UNIVERSITY OF NAIROBI

EVALUATION OF *TRICHODERMA* ENHANCED FERTILIZERS ON GROWTH OF COMMON BEANS (*PHASEOLUS VULGARIS L.*)

BY

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A Thesis Submitted to the Center for Biotechnology and Bioinformatics (CEBIB) in Partial Fulfillment of the Requirement for the award of the Master of Science Degree in Biotechnology.

DECLARATION

This is my original work and has not been presented for the award of any degree at any other University or educational institution.

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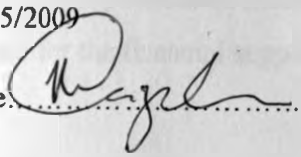
Signature:  .....

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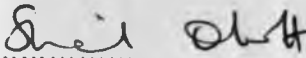
Signature  .....

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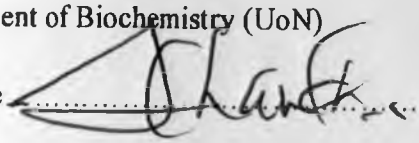
Signature  .....

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DEDICATION

I dedicate this thesis to my grandmother Seamogano Nzwaligwa and my wife Goitsemodimo T. Charles for their relentless support towards this study.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
DF	Degrees of freedom
DNA	Deoxyribonucleic acid
DNTP	Deoxynucleotide triphosphate
CLA	Carnation leaf agar
CMD	Corn meal dextrose
EDTA	Ethylenediaminetetraacetic acid
GC	Guanine-cytosine
ITS	Internal transcribed spacer
Mer	Repeat unit
MGA	Minimal glucose agar
NC	Negative control
NPK	Nitrogen, Phosphorus and Potassium
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PCR	Polymerase Chain Reaction
r DNA	Ribosomal DNA
Sig	Significance.
SNA	Spezeieller naurtoffarmer agar

ABSTRACT

Three *Trichoderma* spp. were isolated from agricultural soil in Embu district, Kenya and identified morphologically as *T. harzianum*, *T. polysporum* and *T. viride*. These isolates were evaluated for their effect on the growth of *F. oxysporum* var *phaseoli*. Evaluation of the effect of *Trichoderma* spp. on *F. oxysporum* var *phaseoli* was carried out by setting up *invitro* experiments and green house experiment. *Invitro* experiments were done by placing 5mm mycelia growth disc cut out from the growing *Trichoderma* spp. isolates and placed on one side of a 9 mm PDA plate, a similar disc of *F. oxysporum* var *phaseoli* isolate was cut out and placed 3 mm opposite the *Trichoderma* spp. mycelia disc. *F. oxysporum* var *phaseoli* without *Trichoderma* spp. was also cultured as a control; all the treatments were in triplicates.

A green house experiment was carried out by planting common bean seeds coated with *Trichoderma* spp. in sterilized soil infected with *F. oxysporum* var *phaseoli*. The beans were immersed in a mixture of gum Arabic and *Trichoderma* spp. and left for 5 hours prior to planting so as to let the mixture attach to seed coat. Seven mixtures of *Trichoderma* isolates were formulated: *T. polysporum*; *T. harzianum*; *T. viride* and in combinations of the species; *T. polysporum* and *T. harzianum*; *T. polysporum* and *T. viride*; *T. harzianum* and *T. viride*; *T. polysporum*, *T.harzianum* and *T.viride*. The soil was placed in 22cm diameter pots which were then applied with manure at a rate of; 0g (control), 38g (without *Trichoderma* spp.) 38g, 28g, 18 g and 9 g (with *Trichoderma* spp.). The same procedure was followed with NPK fertilizer application except that fertilizer rates were; 0g (control), 0.76g (without *Trichoderma* spp.), 0.76 g, 0.57 g, 0.38 g, 0.19 g (with *Trichoderma* spp.).

Invitro experiments of *F. oxysporum* var. *phaseoli* with *T. harziunum*, *T. polysporum* or *T. viride* revealed that each of these *Trichoderma* species inhibited growth of the pathogenic fungi. *T. polysporum* had the highest inhibitory ($P<0.05$) effect at 64% while *T.viride* had the least inhibitory effect (47%). On the other hand a combination of manure and the *Trichoderma* species improved growth of common bean when grown in *F.*

oxysporum infested soil. A combination of manure and *Trichoderma* (in green house) showed a highly significant effects ($P<0.001$) on all the bean growth parameters measured. The combination of *Trichoderma* spp. and fertilizers however did not show significant difference ($P>0.05$) among the different rates of fertilizer in root length and fresh weight. The results reveal the ability of *Trichoderma* spp. to control pathogens and improve plant growth.

CHAPTER ONE

INTRODUCTION

Common bean (*Phaseolus vulgaris L.*) originated in Peru. It is an annual self-pollinated legume which can be grown in many parts of the world and it is, among the pulses, the cheapest source of protein, essential amino acids, lysine and tryptophan in Kenya. Beans are produced mainly at subsistence level by small scale farmers who interplant them with maize but sometimes as monocrops (Okoko *at el.*, 2005). Common beans though versatile, are susceptible to some diseases such as charcoal root, dry root rot, *Fusarium* wilt and poor soil fertility which can greatly reduce the grain yield by 50% per year. Diseases such as dry root, *Fusarium* wilt and charcoal root are common in Kenya and other parts of the East African region and Southern Africa (Allen *et al.*, I996). Common bean root diseases have caused a decline in bean production in Kenya (Otsyula and Buruchara, 2001b). The diseases can be controlled by applying fungicides, using clean seeds, crop rotation and planting resistant varieties. But due to the fact that fungicides are expensive, coupled with the unavailability of resistant varieties and lack of crop rotation, farmers are not able to get the maximum yields from common beans. Soil fertility is critical in determining the ability of the crop to tolerate diseases. In order to improve soil fertility, inorganic fertilizers, manures and biofertilisers have to be applied in the soil. All these forms of fertilizers have advantages and disadvantages, hence the need for an integrated approach where organic fertilizer and inorganic fertilizers are used to ameliorate the soil. Inorganic fertilizers are efficient and readily available to plants immediately when applied to the soil but unfortunately they are not affordable to many farmers and they do not improve soil structure (Roland, 1993). On the other hand farmyard manure is cheap but large quantities of it are needed in order to provide enough nutrients required by the crops. The problem with manure is that such large quantities cannot easily be obtained and if found the nutrients cannot readily be availed to crops- its nutrient release efficiency is very low. The use of biofertilisers is relatively new; beneficial microbes-based fertilizers are becoming popular. Biofertilisers usually work perfectly well when blended with other fertilizers such as manure. The beneficial

microbes have to be multiplied in large quantities, blended with manure then applied to the soil or inoculated on the seeds to be planted (Harman *et al.*, 2004).

A lot of research on testing *Trichoderma* spp. as biocontrol and biofetiliser agent is going on but very little has been done to integrate it into crop farming systems. Biological inocula are environmentally friendly, cheap, rich in nutrients as well as in organic matter and have been shown to be able to control some diseases (Renzel *et al.*, 2000).

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy of *Trichoderma* spp.

Trichoderma is a genus of fungus which belongs to the family Hypocreaceae (Samuels and Dodd, 2002). *Trichoderma* was first anticipated to be a genus of the Kingdom Fungi in 1794 by Perseon and only few species were listed under this genus (Samuels and Dodd, 2002). With the discovery of new descriptors the species now stand at more than 100 (Druzhinina *et al.*, 2006). *Trichoderma* is a filamentous fungus widely found in the many different soils, decaying vegetation and wood (Alexopoulos *et al.*, 1996). The species are characterized by fast growing colonies with a lot of powdery green mold which grows well at 25-30°C but will not grow at 35°C. When the fungus is grown on corn meal dextrose agar (CMD) they show a transparent color while when cultured on potato dextrose agar (PDA) the colony is white. A yellowish pigment may be secreted into the agar especially when grown on PDA and some species produce a characteristics sweet or coconut odor. *Trichoderma* spp. is also characterized by highly branched and difficult to define conidiophores. Phialides are typically enlarged in the middle and may be cylindrical or rarely subglobulose and the conidia typically appear dry but in some species they may be held in drops of clear green or yellow (Samuels *et al.*, 2004). Morphological criteria used in identification of other fungal genera are difficult to apply due to plasticity of the characteristics. The taxonomy and identification of *Trichoderma* spp. has remained a problem since it was first discoverd in 1794 by Persoon (Druzhinina and Kubicek, 2005). Many concepts followed thereafter which had to expand the morphological criteria to accommodate the wide range of morphological variation expressed by some anamorphs of *Trichoderma* spp. The molecular tools have recently been used to investigated the fungal taxonomy such as use of ITS1 and ITS4 primers and random amplified polymorphic DNA (RAPD) (Druzhinina and Kubicek, 2005). Morphological analysis is highly prone to errors and consequently about 50 % of the

Trichoderma spp. deposited in culture collections under names obtained by morphological analysis alone can be wrong (Lu *et al.*, 2004).

2.2 Uses of *Trichoderma* spp.based inoculum in Agriculture.

Trichoderma spp. has been reported to improve plant growth and control fungal diseases in the rhizhosphere of a plant. The species are able to control the population of pathogenic microorganisms in the rhizhosphere. Some minerals can be solubilised by hormonal activities secreted by *Trichoderma* spp. thereby making the minerals available to plant roots hence maximum growth of the plants (Chacón *et al.*, 2007). The discovery of the ability of *Trichoderma* spp. to control diseases and improve plant growth has led to the development of biofertilisers and biocontrol agents. Fungal agents have so far been registered in United States of America and in Europe. *Ampelomyces quisqualis Ces* (AQ10) is one of the fungi which were developed by Ecogen Company in America for control of powdery mildews in vegetable and fruit crops, but primarily in viticulture. Germinating spores suppress the development of powdery mildew by hyper parasitism. *T.harzianum* is recommended for the control of soil inhabiting Botrytis, *Sclerotinia* spp. in vines, vegetables and for the treatment of tree wounds to prevent decay. It is sold by Makhteshim under trade name Trichodex and by NPP as Harzan formulated as a microgranule. There are many other formulations of *Trichoderma* strains from other countries:- Belgium; Root Pro and *Trichoderma* 2000, Israel; Trochoject, Trichopel, Trichodowels and Trichoseal, New Zealand; TUSAL, Spain; Trieco, India; Trichodex). These commercial formulations are available against pathogenic *Fusarium* species (Klokoaar-Smit *et al.*, 2008).

2.2.1 Other biofertilisers in the market.

Biofertilisers are microbial inoculants of microbial-converted organic material which are used to supply nutrients to plants, hence, higher productivity of plants (Renzel *et al.*, 2000). There are common beneficial microbes which are used to formulate biofetilisers such as *Trichoderma* spp, *Rhizobia* spp.

