean P uptake of 32.43 mg/kg and treatment VAM giving a mean P uptake of 32.63 mg/kg. The control treatment yielded the least mean P uptake of 11.36 mg/kg. A combination of VAM and TF gave an average P uptake of 27.17 mg/kg as illustrated in Figure 4.2. On the other hand, mean P uptake with lime as a single effect was non significant. Furthermore, mean P uptake was also insignificant in the interaction of lime and variety.

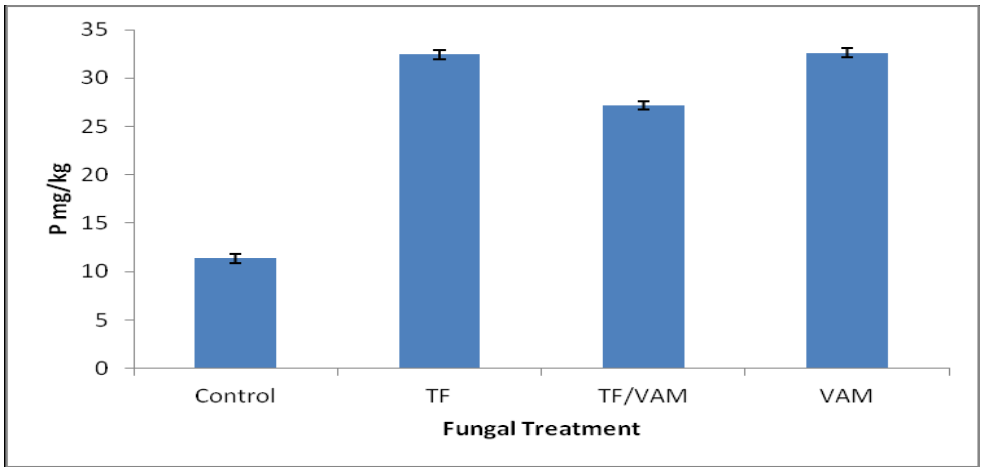

Figure 4.2 Effect of Trichoderma (TF) and Vesicular Arbuscular Mycorrhiza (VAM) on Phosphorus (P) uptake in acid sandy clay loam

4.4 Variation of Phosphorus uptake with Variety

The results in Figure 4.3 show that there was a significant increase in P uptake in all four wheat varies. Therefore, variety had an effect on P uptake. Variety Sahai had the highest P uptake of 29.9 mg/kg followed by Lorrie II with 28.51 mg/kg. The lowest P uptake was observed in variety UNZA WV1 with 17.33 mg/kg

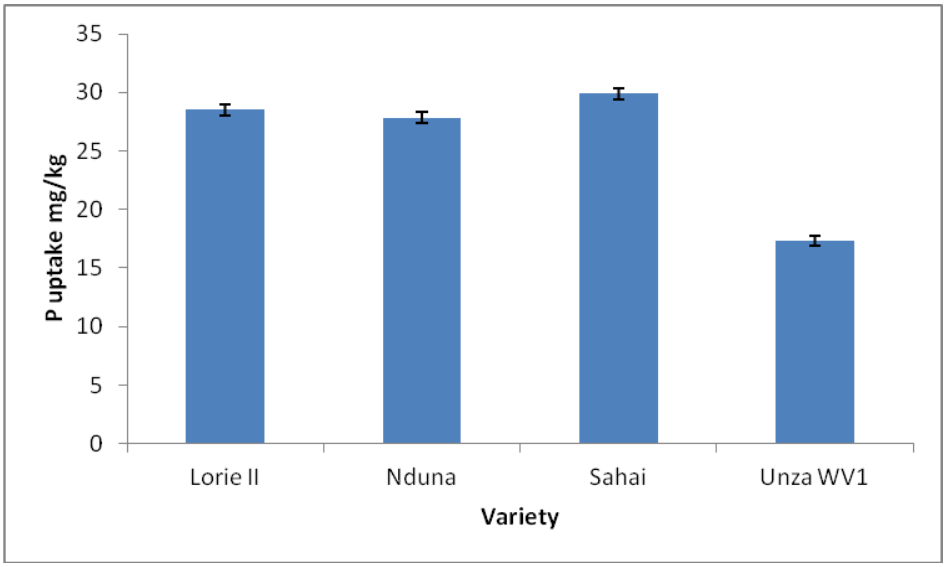

Figure 4.3 Phosphorus uptake across the four wheat varieties

4.5 Change in Phosphorus (P) uptake with variety and fungal treatment

Phosphate uptake significantly (p = 0.001) varied with variety and fungal treatment as seen in Figure 4.4.