There are 7 biofertilisers which have been successfully formulated. These are:

i) Rhizobia which fix nitrogen (form nodules on plants roots), in association with leguminous crop and can fix up to 40-120 kg/ha (Kannaiyan, 2004). It improves soil fertility, plant nutrition, growth and has no negative effect on soil or the environment (Kannaiyan, 2004). Every leguminous crop requires a specific *rhizobium* species (Vessey, 2004).

ii) Azotobacter is a nitrogen fixing bacterium which does not form root nodules or associate with leguminous crops (Kannaiyan, 2004). It is a free-living and associated nitrogen fixer and is used for all types of upland crops, i.e. they cannot survive in wetland conditions (Kannaiyan, 2004). In soils of poor fertility and organic matter, *Azotobacter* needs to be regularly applied. In addition to nitrogen fixation, they also produce beneficial growth substances and beneficial antibiotics that help control root diseases.

iii) *Azospirillum*: also does not form root nodules or associate with legume crops. They are however not free-living and resides inside plant roots where they fix nitrogen and can be used in wetland conditions. They produce beneficial substances for plant growth, besides fixing atmospheric nitrogen. *Azospirillum* does well in soils with organic matter and moisture content and requires a pH level of above 6.0 (Kannaiyan, 2004).

iv) Blue-green algae or cyanobacteria are free-living N-fixing photosynthetic algae that are found in wet aniod marshy conditions (Vaishampayan, 2004). Blue-green algae are so named for their colour, but they may also be purple, brown or red. They are easily prepared on farms but can be used only for rice cultivation where the field is flooded and do not survive in acidic soils (Gopalaswamy, 2004).

v) *Azolla* is a free floating water fern that fixes N in association with a specific species of cyanobacteria, *Anabaena azolla* (Peoples and Craswell, 1993). *Azolla* is a renewable biofetiliser and can be mass-produced on the farm like blue-green

algae. It is a good source of nitrogen and on decomposition, a source of various micronutrients (Samal, 2004). It is also used as green manure in rice fields and is a high quality feed for cattle and poultry (Kannaiyan, 2004).

vi) Phosphate-solubilising micro-organisms: These are a group of bacteria and fungi capable of breaking down insoluble phosphate to make them available to crops. Their importance lies in the fact that barely a third of phosphorus in the soil is actually accessible to the crops the rest is insoluble. They require sufficient organic matter in the soil to be of any great benefit (Vessey, 2004).

vii) *Mychorrhiza* is a term for a number of fungi which form a symbiotic association with the plant. The symbiotic relationship between plants and the fungi (vesicular-arbuscular mycorrhiza or VAM is important in agriculture. Plants with VAM colonies are capable of higher uptake of nutrients and water. VAM strands acts as extensions and bring up water and nutrients from lateral and vertical distances where the plant root system does not reach (Kannaiyan, 2004).

2.3 Application of *Trichoderma* spp. on crops.

Crops such as maize, carrots, cucumber, tomatoes, cabbage, and legumes, when inoculated with *Trichoderma* species improved growth in variables such as root length, germination rate and plant leaves. Yedidial *at el.*,(2001) observed a significant (150 %) increase in number of leaves in tomato crop when *T. harzianum* was used. *Trichoderma* secretes cellulotytic and proteolytic enzymes which aid it in the penetration of the roots. The roots can only allow the *Trichoderma* to penetrate up to the second layer of root cells (Brotman *et al.*, 2008). The presence of *Trichoderma* in the plants cells induces the production of ethylene by plants. Ethylene has been reported to take part in the many metabolic processes in a plant such as germination, fruit setting, senescence, and act as a pathogen repellent. Production of ethylene in higher plants vary with the stages of plants development, it is highly produced during seed germination, abscission of leaves and ripening of some fruits (Matilla, 2000).

2.4 Cultivation and use of common beans *(Phaseolus vulgaris L.)*

2.4.1 Importance of common bean

Common bean (Maharagwe in Kiswahili, Kenya) is a leguminous plant which is widely produced in different parts of the world. It is normally grown for its grains (beans) which have high protein content. Its protein is high in lysine which is not found in maize, rice and cassava, therefore a mixture of rice and beans or maize and beans will complement each other in a diet. The crop is relatively cheap compared to meat, which makes it possible for malnourished people to acquire the proteins from the beans. It has also been reported that beans helps in reducing diseases such as cancer, diabetes due to low fat content and are cholesterol free. Common beans form a basic dish of most Kenyan people; it is a leading source of protein among the pulses. The crop can grow everywhere in Kenya, it can survive very harsh conditions and still produce something for a farmer to harvest (Katungi *et al.*, 2009).

2.4.2 Cultivation of common beans

Beans are versatile leguminous crops which are able to perform well in altitudes ranging from 1000m to 2000m above sea level and they may also grow very well even beyond these altitudes levels. Beans in high altitudes tend to mature later than beans in lower altitudes. Rainfall is required to sustain beans up to maturity ranges from 750-4000 mm annually. Too much rain and drought lead to reduced yields. Temperature is also an important factor which lies within the range of 20-30 °C. Higher temperatures cause dropping of buds and flowers, hence low yields. Beans grow in a wide range of soil types but they grow best in well drained soils, high organic matter and pH range of 6.0 to 7 (Okoko *et al.*, 2005).

Farm yard manure and chemical fertiliser are used to supplement the amount of nutrients required by the crop. Manure is applied at the rate of 10t/ha and fertiliser is applied at 200 kg/ha depending on the nutrient soil analysis results. Beans have to be protected from pest, weeds and diseases. Weeds are controlled by hoeing, uprooting and applying herbicides and pests are only easily controlled by chemicals. Diseases are not easy to control; an integrated approach should be applied. Common beans are affected by

different kinds of diseases aerial (leaves, stems, flowers, braches and grains) and below the ground (roots) (Allen *et al.*, 1996).

Cultural practices which will allow the crop to establish a well developed rooting system so as to explore the maximum amount of moist soil are inevitable. This requires soil that has a good structure, is well drained, has a high water holding capacity and free from compacted layers. Soils with high organic matter content have a higher water-holding capacity. This is in contrast with low organic matter content soils such as those of the tropics which are unstable and are easily damaged by heavy rains and over-cultivation (Roland, 1993). Such soils are very poor in nutrient content and cannot allow crops to achieve good growth during harsh conditions or during low soil moisture content (Roland, 1993). A naturally fertile soil is one in which the soil organisms are releasing inorganic nutrients from the organic reserves at a rate sufficient to sustain rapid plant growth (Wild, 1988). The proliferation of the root diseases has been reported in dry and poor soils (Otsyula and Buruchara, 2001a).

Soil contains microbes, (beneficial and non-beneficial), organic matter, nutrients and available moisture, air and mineral particles. The crop is able to access nutrients by drawing (through roots) macro elements (nitrogen, phosphorus and potassium) and microelements (carbon, zinc, iron, copper and cobalt) from the soil. Deficiency of these nutrients is the main impediment to agricultural production in both rain fed and irrigation agriculture (Gupta, 2010). Agriculture is therefore amassed with chemical based methods in trying to alleviate the constraints caused by lack of nutrients in the soil.

Inorganic fertilizers and manures are used to boost the soil's capacity with available plant nutrients. Fertilizers such as nitrogenous, phosphates and potassium supply essential nutrients for plant growth and are often applied to the soil prior, during and/or after planting (Bagyaraj, 2004). The main reason chemical fertilizers are applied frequently is because they are easily leached from the soil. Fertilizers have been optimized to dissolve quickly and become available to plants roots. However, fertilizer usage among small-scale farmers in tropical Africa is low and largely restricted to higher potential area such

as irrigation schemes and cash crops (Roland, 1993). High cost, poor availability and uncertain returns are among the main reasons for their slow adoption.

The organic matter content of soil is improved by use of organic manure (Gupta, 2010). Organic manures such as crop residues, green manures and composts are used for organic recycling practices which increase the water holding capacity and the cation exchange capacity –CEC (Saha and Muli, 1999). Regular additions of adequate amounts of organic matter to soils reduce the effect of nutrient run off, erosion and gradual deterioration of soil physical properties. Proper practices of organic material usage can be environmental friendly, thereby reducing environmental pollution caused by chemical fertilizers (Gupta, 2010). However, a large quantity (10t/ha) of manure has to be applied to the soil to meet the plant nutrient requirement. Moreover the nutrients from manure are not readily available to plants; further decomposition by microbes is required for the plants to access nutrients from manure.

Plants interact symbiotically with certain beneficial microbes such as *Rhizobia, Azolla, Azotobacteria, Azospirillum*, blue-green algae, phosphate-solubilising micro-organisms and *mychorrhiza* (Vinale *et al.*, 2008). The *Rhizhobia* bacteria form a symbiotic relationship with plant roots and convert atmospheric nitrogen into nitrates needed by plants (Vessey, 2004). Mycorrhiza, a group of fungus which includes *Trichoderma* spp, colonises the rhizosphere and obtains organic nutrients from plants and in turn enhances nutrient uptake during moisture transport in the plants (Hajek, 2004). This improves the rate of seed germination, the growth rate, yield and resistance to diseases, the population of pathogenic microorganisms in the rhizhosphere and by influencing plant physiology through mineral solubilisation or hormone secretion (Chacón *et al.*, 2007; Harman *et al.*, 2004). Another important feature mentioned by (Chacón *et al.*, 2007) is that *Trichoderma* spp. have plant cell wall degrading enzymes, which induce the production of ethylene that guard against pathogens.

2.4.3 Root diseases of common beans

(i) Charcoal root (Ashy stem blight)

The causal fungus (*Macrophommina phaseolina* syn *Sclerotium bataticola*)) commonly inhabit the roots of a wide range of plants species found in Eastern and Southern Africa. Infected seedlings often have dark irregular lesions, which start on the cotyledons followed by sunken cankers with well-defined margins. Infected older plants result in wilting chlorosis, premature defoliation, rotting of the hypocotyls and roots leading to death of a plant. The disease is spread by infected debris, movement of plant residues and infected seeds, on which the fungus can survive for a long time. Infection is high in high temperatures and drought stress. Use of clean seeds, treatment with fungicides and flooding decrease disease severity and improves the crop yield but it comes at a higher cost (Allen *et al*., I996; Otsyula *et al*., 2003).

(ii) *Fusarium* wilt (yellows)

Fusarum oxyporium F. sp. *phaseoli* is widespread across Africa. The foliage infected crops appear yellowish in colour (chlorosis), followed by a permanent wilting of affected crops. It is a true vascular wilt pathogen, which penetrates internal tissues of the root and hypocotyls.

The disease is spread through movement of infected debris, seeds, soil and proliferates well in high temperatures and drought prone areas. The wilt can be controlled by using resistant cultivars and practicing cultural practices that improve soil fertility. Nutrients deficiency in the soil makes it impossible for the crop to tolerate some diseases.