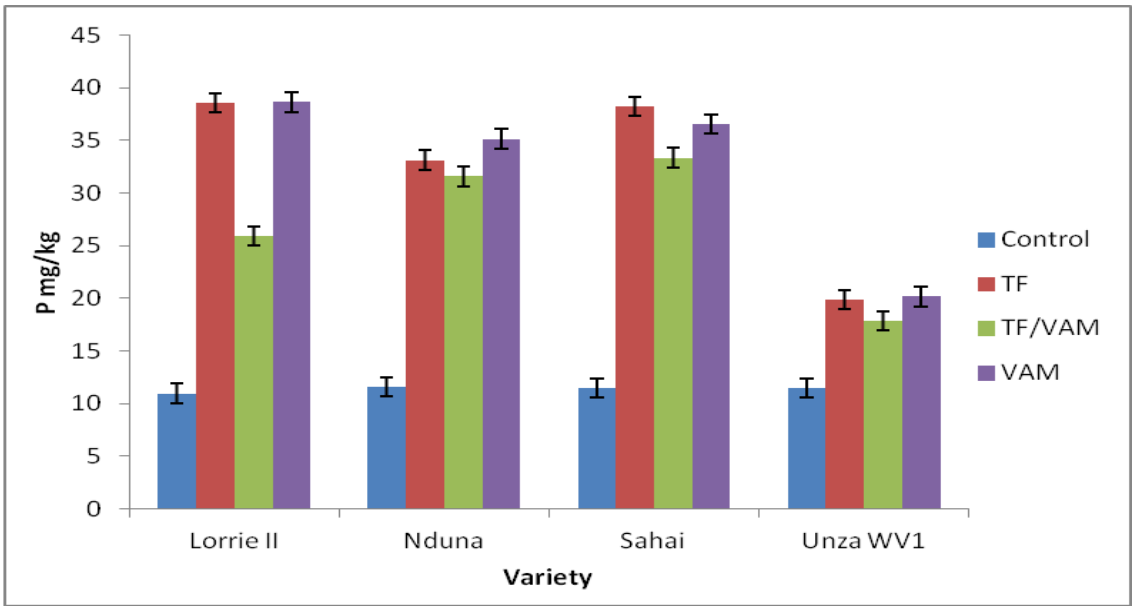

Figure 4.4 Effect of Trichoderma (TF) and Vesicular Arbuscular Mycorrhiza (VAM) on Phosphorus uptake in four wheat varieties.

The highest P uptake was obtained with Lorrie II when treated with TF and VAM (38.53 and 38.62 mg/kg respectively) and Sahai with TF (38.2 mg/kg). This was followed by Sahai with VAM (36.58 mg/kg). The lowest P uptake was obtained with all the varieties with the control treatment with an average P uptake of 11.3 mg/kg. P uptake for the other treatments fell in between as shown in Figure 4.4.

4.6 The Response of Grain Yield to Trichoderma (TF) and Vesicular Arbuscular Mycorrhiza (VAM)

The highest grain yield for fungal treatment was obtained with TF (12.98 ton/ha) and TF/VAM (11.94 ton/ha) then followed by VAM. The control produced the lowest grain yield (3.44 ton/ha) as shown in Figure 4.5.

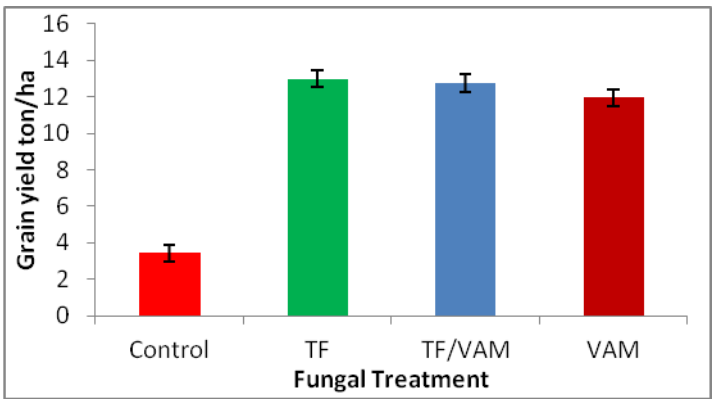

Figure 4.5 Effect of fungal treatment on grain yield

4.7 Variation in Grain Yield with Variety

The highest grain yield with variety as a main effect was obtained with Nduna (10.78 ton/ha) and Sahai (10.54 ton/ha) followed by Lorrie II (10.22 ton/ha). UNZA WV1 gave the least grain yield of 9.57 ton/ha as depicted in Figure 4.6.