(iii) Dry root rot (*Fusarium solani* F.sp. *phaseoli*)

This is a disease which is widespread in Southern and Eastern Africa. The disease does not cause more harm if the crop is vigorously growing but when the crop is under drought stress it causes up to 80% losses. Narrow longitudinal, reddish brown streaks on the hypocotyls and taproot of 7 to 8 days old seedlings are observed. The streaks expand and merge to affect all underground tissues up to the collar, at the soil level. Infected plants often produce numerous adventitious roots. The pathogen spreads at low rate and it is not

found in the seed. The fungus is spread through infected soil particles and it can survive as chlamydospores in infested soil for a very long time. Cultural practices that promote crop vigour may decrease disease damage and also natural biological control can be enhanced by soil amendments (Ramezani, 2008).

2.5 Justification:

Common bean is an annual self-pollinated legume which can be grown in many parts of the world. Among the pulses grown in Kenya, it is the cheapest source of protein, essential amino acids, lysine and tryptophan (Allen *et al.*, 1996). The crop is valued and widely grown in the country because it is drought resistant however more than 80 % of the crop yield is lost due to *Fusarium* wilt. The effect of the diseases is also compounded by low soil fertility in most of local soils. Fungicides are used to reduce the wilt damage to some extent but these chemicals are unaffordable to peasant farmers and the chemicals can also be environmentally damaging (Kimani *et al.*, 1994). Farmyard manure and chemical fertilizers are applied on agricultural soil to provide nutrients for crops though there are advantages and disadvantages associated with them. Farm yard manure provide nutrients for crops and it improves soil fertility but those nutrients are not readily available until the manure is broken down into humus (Ronald, 1999). A large quantity of manure has to be applied on the soil in order to meet the crop nutrient requirements per plant which is difficult for most of the small scale farmers to obtain. Chemical fertilizers on the other hand are applied in small quantity and provide readily available plant nutrients to crops. Unfortunately they are expensive, easily leached from the soil and continued use of chemical fertilizers can affect the ecosystem and soil pH. As a results small scale farmers find it very hard to use either manure or chemical fertilizers because of the above mentioned disadvantages (Kimani *et al.*, 1994). This project therefore, investigates the possibility of using *Trichoderma* spp. To enhance the growth of common beans thus reduce the quantity of fertilizers.

2.6 General objective

To test the effect of *Trichoderma* spp. augmentation fertilizers on growth of common bean (*Phaseolus vulgaris* L.)

2.6.1 Specific objectives:

i. To isolate and identify *Trichoderma* spp. from bean root rhizosphere.

ii. To isolate and identify *Fusarium oxysporum var phaseoli* from infected beans (root rot).

iii. To evaluate inhibition effects of *Trichoderma* isolates on growth of pathogenic *Fusarium oxysporum var phaseoli in vitro.*

iv. To evaluate the growth of common bean inoculated with *Trichoderma* inoculum *in* greenhouse.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Isolation and identification of *Trichoderma* spp. strains from soil

Soil samples were collected from Embu district, Kenya in a small scale farm under a maize-bean intercrop. Sampling points were identified and 3 m and 6 m radius cycle were marked. Within the 3 m radius cycle four soil samples were collected at depth of 0-20 cm and another eight soil samples were collected from 6 m radius cycle. The collected soil samples were then bulked, homogenized and packaged in paper bags. The samples were kept at 2-5°C in the laboratory to reduce microbial activity. The soil sample was sieved and small fine particles. One gram of the sieved the soil was weighed. Ten grams of PDA was dissolved in 255 ml of distilled water to make a solution. The solution was then autoclaved for 20 min at 121psi. The media was left to cool at room temperature and one tablet of 500 mg streptomycin added then poured into twelve 9 mm wide Petri dishes under aseptic condition. The weighed soil was then sprinkled on the set media and the Petri dishes were sealed with a parafilm then incubated in an oven at 30°C for seven days. After seven days a uniformly growing mycelia of fungus was picked with a wire loop (from each plate) in the mixture of soil and isolates based on the morphology appearance, mode of growth, colony morphology, conidia and conidia spore characteristics (Mghalu *et al.*, 2007; Siameto *et al.*, 2011a) and re-cultured it on a new PDA. Three species of *Trichoderma* were identified using taxonomic key of the genus *Trichoderma* (Samuels *et al.*, 2004).

The isolated *Trichoderma* spp. were further cultured in potato dextrose broth (PDB). Thirty grams of PDB was dissolved in 125 ml. The solution was autoclaved for 1hour at 121 psi. The solution was allowed to cool at room temperature and two tablets of 500 mg streptomycin were added then the media was distributed among ten 250 ml conical flasks, poured to cover the base of the flasks. The pure isolates were cultured in the broth and the flasks were sealed with aluminum foil and placed in a shaker for seven days at a speed of 60 rvs/min. Isolates were harvested after seven days by filtration method using the

Whatman filter paper for harvesting in small bottles, put in a freezer and then lyophilized with a Freezemobile 35 XL machine. The isolates were kept in a refrigerator until further use.

3.1.1 Molecular characterization of *Trichoderma* spp. isolated from soils.

Three isolates of *T polysporum*, *T. viride*, and *T. harziunum* obtained from rhizosphere soil of small scale farmers were characterised using ITS-PCR. The genetic differences among three isolates of *Trichoderma* spp. were analyzed with two random primers.

3.1.2 Genomic DNA extraction from *Trichoderma* Isolates

Isolation of fungal genomic DNA was done by growing the fungi for seven days potato dextrose broth at room temperature. Mycelia were harvested by filtering through a Whatman filter paper then collected in 50 ml sample bottles and frozen at -20°C and thereafter the mycelia were lyophilized and kept in ependorf tubes. Fungal genomic DNA was extracted using the phenol-chloroform method; Ten (10) mg of lyophilized mycelium was put in 500 ul extraction buffer (consisting of 1M Tris-HCl pH 8.0; 1M NaCl PH 8.0, 1M EDTA pH 8.0 and 10% sodium dodesyl sulphate) and 20 ul of protenaise K. The mixture was vortexed vigorously for 5 minutes and then incubated in a water bath for 1 hour at 65^0C, after that 500 ul 25:24:1 phenol: chlorophorm: isoamyl was added and mixed gently. It was centrifuged at 1400 rv/m for 15 minutes at 4°C. After centrifugation the upper aqueous layer was transferred to a clean 1.5 ml centrifugation tubes and 500 ul of 25:24:1 phenol chlorophorm isoamyl was added to the supernatant and centrifuged again at 1400 rpm for 15minutes at 4°C.The upper aqueous phase was transferred to 1.5 ml new tubes and the DNA was precipitated with 60 ul 8M sodium acetate and 800 ul absolute ethanol for overnight at -20 °C. The mixture was removed from the freezer, left to thaw at room temperature then centrifuged at 1400 rpm for 15 minutes at 4°C, the pellet was formed at the bottom of the tube and the upper layer was discarded. The pellet was thoroughly washed twice with 500 ul of 70 % ethanol, air dried and dissolved in double distilled water. 5 ul of RNAse was added and left at room

temperature for an hour. The DNA was kept at -20°C until it was further used in polymerase chain reaction (PCR) (Park *et al.*, 2005).

3.1.3 PCR Amplification of ITS Region of *Trichoderma* Isolates

Two pairs of primers (internal transcribed sequences (ITS)) were used in this study; ITS 1 (5-3) for forward and ITS 4 (3-5) for reverse (Table 1) were used to study variation among the isolates

Genomic DNA was amplified by mixing the template DNA (50 ng), with the polymerase reaction buffer, dNTP mix, primers and Taq polymerase. Polymerase Chain Reaction was performed in a total volume of 200 µl, containing 138 µl deionized water, 25µl of 10 X Taq pol buffer, 8 µl of 1 U Taq polymerase enzyme, 10 µl of 10 mM dNTPs all these constituted a master mix for ten reactions. 20 µl of master mix was aliquoted to a PCR tube, 1.5 µl of 100 mM reverse and forward primers and 1µl of 50 ng template DNA were added. PCR was programmed with an initial denaturing at 94 °C for 5 min. followed by 33 cycles of denaturation at 94 °C for 30 sec, annealing at 59 °C for 30 sec and extension at 70 °C for 2 min and the final extension at 72 °C for 7 min in a PTC-100 Peltier Thermal cycler. The PCR product (8 µl) was mixed with 2 µl of loading buffer containing 0.25 % bromophenol blue, 40 % w/v sucrose in water and then loaded in 2 % Agarose gel with 0.1 % ethidium bromide for examination with horizontal electrophoresis (Martin and Rygiewicz, 2005).

Table 1: The nucleotide sequence used for ITS PCR

Primer name	Sequence	Mer	TM (°C)	%GC
ITS primers				
T/ITS 1	TCTGTAGGTGAACCTGCGG	19	63.9	57%
T/ITS 4	TCCTCCGCTTATTGATATGC	20	61.5	45%

3.2. Isolation of *Fusarium oxysporum* from infected bean crop

Roots of infected plants were collected, cut into smaller pieces of about 1cm long and washed in 1% sodium hypochlorite for ten seconds, rinsed twice with sterilized distilled water and dried with sterilized paper towels. The root pieces were transferred to minimal glucose agar (MGA) plates and incubated for five days at room temperature. MGA is used mainly to encourage spore forming in fungus for easier identification. Colonies growing on the plates were scooped and transferred PDA plates for seven days for identification as *Fusarium* spp. Further identification was done using spezeieller naurtoffarmer agar (SNA) and carnation leaf agar (CLA) media (Apendix 3). The pure culture was re-cultured in PDB for seven days, harvested in small bottles, put on a freezer and then lyophilized with a Freezemobile 35 XL machine. The isolate were freeze dried and kept in eppendorf tubes until there were used.

3.3 In vitro tests of *Trichoderma* spp. against *Fusarium oxysporum.*

3.3.1 In vitro tests of *Trichoderma* spp. against *Fusarium oxysporum* on culture media.

A 5mm mycelia growth disc was cut out of the growing *Trichoderma* spp. isolates and placed on one side of a PDA plate, a similar disc of *Fusarium* spp. isolates was cut and placed on the opposite side, and this was done in triplicate. Control plates without *Trichoderma* spp. isolates were also cultured in triplicate. The cultures were incubated at

room temperature for five days (Lubna and Nawar, 2005) and the percentage reduction in growth of the pathogenic fungus due to *Trichoderma* spp. was calculated using the formula:

$$growth\ reduction\ \% = \frac{growth\ in\ check - growth\ in\ treatment}{growth\ in\ check} \times 100$$

3.3.2 *Invitro* test of *Trichoderma* spp. against *F. oxysporum* using slide culture.

A dual culture was set up on a slide in order to allow a proper view of interaction between *Trichoderma* and *Fusarium* spp. Three sterile slide glasses were placed on separate 9 mm Petri dishes and a small amount of PDA was cut and placed on each slide. About 3 mm mycelia discs of 7 days old *Trichoderma* and *Fusarium* spp. were cut out from the growing colonies and placed on the slides. *Trichoderma* spp. was placed 3mm away from the *Fusarium* spp. on the opposite side of the slides. The Petri dishes were closed and then sealed with parafilm and incubated for a week at room temperature. The slides were further stained by lacto phenol blue at the point of contact between *Trichoderma* and *Fusarium* and observed under a light microscope (x 400 magnification) for the presence of mycelia penetration (Siameto *et al.*, 2011b).

3.4. Green house experiment on the effect of *Trichoderma*–augmented fertilizer on growth of common beans

3.4.1 Effects of *Trichoderma* enhanced manure on growth of common beans

Soil was collected from a selected forest land, air dried and then sterilized. The soil was tested for the following chemical properties; pH H_2O, pH 0.01M $CaCl_2$,%N, %C, K, Na (mg/l),CEC (mol/kg) and P(ppm) (Appendix 1). The sterilized soil was mixed with sterile, in 22cm diameter pots, manure was at different rates, 10000 kg/ha, 7500 kg/ha, 5000 kg/ha and 2500 kg/ha. The rates of manure were converted to grams to be able to suit per pot application; 38 g (without *Trichoderma* spp.), 38 g, 28 g, 19 g, 9 g,0 g (all

with *Trichoderma* spp.). The pots were then planted with local bean variety (Mwezi Moja) bought from local seed company, E.A. Seed CO. LTD. coated with *Trichoderma* spp as follows; *Trichoderma* spp. was as follows: 1). *T. polysporum* 2). *T. harzianum*, 3) *T. viride* ,4) *T. hazanium* and *T. viride*; 5) *T. hazanium* and *T. polysporum*, 6).*T. viride* and *T. polysporum* 7).*T. polysporum*, *T. harzianum* and *T. viride.* The different rates of manure and *Trichoderma* spp. constituted the treatments (Table 2). The treatments were arranged in complete randomized design in triplicates. The bean seeds were soaked in 1% sodium hypochlorite (Jik) to wash off any chemical and microorganisms which might be attached on seed cover. *Trichoderma* spp. were inoculated on the rinsed beans at a rate of 20 ml/g of beans then thoroughly mixed with gum Arabic and left to dry under shade for five hours before planting.

3.4.2 Effects of *Trichoderma* enhanced NPK on growth of common beans

The sterilized soil was mixed, in 22 cm diameter pots, with 2:3:2 NPK compound, obtained from Botswana in Game supermarket, at different rates, 200 kg/ha, 150 kg/ha, 100 kg/ha and 50 kg/ha. The rates of NPK were converted to grams to be able to suit per pot application; 0.76g (without *Trichoderma* spp.), 0.76 g, 0.57 g, 0.38 g, 0.19 g, 0 g (all with *Trichoderma* spp.). The pots were then planted with local bean variety (Mwezi Moja) bought from local seed company, E.A. Seed CO. LTD. coated with *Trichoderma* spp. as follows; *Trichoderma* spp. was as follows: 1). *T. polysporum* 2). *T. harzianum*, 3) *T. viride* ,4) *T. hazanium* and *T. viride*; 5) *T. hazanium* and *T. polysporum*, 6).*T. viride* and *T. polysporum* 7).*T. polysporum*, *T. harzianum* and *T. viride.* The different rates of NPK and *Trichoderma* spp. constituted the treatments (Table 3). The treatments were arranged in complete randomized design. The bean seeds were soaked in 1% sodium hypochlorite (Jik) to wash off any chemical and microorganisms which might be attached on seed cover. *Trichoderma* spp. were inoculated on the rinsed beans at a rate of 20 ml/g of beans then thoroughly mixed with gum Arabic and left to dry under shade for five hours before planting. The required moisture and optimum temperature was maintained under green house conditions. The soil both on treatments of manure and NPK were also disease inoculated with *Fusarium oxysporum* at a rate of 10^6 conidia/ml and mixed with

soil in all the pots. Common bean was planted on the 1st May 2011 and harvested after 30 days. Parameters measured include plant height, root length, leaf area index, dry matter (wet and dry mass).

Table 2: Treatment formulations for manure green house experiment.

Treatments	Descriptions	No of repeats
Treamtment1: (Control)	*Trichoderma* spp. + *Fusarium* spp (10^6 conidia/ml).	Triplicates
Treatment 2:	38g without *Trichoderma* spp.+ *Fusarium* spp(10^6 conidia/ml)	Triplicates
Treatment 3:	38g of manure /pot + *Fusarium* spp. and *Trichoderma* spp.	Triplicates
Treatment 4	29g of manure/pot + *Fusarium* spp. and *Trichoderma* spp.	Triplicates
Treatment 5	19 g of manure/pot + *Fusarium* and *Trichoderma* spp.	Triplicates
Treatment 6	9 g of manure/pot + *Fusarium* and *Trichoderma* spp	Triplicates

soil in all the pots. Common bean was planted on the 1st May 2011 and harvested after 30 days. Parameters measured include plant height, root length, leaf area index, dry matter (wet and dry mass).

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Treatment 2:	38g without *Trichoderma* spp.+ *Fusarium* spp(10^6 conidia/ml)	Triplicates
Treatment 3:	38g of manure /pot + *Fusarium* spp. and *Trichoderma* spp.	Triplicates
Treatment 4	29g of manure/pot + *Fusarium* spp. and *Trichoderma* spp.	Triplicates
Treatment 5	19 g of manure/pot + *Fusarium* and *Trichoderma* spp.	Triplicates
Treatment 6	9 g of manure/pot + *Fusarium* and *Trichoderma* spp	Triplicates

Table 3: Treatment formulations for NPK in green house experiment.

Treatments	Descriptions	No of repeats
Treamtment1: (Control)	*Trichoderma* spp. + *Fusarium* spp (10^6 conidia/ml)	Triplicates
Treatment 2:	0.76g of NPK/pot + *Fusarium* spp *(without Trichoderma* spp.*)*	Triplicates
Treatment 3:	0.76g of NPK/pot + *Fusarium* spp and *Trichoderma* spp	Triplicates
Treatment 4	0.57g of NPK/pot + *Fusarium* spp and *Trichoderma* spp.	Triplicates
Treatment 5	0.38g of NPK/pot + *Fusarium* and *Trichoderma* spp	Triplicates
Treatment 6	0.19g of NPK/pot + *Fusarium* and *Trichoderma* spp	Triplicates

CHAPTER FOUR

RESULTS

4.1. Identification of *Trichoderma* spp. Isolated from soil

Three *Trichoderma* spp. were identified in this study as *T. polysporum*, *T. harzianum* and *T.viride* (Plate 1). *T. polysporum* (Plate 1a) was characterized by green conidia as well as species with white or yellow in mass. The fungi have relatively short and broad phialides arising from often clustered wide branches of the conidiophores. Conidiophores on corn meal dextrose (CMD) typically consist of an elongated, spiral, smooth or warted, sterile central axis from which lateral branches arise near the base. Fertile branches are progressively longer with distance from the tip of the sterile hair, comprising of one or a few broad cells from which phialides arise (Samuels *et al.*, 2004).

T. harzianum (Plate 1b) is characterized by smooth, green and subglobose to ovoid conidia. Conidiophores are typically with paired branches forming over 150 µm of the length of terminal branches. Within these systems, longest branches form near the base of the system and nearest the main axis. Branches toward the tip and secondary branches tend to be held at 90° with respect to the axis from which they arise; further from the tip of the branching system the angle of branching tends to be less than 90° with respect to the axis above. Cells support the phialides equivalent in width to, or at most only slightly wider than, the base of phialides arising from them (Samuels *et al.*, 2004).

T.viride (Plate 1c) is characterized by dark green subglobose and conspicuously tuberculate conodia. Conidiophores on CMD are typically comprised of fertile central axis or the central axis 100-150 µm long and flexuous, with lateral branches paired or not and typically arising at an angle at or near 90° with respect to its supporting branch, sometimes lateral branches are widely-spaced intervals when near the tip of the conidiophore and arising at closer intervals when more distant from the tip; phialides arising singly from the main axis or in whorls of 2-3 at the tips of lateral branches or at the tip of the conidiophore (Lieckfeldt *et al.*, 1999; Samuels *et al.*, 2004).

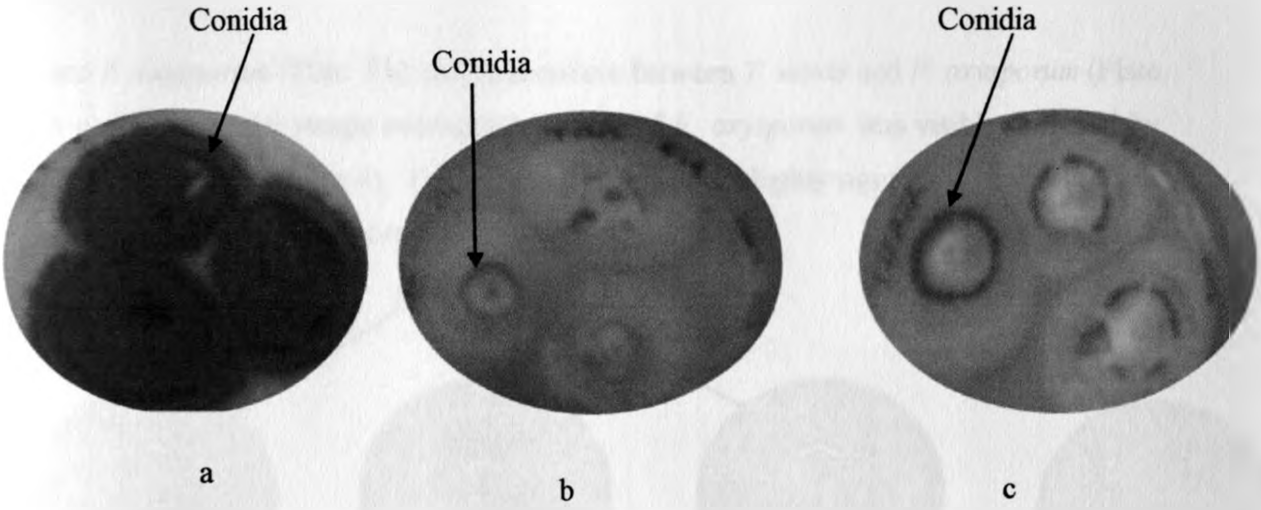

Plate 1: Characteristic of isolated *Trichoderma* spp. features growing on potato dextrose agar

(a) *Trichoderma polysprum* conidia, (b) *Trichoderma viride* conidia and *(c) Trichoderma harzianum* conidia.

4.2 Isolation of *Fusarium oxsysporum* from infested beans

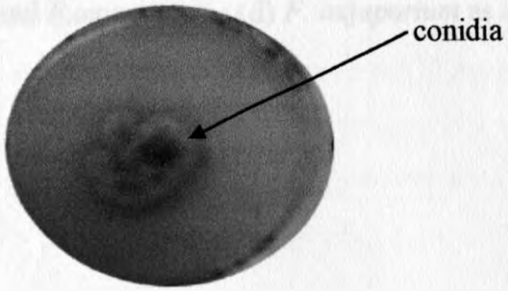

Plate 2 *Fusarium oxysporum* F.sp.*phaseoli* growing on potato dextrose agar.