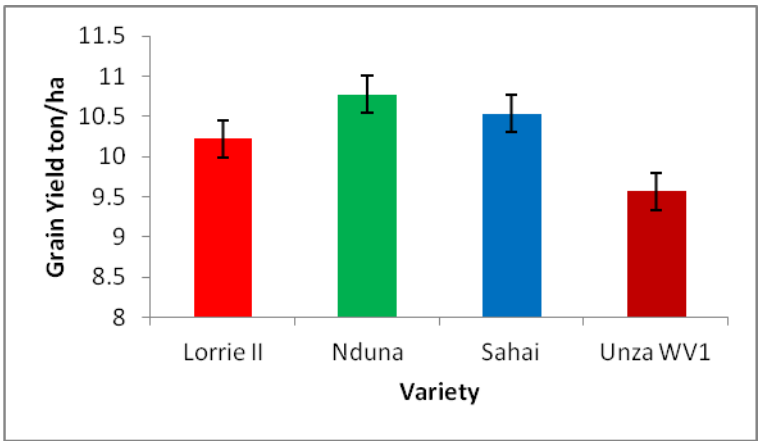

Figure 4.6 Mean grain yield obtain for the four wheat varieties

4.8 The Effect of Variety with Lime on Grain Yield

The results in Figure 4.7, indicates that the highest grain yield was obtained by treatment; Lorrie II with lime (10.65 ton/ha), Nduna with lime and no lime (10.74 and 10.81 ton/ha respectively) and Sahai with lime and no lime (10.40 and 10.68ton/ha respectively).This was followed by Lorrie II with no lime (9.89 ton/ha). The lowest grain yield was obtained with treatment UNZA WV1 with lime (9.22 ton/ha).The use of lime as a main effect was insignificant on grain yield.

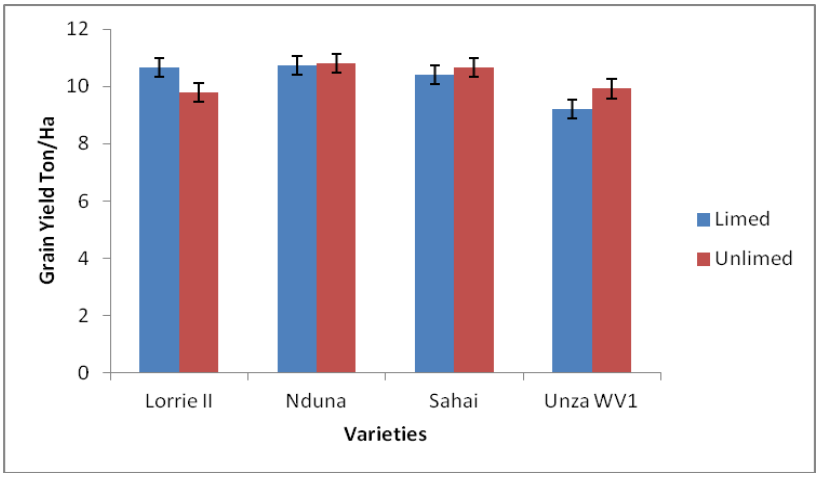

Figure 4.7 Mean grain yield variations for four wheat varieties in limed and unlimed soils

4.8.1 The Response of Grain Yield to Trichoderma (TF) and Vesicular Arbuscular Mycorrhiza (VAM) with Lime

Grain yield varied significantly ($p = 0.001$) with lime and fungal treatment. The highest grain yield was obtained in unlimed soil with TF (13.82 ton/ha) and TF/VAM (13.01 ton/ha). This was followed by treatment TF in limed soils (12.13 ton/ha), TF/VAM in limed soils (12.45 ton/ha) and VAM in limed soils (11.26 ton/ha).

The control treatment gave the lowest grain yield of 1.733 ton/ha in unlimed soils. The grain yield for the other treatments fell in between as illustrated in Figure 4.8

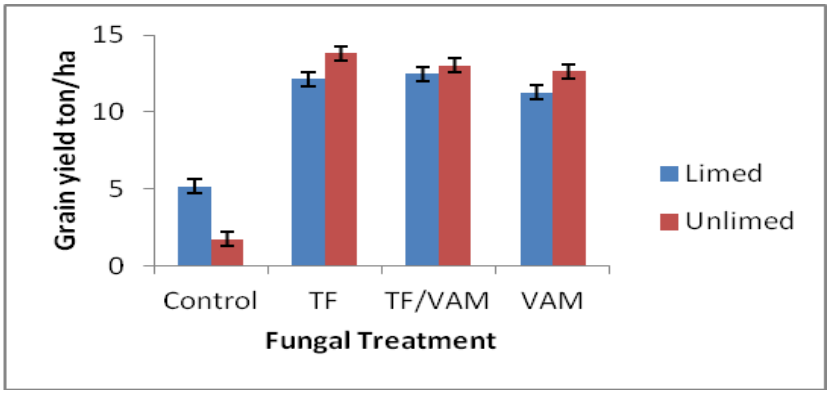

Figure 4.8 Effect of fungal treatment on grain yield

4.8.2 Response of Grain Yield to Variety with Trichoderma (TF), Vesicular Arbuscular Mycorrhiza (VAM) and Trichoderma/Vesicular Arbuscular Mycorrhiza (TF/VAM)

The results in Figure 4.9 show the interaction between Variety and fungal treatment. The highest grain yield was obtained when Nduna was treated with TF/VAM (13.94 ton/ha) and Sahai with TF/VAM and TF (13.4 and 13.64 ton/ha respectively).