4.3 Growth inhibition of *F. oxysporum* by *Trichoderma* spp in vitro

All isolates of *Trichoderma* spp. that were tested inhibited growth of *F. oxysporum* with *T. polysporum* giving the highest inhibition (63.4 %) while *T. viride* the lowest at 47.73 % (Table 5). The process of inhibition was competitive among the species and also parasitic (Plate 3; Plate 5) as shown by a clear zone between dual culture of *T. harzianum*

and *F. oxysporum* (Plate 3 b) and dual culture between *T. viride* and *F. oxysporum* (Plate 3 c). Under a microscope micrograph growth of *F. oxysporum* was visibly inhibited by *Trichoderma* spp. (Plate 4). *Trichoderma* spp. caused a highly significant ($P<0.01$) effect on the growth of *F. oxysporum* F. sp. *phaseoli* (Table 4).

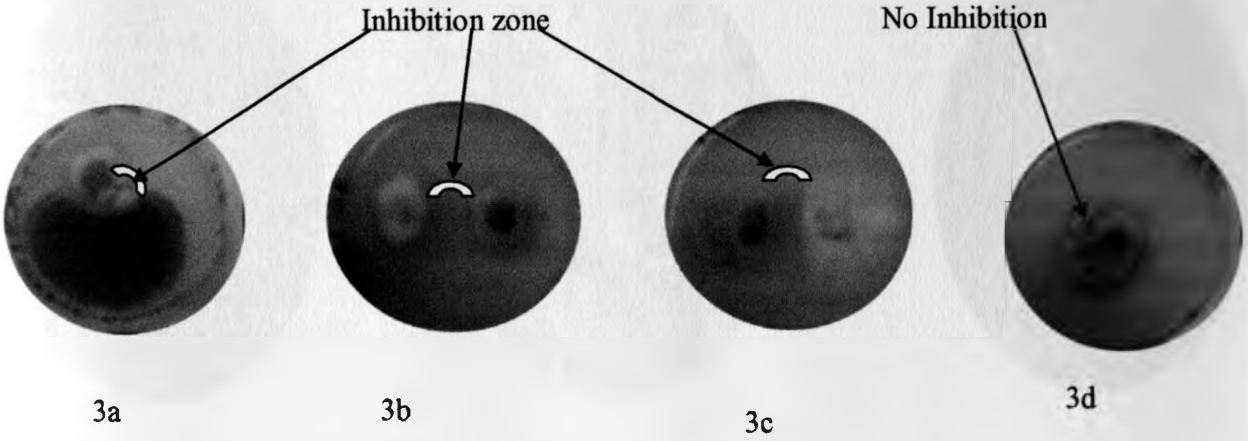

Plate 3: Growth inhibition of *F. oxysporum by Trichoderma* spp. on seven days old cultures.

(a) *F. oxysporum* by *T polysprum* ; (b)*T. harzianum* and *Fusarium oxysporum*; (c) *T.viride* and *F.oxysporium* ; (d) *F. oxysporium* as a control.

4.3.1 Light microscopic image of interaction between *Trichoderma* spp. and *Fusarium oxysporum*

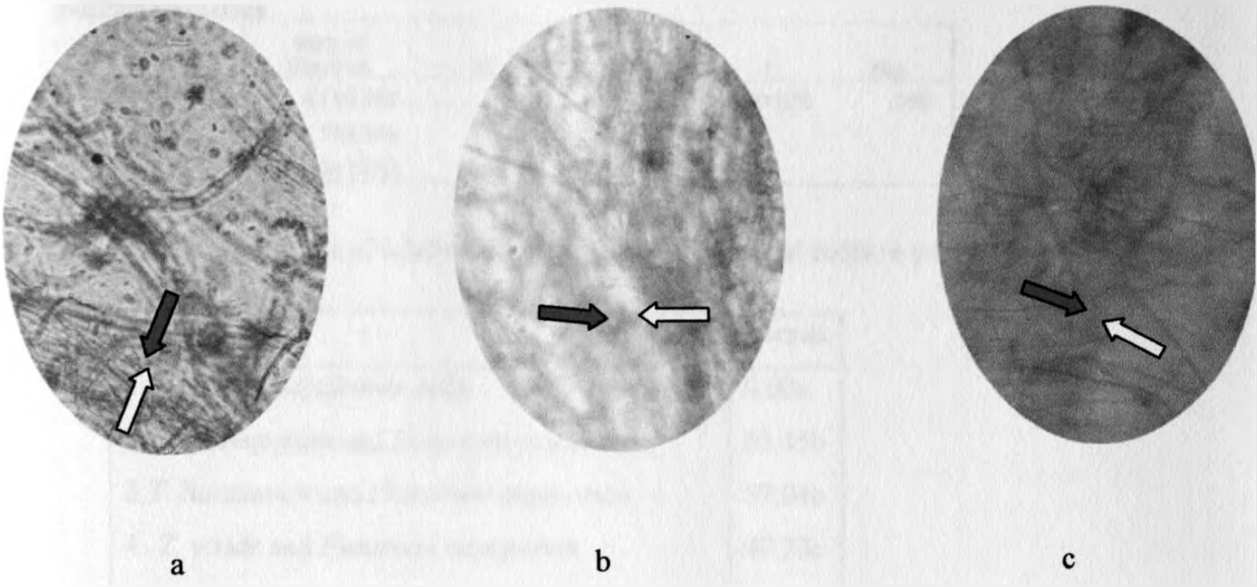

Plate 4: The light microscopic image of interaction between *Trichoderma* spp. and *Fusarium oxysporum.*

(a) *F. oxysporum* by *T polysprum*; (b) *T.harzianum* and *Fusarium oxysporum*; (c) *Trichoderma viride* and *F.oxysporium*

The red arrow indicates the inhibition zone by *Trichoderma* spp. Preventing *Fusarium* spp. (white arrow) from further growing.

Table 4: Inhibition of growing *Fusarium oxysporum* F. sp. phaseoli by *Trichoderma* spp.

ANOVA

Inhibition percentage

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4716.962	3	1572.321	100.675	.000
Within Groups	124.942	8	15.618		
Total	4841.903	11			

Table 5: Comparisons of inhibition means in various dual culture treatments

Treatments	Means
1.*Fusarium oxysporum* only	0.00a
2. *T. polysporum* and *Fusarium oxysporum*	63.45b
3.*T. harzianum* and *Fusarium oxysporum*	57.04b
4. *T. viride* and *Fusarium oxysporum*	47.73c

Means (Duncan's mean) separation with the same letter are not significantly different at ($P<0.05$)

Table 6: Effects of *Trichoderma* spp. and manure rate on growth of common beans

Treatments	Plant growth parameters				
	Plant height(cm)	Root length(cm)	Fresh weight(g)	Dry weight(g)	Leaf area index
Control	10.9d	3.5d	0.9c	0.2c	0.1b
T. polysporum	29.8cb	13.3bc	3.1b	0.5ab	0.6a
T. harzianum	37.7abc	18.2abc	4.0b	0.9abc	0.3a
T. viride	25.3c	16.3bc	3.0b	0.8abc	0.5a
T. polysporum x*T. Harzianum*	33.6abc	13.7bc	4.0b	1.3abc	0.4a
T. polysporum x *T. viride*	24.1c	9.0bc	3.4b	1.0ab	0.3a
T. viride x*T. harzianum*	41.8ab	21.3dc	5.0b	1.6ab	0.5a
T.polysporum x *T. Harzianum* x *T.viride*	45.8ab	27.0a	6.6a	1.1ab	0.3a
Source of variance					
Trichoderma spp.*(P0.05)*	0.025*	0.003**	0.010*	0.051*	0.2461

Means with same letters (within a column) are not significantly different at (* P<0.05) and ** highly significant at (P<0.01).

Trichoderma harzianum had the highest plant height (37.7cm) and longest root length (18.2 cm) compared to other individual *Trichoderma* spp.(*T. viride* and *T polyporum*) plant height and root length under *T. polysporum* was 29.8 cm and 13.3 respectively while under *T. viride* plant height and root length was 25.3 cm and 9.0 cm respectively (Table 6). The combination of *T. polysporum*, *T. harzianum* and *T. viride* had the highest

effect on plant height (45.8 cm), root length (27 cm), fresh weight (6.6 cm), dry weight (1.1 cm) but was lower in leaf area index (0.3). The combination between *T. viride* and *T. polysporum* had the least effect on plant height (24.1 cm), root length (9 cm) but with fresh weight (3.4 cm), dry weight(1.0 cm) and leaf area (0.3) had no significant ($P<0.001$) difference among the other *Trichoderma* spp. combination (Table 6).

Table 7: Effects of Manure rates on growth of common beans

	Plant growth parameters				
Treatments	Plant height(cm)	Root length(cm)	Fresh weight(g)	Dry weight(g)	Leaf area index
Control	6.9c	3.2d	1.0c	0.2c	0.1c
Manure rate(38g nc)	1.0c	3.5d	0.9c	0.2c	0.1c
Manure rate(38g)	63.9a	29.3a	6.5a	1.8a	0.7a
Manure rate(28g)	37.1b	17.5bc	5.1ab	1.3ab	0.6ab
Manure rate(18g)	34.2b	21.3b	4.6b	1.0b	0.5b
Manure rate (9g)	24.7b	13.4c	3.5b	0.9b	0.4b
Source of variance					
Manure rates.(P<0.05)	0.000*	0.000**	0.000**	0.000**	0.000**

Means with same letters are not significantly different at (* P<0.05) and ** highly significant at (P<0.01).38gnc= manure at higher rate without *Trichoderma* spp.

The highest rate (38 g) of manure had the highest effect on plant height (63.9 cm), root length (29.3 cm), fresh weight (6.5 g), dry weight (1.8 g) and leaf area index (0.1) while

the controls had the least effect on plant height (6.9 cm), root length (3.2cm), fresh weight (0.1), dry weight (0.2 cm) (Table 7).

Table 8: Effects of *T. polysporum* enhanced manure on the growth common beans

Treatments	Plant growth parameters				
	Plant height(cm)	Root length(cm)	Fresh weight(g)	Dry weight(g)	Leaf area index
T. polysporum x38g(nc)	1	3.5	0.7	0.1	0.1
T. *polysporum* x0g	6.3	3	1.2	0.04	0.03
T. polysporum x38g	54.8	19.7	4.5	2.3	0.8
T. polysporum x28g	42.6	24.7	4.1	0.6	1
T. polysporum x18g	35.7	17	4.2	1.9	0.5
T. polysporum x9g	6.1	2.3	1.2	0.5	0.5
Source of variance					
T. polysporum x manure rates*(P<0.05)*	0.000*	0.002**	0.006**	0.005**	0.136

Means are significantly different at (*P<0.05) or**highly significant at (P<0.01)

The results indicated that *T. polysporum* and manure at the rate of (38 g) had highest effect on growth parameters and least effect on growth parameters were recorded under control and at a lower rate of manure of (9 g) (Table 8). *T. harzianum* and 28 g manure rate had a highest effect on all the growth parameters while *T. harzianum* and 18 g manure rate had the least effect on the growth parameters but much better than the controls. The combination between *T. viride* and 38 g manure rate had the highest effect on growth parameters; plant height (86.8 cm), root length (54 cm), fresh weight (9.6 g), dry weight (3.2 g) and leaf area index (0.8).