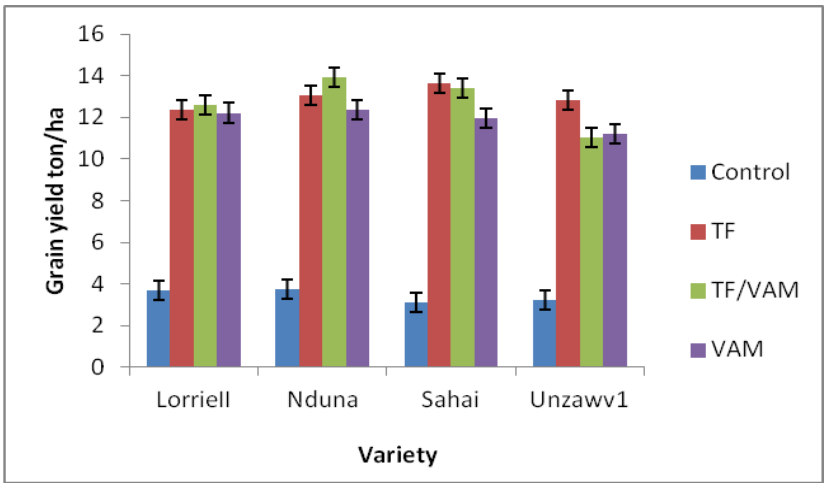

Figure 4.9 Mean grain yield of four wheat varieties treated with Trichoderma (TF) and Vesicular Arbuscular Mycorrhiza (VAM)

This was followed by Lorrie with TF and TF/VAM (12.38 and 12.6 ton/ha respectively), Nduna with TF and VAM (13.05 and 12.36 ton/ha respectively) and UNZA with TF (12.83 ton/ha). The lowest grain yield was obtained across all the wheat varies with the control giving an average yield of 3.4 ton/ha.

5.0 DISCUSSION

5.1 Response of Root Development to treatment with Trichoderma (TF) and Vesicular Arbuscular Mycorrhiza (VAM)

The increased root volume observed with the treatment of wheat varieties with TF, VAM and TF/VAM as compared with plants not treated was due to the ability of the Trichoderma and VAM to extend and increase the root system for the purpose of efficient uptake of mineral nutrients. Smith and Read (1997) reported that VAM grow in the cortical root tissues and also grow out from the roots into the surrounding soil, forming an external hyphae network which increases uptake of mineral nutrients and consequently promotes plant growth. Furthermore, Ghahfarokhy *et al,* 2011 reported that comparison of the root in control and root colonized with VAM and *Trichoderma* species showed that colonization of root with VAM fungi and *Trichoderma* promote massive root growth which intern help in absorption of nutrients. Hence, this is consistent with the observations and results of the present study.

Varma *et al.,* (1999) also reported that *Trichoderma* species have the ability to coil around roots of most plants and penetrate into their hyphae and cells. They also enhance progressive growth in mycelium. Versicular arbuscular mycorrhiza has a wider host range and exerts several positive effects on colonized host plants when grown in pot cultures (Singh *et al*., 2000). Versicular arbuscular mycorrhiza has been reported to be involved in the improvement of growth and biomass production in a range of hosts such as monocots and dicots, shrubs and trees, medicinal plants (Giovannetti and Mosse, 1998) and several economically important crops (Varma *et al.*, 1999). Also, it has been

proven that VAM has an inductive effect on the growth of terrestrial orchids (Blechert *et al.,* 1999). Previous studies showed that VAM *and Trichoderma spp* significantly reduce negative effect of leaf pathogen (*Blumeria graminis* f. sp. *tritici*), stem base (*Pseudocercosporella herpotrichoides*), and root (*Fusarium culmorum*) pathogen on wheat and increase growth, development and biomass root. *Trichoderma spp* increase disease resistance, salt stress tolerance and higher yield in barley (Waller *et al*., 2005). Hence findings of this experiment are in agreement with those of the above mentioned authors. *Trichoderma* is able to biologically control multiple number of plant pathogens (Agrios, 1997). Giovannetti and Mosse, (1998) proved that *Trichoderma* species could control *Botrytis cinerea* in wine grapes. El-Katatny *et al.,* (2000) indicated the suppressive impact of *T. harizanum* on the activity of *Sclerotium rolfsii* in agricultural and horticultural crops. The mycoparasitic activity of *T. viride* on the mycelia of *Ceratocystis paradoxa* has been also proven (Eziashi *et al*., 2007).

5.2 Effect of Trichoderma (TF) and Vesicular Arbuscular Mycorrhiza (VAM) on Phosphorous Uptake by Four Wheat Varieties and Interaction of Fungi on Host Plant

The significant increase in the uptake of P as shown in the result section was attributed to root colonization by VAM and Trichoderma spp. The improved growth of the roots enabled them to grow deep into the soil to reach out for more plant nutrients necessary for plant growth. Root colonization by VAM improves P uptake per unit of root length due to the enhancement of root surface area by hyphal growth (Smith and Read, 2002).