Table 9: Effects of *T. harzianum* augmented manure rates on the growth of common beans

Treatments	Plant growth parameters				
	Plant height(cm)	Root length(cm)	Fresh weight(g)	Dry weight(g)	Leaf area index
T. harzianum x38g(nc)	1	3.5	0.9	0.2	0.1
T. *harzianum* x0g	19.3	3	1.3	0.6	0.2
T. harzianum x38g	66	20.1	4.5	2.2	0.9
T. harzianum x28g	76.7	24.1	4.1	1.2	0.3
T. harzianum x18g	5	17	4.2	0.4	0.4
T. harzianum x9g	10	2.5	1.2	0.1	0.1
Source of variance					
T. harzianum x manure rates(P<0.05)	0.000*	0.002**	0.006**	0.005**	0.136

Means are significantly different at (* P<0.05) or** highly significant at (P<0.01).

The *T.harzianum* enhanced manure rates increased growth in beams better than the controls. *Combination* of *T. polysporum* x *T. harzianum* and manure rates showed a steady increase on the growth parameters of common beans. Manure 28 g and *T.harzinum* had the effect on growth parameters; plant height (76.7 cm), root length (24.1 cm), fresh weight (4.1 g), dry weight (1.2 g) but influenced leaf area index (0.3) (Table 9).

Table 10: Effects of *T.viride* enhanced manure rates on growth of common beans

Treatments	Plant growth parameters				
	Plant height(cm)	Root length(cm)	Fresh weight(g)	Dry weight(g)	Leaf area index
T. viride x0g	0.1	3.5	0.9	0.2	0.1
T. *viride* x38g(nc)	6.7	4.3	2.4	0.6	0.2
T. viride x38g	86.8	54.7	9.6	3.2	0.8
T. viride x28g	3.3	2.8	1.1	0.07	0.3
T. viride x18g	28.2	16.3	2.5	0.7	0.6
T. viride x9g	3.8	3.2	1.6	0.4	0.4
Source of variance					
T.viride x *manure rates*(P<0.05)	0.000*	0.002**	0.006**	0.005**	0.136

Means are significantly different at (* P<0.05) or** highly significant at (P<0.01).

T.viride and manure rates have improved crop growth based on the rate of manure except for *T.viride* and manure rate 28 g. *T.viride* and manure rate 28g did not increase growth on plant height (3.3 cm); root length (2.8 cm) but dry weight (1.1), fresh weight (0.07) and leaf area index (0.3) was significantly (P>0.05) different from other treatments combinations (Table 10).

Table 11: Effects of *T. polysporum* x *T.harzianum* enhanced manure rates on growth of common beans

Treatments	Plant growth parameters				
	Plant height(cm)	Root length(cm)	Fresh weight(g)	Dry weight(g)	Leaf area index
T. polysporum x *T. harzianum* x 0g	1	3.5	0.8	0.2	0.1
Manure only 38g	22.4	13.6	3.2	1.1	0.3
T. polysporum x *T. harzianum* x 38g	66	17.7	4.4	1.8	0.6
T. polysporum x *T. harzianum* x 28g	55.8	17.7	8.4	2.2	0.3
T. polysporum x *T. harzianum* x 19g	26.5	16	4.5	1.3	0.6
T. polysporum x *T. harzianum* x 9g	19.9	17	2.7	0.8	0.4
Source of variance					
T. polysporum x *T. harzianum* x *manure rates*(P<0.05)	0.000*	0.002**	0.006**	0.005**	0.136

Means are significantly different at (* P<0.05) or** highly significant at (P<0.01).

There was a highly significant (P<0.001) plant growth increase due to combination of *T. polysporum*, *T.harzianum* and manure rates (Table 11) but leaf area index.

Table 12: Effects of *T.polysporum* x *T.viride* augmented manure rates on growth of common beans

Treatments	Plant growth parameters				
	Plant height(cm)	Root length(cm)	Fresh weight(g)	Dry weight(g)	Leaf area index
T. polysporum x *T. viride* x 38g(nc)	1	3.5	0.8	0.2	0.1
T. polysporum x *T. viride* x 0g	0.3	0.2	0.1	0.04	0.02
T. polysporum x *T. viride* x 38g	51.3	20.3	7.5	2.3	0.5
T. polysporum x *T. viride* x 28g	3	8.7	2.5	0.6	0.2
T. polysporum x *T. viride* x 19g	15.3	0.8	0.5	0.07	0.1
T. polysporum x *T. viride* x 9g	50.9	15	6.5	1.9	0.7
Source of variance					
T. polysporum x T.viride x manure rates(P<0.05)	0.000*	0.002**	0.006**	0.005**	0.136

Means are significantly different at (* P<0.05) or** highly significant at (P<0.01).

The influence of *T. polysporum* x *T.viride x manure* did not follow a definite gowth trend; *T. polysporum* x *T. harzianum* x 38 g and *T.polysporumx T.viride* x 9 g had the highest plant height, root length, dry weight,fresh weight and leaf area index (Table 12).

Table 13: Effects of *T. polysporum* x *T.viride* x *T.harzianum* enhanced manure rates on growth of common beans

Treatments	Plant growth parameters				
	Plant Height (cm)	Root Length (cm)	Fresh Weight (g)	Dry Weight (g)	Leaf area Index
T. polysporum x *T.viride* x *T.harzianum* x 0g	1	3.5	0.8	0.2	0.1
Manure only 38g	13.6	4.5	1.9	0.3	0.1
T. polysporum x *T.viride* x *T.harzianum* x 38g	59.2	36.3	8	1	0.6
T. polysporum x *T.viride* x *T.harzianum* x 28g	64.3	27.3	7	1.1	0.8
T. polysporum x *T.viride* x *T.harzianum* x 19g	69.2	52	11.3	2.5	0.6
T. polysporum x *T.viride* x *T.harzianum* x 9g	22.7	14.7	4.6	0.6	0.3
Source of variance					
T. polysporum x T. harzianumx manure rates (P<0.05)	0.000*	0.002**	0.006**	0.005**	0.136

Means are significantly different at (* P<0.05) or** highly significant at (P<0.01).

The results indicated that the effect of combination of the three species together with manure rates improve plant growth parameters as compared to species and manure alone (Table13).

4.4.1 The effects of *Trichoderma* spp. enhanced manure rates on growth parameters of common beans.

Over 80cm plant height has been recorded under the interaction between 28 g manure rate and *T. viride*. Other interactions had a shorter plant height which dropped to 20 cm in some treatments. *T. polysporum* x *T. harzianum* x *T. viride* and all the manure rates had a steady plant height which ranged from 60 cm at higher rate of manure to 20 cm at a lower rate of manure (Table 13). Root length was longest (60 cm) under interaction between *T. viride* and manure rate 38 g and the shortest (10 cm) under interaction between *T. viride* and manure rate 9 g (Table 12). Combination between *T. polysporum* x *T. harzianum* and manure rates had the least root length but it had a descending pattern with little variation from higher rate to lower rate of manure (Table 11). The highest fresh weight of about 10 g was obtained between *T. polysporum* x *T. viride* x *T. harzianum* x 18 g manure rate and between *T. viride* and manure rate 38 g (Table 13, Table 12). *Trichoderma polysporum* and all manure rates had the least fresh weight which was descending from higher rate to lower rate of manure (Table 8) Results indicate that dry weight was significantly($P<0.01$) influenced by a combination between *T. viride* and 38 g manure rate and the interaction between *T. polysporum* and manure rates (Table 10). The heaviest dry weight of 3.2 g was recorded in the combination between *T. viride* and 38 g manure (Table 10); whereas the lighter dry weight was influenced by the combination between *T. polysporum* and manure rates 28 g (Table 8). Leaf area index results reveal that combination between *T. harzianum* and 38 g manure rate had the highest effect on the growth parameters,leaf area index 0.9 was recorded and then it dropped to its lowest at 0.2 (Table 9). *T.polysporum* x *T. viride* and all the manure rates had the least leaf area index except it went up to 0.7 under interaction between *T. polysporum* x *T.viride* x manure rate 19 g (Table 13). Controls, *Trichoderma* spp. only and manure rate 38 g did not improve plant growth compared to combination ot the three species (Table 6, Table 7).

Table 15: Effects of NPK fertilizer rates on growth of common bean

Treatments	Plant growth parameters				
	Plant height(cm)	Root length(cm)	Fresh weight(g)	Dry weight(g)	Leaf area index
Fertilizer rate 0g	7.5c	2.5d	0.8d	0.1d	0.1c
Fertilizer rate 0.76g(nc)	11.0c	3.9d	1.1d	0.3d	0.1c
Fertilizer rate 0.76g	55.9a	6.9a	4.9b	1.6a	0.6a
Fertilizer rate 0.57g	42.1b	13.1ab	2.5bc	0.7bc	0.5ab
Fertilizer rate 0.38g	39.5b	11.9b	2.9b	0.9b	0.4b
Fertilizer rate 0.19g	31.4b	8.9bc	1.6cd	0.7cd	0.5ab
Source of variance					
Fertilizer rate (P<0.05)	0.000*	0.000**	0.000**	0.000**	0.000**

All means with same letters are not significantly different at (P<0.05) and ** highly significant at (P<0.01).

0.76 g fertilizer rate with *Trichoderma* spp. had a significant effect on growth parameters followed by subsequent rates 0.57 g, 0.38 g and 0.19 g but controls had the least effect on the growth parameters (Table 15).

Table 16: Effects of *T.polysporum* enhanced NPK fertiliser rates on growth of common bean

Treatments	Plant growth parameters				
	Plant height(cm)	Root length(cm)	Fresh weight(g)	Dry weight(g)	Leaf area index
T. polysporum x 0.78g(nc)	11	3.9	1.1	0.3	0.1
T. polysporum x 0g	12.7	4.7	1.7	0.3	0.3
T. polysporum x 0.78g	60.3	24.3	7.8	0.6	0.5
T. polysporum x 0.57g	32	14	3.2	0.7	0.3
T. polysporum x 0.38g	45.3	10	2.8	0.9	0.6
T. polysporum x 0.19g	68.7	13.7	2.9	1.2	0.9
Source of variance					
T. polysporum x Fertiliser rate *(*P<0.05*)*	0.037*	0.558	0.798	0.002**	0.032*

Means are significantly different at (* P<0.05)

A combination between *T. polysporum* x 0.19 g and *T. polysporum* x 0.76 g had a significant effect on growth parameters but root length was not significantly influenced by the treatment. *T. polysporum* alone (control) and fertilizer alone (0.76 g) did not improve plant growth (Table 16).