According to Abbot and Robson (1982) and Mengel and Kirkby (1987) there are three possible means by which mycorrhizal fungi help to enhance P uptake: (i) by increasing the root surface in contact with the soil volume, (ii) by extending the period during which roots remain active in absorbing nutrients and (iii) by increasing nutrient translocation into the shoots, straight into the vacuole through fungal external mycelium. All the three have the potential to contribute to the increase in P accumulation because each one enhances P uptake. Further more, Johanssen *et al*, (1993) reported increased P uptake in the whole plant of corn inoculated with *Glomus intraradics* or *Gigaspora margarita*. The results of this study support the findings of Johanssen *et al*., (1993).

The treatment VAM/TF gave lower P uptake than TF and VAM probably due to level of competition between the two fungi which might have led to reduced uptake of mineral nutrients including P. Jensen and Wolffhechel (1995) reported that Trichoderma species were widely recognized as a potential biocontrol agent of several soil borne plant pathogens because of its antagonistic nature to certain pathogens. However, an increasing number of reports support the concept that establishment and functioning of the VAM symbioses are affected by a range of soil microorganisms that may act either supportively or detrimentally (Paulitz and Linderman, 1991). VAM fungi may also contribute to protection of the host plant against soil borne plant pathogens (Jalali and Jalali, 1991). Combinations of VAM fungi and biocontrol agents like *T. harzianum* and other *Trichoderma species* could, therefore, provide levels of disease control which are superior to the effects of the organisms when they are used alone (Linderman, 1994), although previous results (Nemec *et al*, 1996) are contradictory.

The nature of the interactions between VAM fungi and biocontrol agents is important for such additive or synergistic effects. The effects of fungi belonging to the genus *Trichoderma* on spore germination and hyphal growth of *Glomus mosseae* have been examined in vitro, and contradictory results have been obtained (McAllister *et al*., 1994). However, the results from other pot experiments suggest that *Trichoderma* species suppress VAM root colonization (Siddiqui and Mohmood, 1996), although this depends on the timing of inoculation (McAllister et al, 1994) and the host plant species (Dhillion, 1994). On the other hand, adverse effects of VAM fungi on the population density of *Trichoderma koningii* have also been observed (McAllister *et al*, 1994). The possible effects of the saprophytes on VAM spore germination and root colonization cannot be clearly distinguished from effects on the outgrowth and functioning of the external mycelium. In addition, the majority of these studies have focused on the effect on the host plant rather than on measuring the biomass and specific activity of the organisms involved. Consequently, specific interactions between the external mycelia of VAM fungi and saprotrophic microorganisms are poorly understood.

Therefore, TF and VAM may be used to control wheat fungal diseases common in Zambia like the *Take-all of wheat.* This is the most deadly root disease of wheat in Zambia. Freeman and Ward, (2004) reported that this diseases causes stunting and nutrient-deficiency symptoms in the tops, and progresses upward into the bases of the stems. It also disrupts the flow of water to the tops and causes premature death of the plant (Cook, 2003).

5.3 Effect of Vesicular Arbuscular Mycorrhiza (VAM) and Trichoderma (TF) Inoculation on Grain Yield

Treating wheat with TF and VAM resulted in a significant increase in the grain yield. This was attributed to the enhanced P uptake by the *Trichoderma* species and VAM. The improved grain yield with fungal-inoculants was due to the absorption of more nutrients by wheat plants and control of pathogens by *Trichoderma* species. Furthermore, VAM treatments (Manske *et al.*1998 and Behl *et al.,* 2003) have also provided access to more soil volume as extra metrical hyphae of VAM fungi enlarge the effective surface outside of the roots. Fungal inoculation increased sink size by increasing either panicle number or spikelet number per panicle. Yanni *et al.,* (1997) also reported higher grain yield following inoculation with VAM in a field experiment in Egypt. The importance of additive effects of fungal-inoculants was reported by earlier workers for component traits like plant height (Katiyar and Ahmad, 1996), spike length, grain weight (Walia *et al*., 1991), flag leaf area and grains per spike.

The growth-promoting activities (GPA) of fungal inoculants on crop plants may be manifested in several ways. For example, their production of iron-sequestering siderophores and antimicrobial compounds may hinder colonization of hosts by phytopathogens, thereby suppressing the diseases they cause (Khavazi *et al*., 2005). Other mechanisms of GPA include the induction of host systemic disease resistance, N_2 fixation, solubilization of precipitated mineral nutrients and production of plant growth regulators (Bashan *et al*., 1990). These induce additional root hair and lateral root formation and enhance plant's ability to take up additional nutrients and water from soil

and increase plant yield. It has been reported that VAM symbiosis increased photosynthesis and the rates of photosynthetic storage (Singh and Singh, 1992). It has been further proved that concentration of chlorophyll in VAM plants is higher than plants without VAM (Khavazi *et al*., 2005). Such plants are therefore likely to produce more and larger grains and result in increased economical yield. De Jong and Phillips (1981) reported higher leaf photosynthesis and increased leaf N content in Alaska pea (*Pisum sativum* L.) following fungal inoculation. In their study, both leaf N content and photosynthetic rate increased linearly with symbiotically fixed N_2 (De Jong and Phillips 1981).