Table 17: Effects of *T. harzianum* enhanced NPK fertiliser rates on growth of common beans

	Plant growth parameters				
Treatments	Plant height(cm)	Root length(cm)	Fresh weight(g)	Dry weight(g)	Leaf area index
*T. harzianum*x 0.78g(nc)	11	3.9	1.1	0.3	0.1
*T. harzianum*x 0g	1.4	0.5	0.6	0.1	0.2
*T. harzianum*x 0.78g	59.7	18	6.1	1.7	0.7
*T. harzianum*x 0.57g	21.3	6.4	2.1	0.5	0.3
*T. harzianum*x 0.38g	57	16	6.6	1.7	0.6
*T. harzianum*x 0.18g	18.7	7.3	1.7	0.4	0.1
Source of variance					
T. harzianum x Fertiliser rate *(*P<0.05*)*	0.037*	0.558	0.798	0.002**	0.032*

Means are significantly different at (*P<0.05).

The effect of *T. harzianum* x 0.76 g fertiliser had a highest effect on plant growth parameters; plant height (59.7 cm), root length (18 cm), fresh weight (6.1g), dry weight (1.7 g) and leaf area index (0.7). *T. harzianum* x 0.38 g had increased plant growth compared to other rates and controls (Table 17).

Table 18: Effects of *T.viride* augmented NPK fertiliser rates on growth of common beans

Treatments	Plant growth parameters				
	Plant height (cm)	Root length (cm)	Fresh weight(g)	Dry weight(g)	Leaf area index
T. viride x 0.78g(nc)	11	3.9	1.1	0.3	0.1
T. viride x 0g)	20.2	0.7	0.1	0.1	0.1
T. viride x 0.78g	72.3	14	3.8	1.1	0.8
T. viride x 0.57g	42	15	2.1	0.9	0.5
T. viride x 0.38g	66.7	15	3.2	1.1	0.6
T. viride x 0.19g	43.7	12	1.9	0.7	0.4
Source of variance					
T. viride x Fertiliser rate *(*P<0.05*)*	0.037*	0.558	0.798	0.002**	0.032*

Means are significantly different at (* P<0.05).

Some plant parameters were significantly influenced by *T. viride* x 0.76; plant height (72.3 cm), dry weight (1.1 g) and leaf area index (0.8). *T. viride* x 0.38 g also had better influence compared to the control and other treatments (Table 18).

Table 19: Effects of *T.polysporum* x *T. harzianum* enhanced NPK fertiliser rates on growth of common beans

	Plant growth parameters				
Treatments	Plant height(c)	Root length(c)	Fresh weight(g)	Dry weight(g)	Leaf area index
T. polysporum x *T. harzianum* x0.76g(nc)	11	3.9	1.1	0.3	0.1
T. polysporum x *T. harzianum* x 0g	5.3	1.7	0.4	0.1	0.1
T. polysporum x *T. harzianum* x 0.76g	45.7	17.7	2.9	1.2	0.6
T. polysporum x *T. harzianum* x 0.57g	44	16.3	2.1	0.5	0.4
T. polysporum x *T. harzianum* x 0.38g	23.7	9.3	1.4	0.6	0.3
T. polysporum x *T. harzianum* x 0.19g	51.3	13.8	2.9	1.1	0.8
Source of variance *T.polysporum* x*T. harzianum* x Fertiliser rate (P<0.05)	0.037*	0.558	0.798	0.002*	0.032*

Means are not significantly different at (* P<0.05).

T. polysporum x *T. harzianum* x 0.19 g had a greater effect on some growth parameters; plant height (51.3 cm), fresh weight (2.9 g), leaf area index (0.8). *T. polysporum* x *T. harzianum* x 0.76 g and *T. polysporum* x *T. harzianum* x 0.57 g the highest effect on root

length which recorded 17.7 cm and 16.3cm respectively. Both the controls did not improve plant growth (Table 19).

Table 20: Effects of *T.polysporum* x *T.viride* enhanced NPK fertiliser rates on growth of common beans

Treatments	Plant growth parameters				
	Plant height(c)	Root length(cm)	Fresh weight(g)	Dry weight(g)	Leaf area index
T. polysporum x *T. viridex* 0.76g(nc)	11	3.9	1.1	0.3	0.1
T. polysporum x *T. viridex* 0g	7.3	1.6	0.1	0.1	0.01
T. polysporum x *T. viridex* 0.76g	61.7	13.7	0.2	0.4	1.1
T. polysporum x *T. viridex* 0.57g	42.3	15	0.8	0.7	0.7
T. polysporum x *T. viridex* 0.38g	22.3	6	0.8	0.3	0.4
T. polysporum x *T. viridex* 0.19g	2	0.4	1.1	1.1	0.1
Source of variance					
T. polysporum x *T. viride* x Fertiliser rate (P<0.05)	0.037*	0.558	0.798	0.002**	0.032*

Means are not significantly different at (* P<0.05).

Combination between *T. Polysporum*, *T. viride*, and fertilizer rates has significantly improved some plant growth parameters. *T. polysporum* x *T. viride* x 0.76 g recorded 61.7 cm plant height and *T. polysporum* x *T. viridex* 0.19g had the least plant height (2 cm). The control (firtiliser only) had relatively longer root (3.9cm) unlike *T. polysporum* x *T. viridex* 0.19 g which had 0.4 cm for root length (Table 20). The results reveal that

some plant parameters are significantly influenced by *T. polysporum* x *T. viride* x *T.harzianum* x manure rates.

Table 21: Effects of *T.polysporum* x *T.viride* x *T.harzianum* enhanced NPK fertilizer rates on growth of common beans

Treatments	Plant growth parameters				
	Plant height (cm)	Root Length (cm)	Fresh Weight(g)	Dry Weight (g)	Leaf area Index
Control (0.76g)	11	3.9	1.1	0.3	0.1
T. polysporum x *T. viride x T. harzianum*x 0g	23.7	9.6	2.2	0.7	0.3
T. polysporum x *T. viride* x *T. harzianum*x 0.76g	50	15	1.9	1.2	0.5
T. polysporum x *T. viride* x *T. harzianum*x 0.57g	56.3	12	1.8	0.7	0.5
T. polysporum x *T. viride* x *T. harzianum*x 0.38g	50.3	22.3	2.3	0.8	0.6
T. polysporum x *T. viride* x *T. harzianum*x 0.19g	11.3	5.7	0.5	0.2	0.3
Source of variance					
T. polysporum x *T. viride* x Fertiliser rate *(*P<0.05*)*	0.037 *	0.558	0.798	0.002**	0.032*

Means are not significantly different at (* P<0.05)

The results indicate that combination of the *T. polysporum* x *T. viride* x *T. Harzianum*x 0.57 g had the highest influence on plant height (56 cm.). The longest root (22.3 cm) is found in *T. polysporum* x *T. viride* x *T. harzianum*x 0.38 g. *T. polysporum* x *T. viride* x *T. Harzianum* x 0.19 had least plant height (11.3 cm), root length (5.7 cm), fresh weight (0.5 g), dry weight (0.2) (Table 22).

4.5. *Trichoderma* spp. enhanced NPK fertilizer rates on growth parameters of common beans.

The results shows that interaction between NPK fertilizer and *Trichoderma* spp. had recorded different plants heights based on the rate of the fertilizer (Table 14) *T. viride* and NPK had the highest plant height from 72 cm to 42 cm (Table 18). *T. polysporum* x *T. viride* and all the NPK rates had a linear curve descending from higher rate of NPK to a lower rate on its effect of plant height (Table 20). Root length of 24 cm was longest under the influence of *T. polysporum* and 0.76 g NPK fertilizer, the shortest root was recorded under combination between 0.19 g NPK and *T. polysporum* x *T. viride* (Table 16). Combination between *T. polysporum* x *T. viride* and NPK fertilizer 0.76 g had the highest fresh weight (10 g) and interaction between *T. harzianum* x *T. viride* and NPK rates had the smallest fresh weight (Table 20). Dry weight of common beans among the treatments was relatively similar except under the combination between *T. polysporum* x *T. viride* and NPK rate 0.76 g which had 4.0g (Table 21). Leaf area index (1.1) was recorded under interaction between *T. polysporum* x *T. viride* and 0.76 g NPK fertilizers rate (Table 20). The effect of the same interaction dropped steadily towards the lower rate of NPK which had the smallest leaf area index at 0.19 g NPK and *T. polysporum* x *T. viride* (Table 20). The combination between NPK and *Trichoderma* spp. increased plant growth better than controls in both growth parameters measured.

4.6 Results of Molecular characterization of *Trichoderma* spp. by ITS 1 and ITS 4 primers

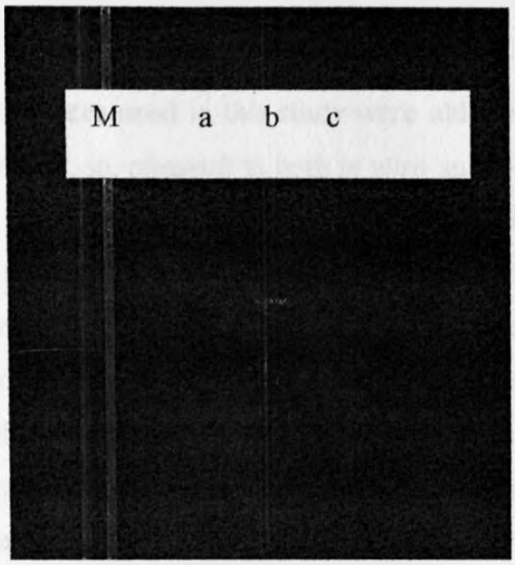

Plate 5: Molecular characterization of *Trichoderma* spp by ITS 1 and ITS 4 primers

The results shows (M) the 100 bp ladder, 600 bp amplicon of ITS1 and ITS 4 for (a) *T.polysporum*, (b)*T. harzianum* and (c)*T.viride* respectively.

CHAPTER FIVE

DISCUSSION

Trichoderma spp. isolates used in this study were able to effectively inhibit growth of *Fusarium oxysporum* F. sp. *phaseoli* in both *in vitro* and *in vivo* trials. *Trichoderma* spp. promoted growth of beans when they were combined with different manure quantities. Many microbes such as *Trichoderma* spp. are well known for producing growth promoting hormones Yaqub and Shahzab, (2008) and biocontrol effect in plants. Harman *et al.*,(2004) proposed that biocontrol agents are effective as seed treatments because they colonise roots and increase root mass, health and therefore increased yield. The findings of in this study are supported by several other reports which indicate that *T. harzianum* can effectively increase plant growth (Chen *et al.*, 1999; Druzhinina *et al.*, 2010).

5.1.0 Isolation *Trichoderma* spp. and *Fusarium* spp.

Three *Trichoderma* species were isolated from soil samples. The *Trichoderma* spp. isolated includes *T. harzianum*, *T.viride* and *T. polysporum*. *F. oxysporum* was also isolated alongside the *Trichoderma* spp. Morphological characteristics of the species were largely used to identify the species by the use of microscopy and also growth characteristics of the species (Plate 1, a, b, c) (Samuels *et al.*, 2004). Further identification of the species was also conducted by use of molecular analysis by the use ITS1 and ITS4 primers, the regions amplified were 600 bp.