A close relationship between photosynthetic rate and leaf N content was reported for both greenhouse and field-grown rice plants (De Jong and Phillips, 1981). In a greenhouse experiment, VAM increased wheat grain yields by 12.6 to 14.0% at N fertilizer rates of 60 to 120 kg /ha (De Jong and Phillips 1981). In a field experiment in Iran, yield improvements of more than 20% have been obtained for wheat as a result of mycorrhiza inoculation (Biswas *et al*., 2000). Narula *et al.*, 2002 reported a net saving of 25-30 kg nitrogen by using VAM inoculants on wheat. The productiveness of rhizosphere for VAM may be attributed to favorable influence exerted by root exudates that contain amino acids, carbohydrates, organic acids, growth promoting substances and also phytohormones (Narula *et al.,* 2002). It is well known that wheat roots secrete carbonaceous exudates, which could help in proliferation of VAM (Biswas *et al*., 2000). However, intense VAM infected roots even at moderate nutrient deficiency are important during early plant growth when roots are too small to provide a high demand for minerals for shoot growth. Brennan (1992) reported that phosphate utilization

efficiency in grain yield production was more enhanced (average 13%) than N utilization efficiency (5%).

Biswas *et al*., (2000) suggest that certain strains of VAM can promote wheat growth and yield through mechanisms that improve single leaf net photosynthetic rate rather than biological N_2 fixation. Narula *et al., (*2002) observed a significant interaction between the inoculants and P uptake and yield of wheat.

5.4 Effect of Trichoderma (TF) and Vesicular Arbuscular Mycorrhiza with Variety in Lime on Grain yield and Phosphorus (P) uptake

Grain weight varied with variety in limed and unlimed soils. In unlimed soils grain yield was low because of the unavailability of P and other mineral nutrients necessary for plant growth and development. However, when TF and VAM was added to variety in unlimed soils, grain yield was observed to have increased due to the ability of TF and VAM to colonize the roots of wheat thereby enhancing uptake of P and other mineral nutrients. TF and VAM have the ability to survive and multiply in acidic soils, hence improving P uptake and grain yield across all the four wheat varieties. Harman and Taylor (1988) reported that it was feasible to increase biological control activity and competitive potential of *Trichoderma* strains through acidification of soil and spermosphere. Acidic condition may increase mycelial growth (Chet and Baker, 1980; Danielson and Davey 1973, Hadar *et al*., 1984), production and activity of antimicrobial compounds such as antibiotics and lytic enzymes (Chet and Baker, 1980). Alkaline soil decreases conidial germination of *Trichoderma* spp and lead to a decreased bio-control

activity of *T. harizanum* (Paulitz and Linderman, 1991). As such a healthy plant free from diseases caused by various pathogens will be productive and will be able to record high P uptake and yields as it has been the case in this study. There was a further differential response of grain yield with lime application. Varieties such as Lorrie II gave a higher grain yield with lime where as UNZA WV1 gave higher yield with no lime. However, the yield of Sahai and Nduna remained constant irrespective of lime or no lime. Therefore varieties such as Sahai and Nduna may be considered as non- acid sensitive varieties which simply mean that their yields are not affected by acidic conditions of the soil.

The genetic and physiological basis of Aluminium tolerance has been investigated in several crops and model plant species in which both Al sensitivity and tolerance has been observed. As proposed by Matsumoto (2000), the tolerance strategies identified can be separated into those involved in exclusion of Al from the root apex and mechanisms that allow the plant to tolerate Al within cells. A wealth of studies provide very strong evidence that Al-tolerant genotypes of wheat, corn, sunflower, soybean and common bean, among others, exclude Al from roots by excretion of organic acids that chelate Al (Matsumoto, 2000).

6.0 CONCLUSION

The study has shown that Vesicular Arbuscular Mycorrhiza (VAM) and Trichoderma fungus (TF) significantly increased P uptake and grain yield in the four wheat varieties (Sahai, Nduna, UNZAWV1 and Lorrie II). Phosphorus uptake and grain yield was also observed to be higher when treated with individual TF and VAM than when treated with a combination of VAM and TF. The grain yield and P uptake for all four wheat varieties was still high irrespective of the liming when wheat varieties were treated with VAM and *Trichoderma* species. This is due to ability by Trichoderma spp and VAM to survive and increase nutrient uptake in acidic soils. The poor performance of control plants was perhaps due to the aluminium toxicity and unavailability of phosphorus and zinc.

7.0 RECOMMENDATION

Based on the findings of the present work, it can be recommended that Vesicular Arbuscular Mycorrhiza (VAM) and Trichoderma (TF) application to wheat would increase plant performance with respect to P uptake and grain yield.

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8.0 APPENDICES

Appendix I Result for P, Grain Weight, Tillers, and Yield of the four Wheat Varieties Used in the Study

Lime	Variety	ftrt	Rep	Proot	Pstover	Pgrain	Ptotal mg/kg	Gweight ton/ha
Limed	Nduna	TF	1	2.1	10.2	20.165	32.465	12
Limed	Nduna	TF	2	1.9	10.25	19.619	31.769	13
Limed	Nduna	TF	3	2	10.8	20.575	33.375	11.4
Limed	Nduna	VAM	1	3.1	11.3	20.426	34.826	12.7
Limed	Nduna	VAM	2	3.2	11.8	20.575	35.573	12
Limed	Nduna	VAM	3	3.1	11.5	18.697	33.297	11.98
Limed	Nduna	TF/VAM	1	3.5	11.9	23.851	39.251	12.55
Limed	Nduna	TF/VAM	2	3.4	10.3	16.679	30.379	14.12
Limed	Nduna	TF/VAM	3	3.8	9.98	14.524	28.304	12.94
Limed	Nduna	Control	1	1.2	4.1	7.01	12.402	1.9
Limed	Nduna	Control	2	1.1	4.2	5.7	11	2.3
Limed	Nduna	Control	3	1.3	4	4.8	10.103	2.1
Limed	Sahai	TF	1	3.6	13.7	20.074	37.374	12.45
Limed	Sahai	TF	2	3.4	13.6	19.489	36.489	11.76
Limed	Sahai	TF	3	3.8	13.67	21.269	38.739	12.23
Limed	Sahai	VAM	1	3.1	12.54	18.876	34.516	11.09
Limed	Sahai	VAM	2	2.9	12.601	22.39	37.891	10.67
Limed	Sahai	VAM	3	2.8	12.596	21.327	36.723	11.44
Limed	Sahai	TF/VAM	1	2.1	11.1	19.387	32.587	13.72
Limed	Sahai	TF/VAM	2	2.3	10.9	21.656	34.856	13.5
Limed	Sahai	TF/VAM	3	2.5	10.899	16.674	30.273	13.8
Limed	Sahai	Control	1	1.2	4.9	5.707	11.807	1.5
Limed	Sahai	Control	2	1.4	4.89	4.974	11.237	1.8
Limed	Sahai	Control	3	1.3	4.5	5.3	11	1.3
Limed	Lorrie II	TF	1	2.78	13.67	21.481	37.931	12.45
Limed	Lorrie II	TF	2	2.98	13.75	20.623	37.353	12.7
Limed	Lorrie II	TF	3	3.2	13.801	21.828	38.829	11.2
Limed	Lorrie II	VAM	1	3.6	14.2	20.463	38.263	12.3
Limed	Lorrie II	VAM	2	3.8	14.4	20.581	38.781	10.1

Limed	Lorrie II	VAM	3	3.89	14.7	18.629	37.219	12.6
Limed	Lorrie II	TF/VAM	1	3	10.5	11.989	25.489	12.8
Limed	Lorrie II	TF/VAM	2	2.76	9.9	15.307	27.967	13.6
Limed	Lorrie II	TF/VAM	3	2.9	9.8	10.237	22.937	13.3
Limed	Lorrie II	Control	1	1.02	4	6.18	11.2	1.9
Limed	Lorrie II	Control	2	1.1	3.8	4	10.092	1.6
Limed	Lorrie II	Control	3	1.01	3.8	5.19	10	1.8
Limed	Unza wv1	TF	1	2.1	7.9	10.658	20.658	12.3
Limed	Unza wv1	TF	2	2.09	7.1	9.566	18.756	12
Limed	Unza wv1	TF	3	2.2	7.5	9.573	19.273	12.1
Limed	Unza wv1	VAM	1	2.5	8	10.189	20.689	8.39
Limed	Unza wv1	VAM	2	2.48	8.1	10.165	20.745	11.9
Limed	Unza wv1	VAM	3	2.61	8	9.362	19.972	9.9
Limed	Unza wv1	TF/VAM	1	2.21	5	11.748	18.958	9.3
Limed	Unza wv1	TF/VAM	2	2.32	7	8.105	17.425	10.2
Limed	Unza wv1	TF/VAM	3	2.12	8	8.645	18.765	9.8
Limed	Unza wv1	Control	1	0.998	3.8	5.058	9.856	1.7
Limed	Unza wv1	Control	2	1.089	3.5	4.869	9.458	1.4
Limed	Unza wv1	Control	3	1.001	3.2	6.602	10.803	1.5
Unlimed	Nduna	TF	1	3.1	10	20.464	33.564	14.6
Unlimed	Nduna	TF	2	2.9	10.1	19.967	32.967	13.5
Unlimed	Nduna	TF	3	3	10.2	21.373	34.573	13.8
Unlimed	Nduna	VAM	1	3	11.3	21.382	35.682	12.4
Unlimed	Nduna	VAM	2	3.2	10.9	22.635	36.735	13.3
Unlimed	Nduna	VAM	3	3.1	11	20.629	34.729	11.8
Unlimed	Nduna	TF/VAM	1	3.3	11.09	16.131	30.521	14.9
Unlimed	Nduna	TF/VAM	2	3.1	10.99	17.847	31.937	13.9
Unlimed	Nduna	TF/VAM	3	3.3	10.918	14.816	29.034	15.2
Unlimed	Nduna	Control	1	1.02	4.2	8.62	13.84	4.39
Unlimed	Nduna	Control	2	1.1	4.1	5.775	10.975	6.43
Unlimed	Nduna	Control	3	1.03	4	6.004	11.034	5.4
Unlimed	Sahai	TF	1	3.2	12.7	22.834	38.734	14.9
Unlimed	Sahai	TF	2	3.1	12.6	22.248	37.948	15.4
Unlimed	Sahai	TF	3	3.3	12.67	24.267	39.937	15.1
Unlimed	Sahai	VAM	1	3	11.54	21.075	35.615	12.5
Unlimed	Sahai	VAM	2	2.9	11.601	22.428	36.929	13.3
Unlimed	Sahai	VAM	3	3	11.596	23.225	37.821	12.9
Unlimed	Sahai	TF/VAM	1	2.5	10.1	21.295	33.895	13.4
Unlimed	Sahai	TF/VAM	2	2.3	9.9	23.785	35.985	13.2
Unlimed	Sahai	TF/VAM	3	2.4	9.899	20.173	32.472	12.8

Unlimed	Sahai	Control	1	1.2	3.9	7.802	12.902	4.6
Unlimed	Sahai	Control	2	1.1	3.89	6.045	11.035	5.1
Unlimed	Sahai	Control	3	1.06	3.5	6.247	10.807	4.4
Unlimed	Lorrie II	TF	1	3.78	12.67	22.382	38.832	13.1
Unlimed	Lorrie II	TF	2	3.98	12.75	21.505	38.235	11.9
Unlimed	Lorrie II	TF	3	3.6	12.801	23.581	39.982	12.9
Unlimed	Lorrie II	VAM	1	3.33	13.52	22.476	39.326	13.22
Unlimed	Lorrie II	VAM	2	3.298	13.4	23.273	39.971	11.34
Unlimed	Lorrie II	VAM	3	3.1999	13.6	21.382	38.182	13.76
Unlimed	Lorrie II	TF/VAM	1	3.2	10.4	13.241	26.841	12.3
Unlimed	Lorrie II	TF/VAM	2	2.976	9.9	15.663	28.539	11.3
Unlimed	Lorrie II	TF/VAM	3	2.9	9.8	10.993	23.693	12.33
Unlimed	Lorrie II	Control	1	1.19	3.7	7.128	11.947	4.1
Unlimed	Lorrie II	Control	2	1.21	3.6	8.285	13.095	5.5
Unlimed	Lorrie II	Control	3	1.098	3.8	4.529	9.427	7.2
Unlimed	Unza v1	TF	1	2	7.8	11.065	20.865	13.9
Unlimed	Unza wv1	TF	2	2.09	7.9	9.567	19.557	13.2
Unlimed	Unza wv1	TF	3	2.1	7.4	10.627	20.127	13.5
Unlimed	Unza wv1	VAM	1	2.1	8.01	9.858	19.968	10.9
Unlimed	Unza wv1	VAM	2	2.3	7.98	8.394	18.674	11.7
Unlimed	Unza wv1	VAM	3	2.61	7.02	11.296	20.926	14.4
Unlimed	Unza wv1	TF/VAM	1	2.11	6.1	8.785	16.995	13.2
Unlimed	Unza wv1	TF/VAM	2	2.232	6.9	7.81	16.942	12.2
Unlimed	Unza wv1	TF/VAM	3	2.012	6.89	9.057	17.959	11.4
Unlimed	Unza wv1	Control	1	0.899	4.3	8.017	13.216	4.1
Unlimed	Unza wv1	Control	2	1.009	4.4	6.936	12.345	5.7
Unlimed	Unza wv1	Control	3	1	4.2	7.78	12.98	4.9

Appendix II ANOVA for Grain Weight

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	0.5261	0.2630	0.40	
Rep.*Units* stratum					
lime	1	0.0477	0.0477	0.07	0.788
ftrt	3	1507.7621	502.5874	769.87	<.001
Variety	3	19.7691	6.5897	10.09	<.001
lime.ftrt	3	100.1163	33.3721	51.12	<.001
lime.Variety	3	7.8968	2.6323	4.03	0.011
ftrt.Variety	9	21.0633	2.3404	3.59	0.001
lime.ftrt.Variety	9	14.3010	1.5890	2.43	0.019
Residual	62	40.4747	0.6528		
Total	95	1711.9570			

Appendix III ANOVA for P uptake

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	12.425	6.213	2.49	
Rep.*Units* stratum					
Lime	1	0.019	0.019	0.01	0.931
ftrt	3	7224.357	2408.119	964.35	<.001
Variety	3	2400.517	800.172	320.44	<.001
lime.ftrt	3	16.999	5.666	2.27	0.089
lime.Variety	3	10.629	3.543	1.42	0.246
ftrt.Variety	9	1129.239	125.471	50.25	<.001
lime.ftrt.Variety	9	11.583	1.287	0.52	0.858
Residual	62	154.822	2.497		