5.1.1 Antagonistic effects of *Trichoderma* spp. on *F. oxysporum*

Invitro experiment of *F.oxysporum* and *T.polysporum*, *T.harzianum* and *T.viride* revealed that each of these *Trichoderma* spp. significantly ($P<0.05$) inhibited growth of *F. oxysporum* each to different extent (Table 4). Maximum pathogen growth inhibition occurred with *T. polysporum* (63.4 %) followed by *T. harziunum* (57.04 %) and *T. viride* (47.73 %) respectively (Table 5). These suppression rates were however lower than those reported by Rajeswari and Kannabiran, (2011) who found that *T. harzianum* and *T. viride* were able to antagonize *F. oxys*porum at 89.4 % and 85.7 % respectively. According to Vinale *et al.*, (2008) *Trichoderma* spp. is able to achieve such antagonism on the growth of *Fusarium* spp. through different means such as competition, antibiosis, parasitism and systematic induced resistance, thereby reducing the population density of the *F. oxsyporum.*

5.1.2. *Trichoderma* enhanced manure

The *Trichoderma* spp. enhanced manure at different rates improved the health and growth of common beans planted in *F. oxysporum* infested soil (Table 6 and Appendix 2). There was a significant difference ($P<0.05$) within the difference species on growth parameters of the common beans (Table 6). *T. viride* and T. *polysporum x T. viride* had the least effect on crop growth while a combination of all the three species had the highest effect on growth on the beans. These results show that application of *Trichoderma* spp. in a *Fusarium* infested soil makes the crops survive the *Fusarium* wilt diseases.

A significant difference ($P<0.001$) was also observed on the effect of different *Trichoderma* spp on the growth parameters of beans at various rates of manure application (Table 7). At 38 g of manure without *Trichoderma* spp., plant growth was significantly suppressed. The bean plants could not survive in *Fusarium* infested soil (Appendix 2).

T. polysporum augmented manure at different rates yielded highly significantly ($P<0.001$) results which followed a steady growth trend from the highest rate of manure

(38 g) having the highest growth to the lowest rate (9 g) did not increase growth. The controls, manure alone (38 g) and *T. polysporum* alone had not improved growth (Table 8). These results therefore indicate that manure provided *T. polysporum* with the right substrate which in turn provided nutrients for the crop. *T. polysporum* thereafter was able to grow faster and shield the crop roots (Table 8) against pathogens hence the healthy crop (Chen *et al.*, 1999).

Trichoderma harzianum applied to the soil without manure did not significantly improve crop growth as compared to *T. harzianum* and 38 g of manure which had the greatest improvement among the *T.harzianum* combinations (Table 9). The results in the combination between *T. harzianum* and manure did not follow a definite trend like *T. polysporum*. The highest growth was recorded at the second highest rate of manure (28 g) while the lowest was at 18 g per pot but they all were better than the control (1.0 cm) (Table 9).

T. viride also improved crop growth based on its combination with manure rates and also at a higher rate of manure and when applied alone (Table10). *T. viride* had a sporadic effect to the plant growth parameters; the second largest rate of manure (28 g) had one of the least growth (3.7 cm of plant height) (Table 10). This form of results may be attributed to other pests such as snails which fed on seedling leaves.

Combinations of the species increased their effects on the growth of common beans. The combination of manure and *T. polysporum* x *T. harzianum* had shown a highly significant ($P<0.001$) improvement of crop growth from higher manure rate and very poor growth at zero rate. *T. polysporum* x *T. harzianum* and manure rates also improved crop growth well above 60 cm for plant height compared to the control and 9 g manure rate which had 1.0 and 19.9 respectively (Table 11). The two species were able to synergize and quickly grow fast and colonize the whole rhizhosphre. A combination of *T. polysporum* x *T. viride* species also did not improve growth on their own but did well when combined with manure, they improved plant height and root mass (Table 12). There was a significant difference at ($P<0.05$). The results did not follow a definite trend; at lower rate

of manure the combination had a high effect compared to 28 g and 18 g which had the least growth. The crops might have been affected by other environmental adversities such as pests like slugs which were really feeding on the leaves. The three species combined together with the manure rates had a steady growth rate from higher rate of manure to lower rate of manure. Our findings shows that these three species (*T. polysporium, T. viride and T. harzianum*) can be used together and perform fairly well unlike when they are on their own. The results as alluded above are not new. Species such as *T. harzianum* which has been reported to promote plant growth (Ramezani, 2008). In this study the *Trichoderma* spp. *T. viride*, *T. harzianum* and *T. polysporum* improved crop growth at different levels but *T. harzianum* was more effective than others. (Mohammed *et al.*, 2008) reported that *T.viride* effectively increased plant growth of tomato plant and successfully protected the crop against *Rhizoctonia solani.* Our results concur with those of (Mohammed *et al.*, 2008), even though they worked on tomato against *Rhizoctonia solani. Trichoderma viride* suppressed Rhizoctonia disease and improve crop growth as it did against *Fusarium* disease in our trials.

5.1.3 *Trichoderma* augmented NPK fertilizer rates.

Trichoderma spp. did not fully influence the growth of bean crops under the different rates of fertilizer (Table 14). *Trichoderma viride* managed to influence growth more than other species though the difference is minimal (Table 14). The combination of *Trichoderma* spp. did not increase plant growth. Significant growth was recorded under plant height and fresh weight (Table 14). Root length was not significantly affected by the presence of *Trichoderma* spp. and their combination, which echoed the results of Gutpa, (2010) who reported that fertilizer and *Trichoderma* spp. did not interact well. Fertilizer rate had significantly influenced bean growth ($P<0.00$, Table 15). Plant height, leaf area index and dry weight plant growth parameters were significantly ($P<0.05$) influenced by interaction between *T. polysporum* and fertilizer rates (Table 16). The highest growth (Table 16) was recorded under lower rate of fertilizer and the least growth was noted in the fertilizer only without *T. polysporum* treatments. Just like other combinations of fertilizer rate and *Trichoderma* species, *T. polysporum* was not able to

improve root length (13.3 cm).The results in (Table 17) were not stable; there is no conclusive pattern which can be followed on how the combination between *T. harzianum* and fertilizer influenced crop growth. Root length and fresh weight were not significantly ($P>0.05$) influenced by interaction between *T. viride* and fertilizer rate (Table 18), but *T. viride* managed to interact better than any of the tested species, a small trend can be followed (Table 18). The interaction between *T. polysporum*, *T. harzianium* and fertilizer rate had a significant effect on plant height, dry weight and leaf area index but not root length and dry weight (Table 19). The results also show the highest growth was recorded under low rate of fertilizer (Table 19) and therefore may explains why fertilizer cannot interact with the *Trichoderm* spp. The combination of *T. polysporum*, *T.viride* and the fertilizer seems to refute that assumption, the high rates of fertilizers did well as compared to the lowest rate (Table 20) and the same results are also shown in the combination of both the species and fertilizer rate (Table 21). Combination of all the species seems to work well more than the either of the species, which therefore the species tends to do better in company each other. The results of this study therefore shows that fertilizer do not do very well in promoting plant growth, more especially root length. Similar results were also reported by Gupta (2010) that *Trichoderma* spp. has biocontrol properties and improves plant growth. The inorganic fertilizer is not able to provide proper substrate for the *Trichoderma* spp. (Vinale *et al.*, 2009). *Trichoderma* spp. can only be applied only if the inorganic fertilizers are at lower rate or 15 days after planting (Table 16 and 19) (Gupta, 2010).

5.2 Conclusion:

The results reveal that the combination of the three species with manure can significantly reduce the use of manure per plot by more than 25 %. To improve the efficiency of manure it is therefore important to blend it with *Trichoderma* spp. based fertilizers. The isolated species can be formulated into biofertilisers and mixed with manure to supplement soil nutrients. The *Trichoderma* based fertilizer trials effectively controlled *Fusarium* disease.

5.3 Recommendations:

Plants with available nutrients tend to survive diseases attack unlike the starved ones. *Trichoderma* enhanced manure at the recommended rate of manure and *Trichoderma* enhanced manure at 25 % reduced manure rate improved growth of plant. *Trichoderma* spp do not have any significant plant growth when combined with chemical fertilizers. Therefore *Trichoderma* spp. and fertilizers should not be mixed during planting. *Trichoderma* spp. can be introduced later after planting when the inorganic fertilizers have been utilized by the plant. Future study should focus on the uptake of fertilizer and manure by the plant. This analysis will give guidance on how much fertilizer should be supplied in subsequent plantings. Routine soil analysis before planting is important to determine the levels of *Trichoderma* spp. in the soil and equate it to the nutrient requirements. A mixture of fertilizer and biofertilisers is not recommended because it does not improve plant growth.

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APPENDICES

Appendix 1: soil and manure (used for green house experiments) analysis results

Lab No	Sample Description	pH		%		Cmol/kg			ppm	
		H_2O	0.01M $CaCl_2$	C	N	K	Na	CEC	ZN	P
608	Manure	8.25	7.98	58.01	1.64	41.0	4.25	24.0	Trace	900
609	Soil	7.45	6.64	1.01	0.17	0.4	0.25	18.4	Trace	4.00

Appendix 2; Photographic picture of beans plants during growing and harvesting. picture (a (i) shows the manure experiment and *Trichoderma* spp. in a *Fusarium oxysporum* infected soil; a(ii) manure and *Trichoderma* spp.; a(iii). manure only without *Trichoderma* spp.; a(iv) *Trichoderma* spp. only without manure and a(v).growth of roots of beans with manure and *Trichoderma* spp. b(i) shows the fertilizer experiment and *Trichoderma* spp. in a *Fusarium oxysporum* infected soil; b(ii)growth of beans with fertilizer and *Trichoderma* spp.; b(iii). Growth of beans with fertilizer only without *Trichoderma* spp.; b (iv).growth of roots of beans with fertilizer and *Trichoderma* spp. (c) shows the effect a control without the combination of fertilizers and *Trichoderma* spp.(controls).

Appendix 3: Procedure for growth media preparation

PDA: Potato dextrose agar

Add 200 g scrubbed and diced potatoes to 1 liter water and boil for 1 h. Let it pass through a fine sieve, add 15 g agar and 20 g glucose (= dextrose) and boil until dissolved. pH 5.6 ± 0.1. Also commercially available

Preparation Potato dextrose broth (PDB)

Potato infusion from 200g 4g

Dextrose 20g

Distilled water 1000ml

Distilled water 1000ml

SNA: Spezeieller naurtoffarmer agar

KH_2PO_4 1 g

KNO_3 1 g

$MgSO_4.7H_2O$ 0.5 g

KCl 0.5 g

Glucose 0.2 g

Sucrose 0.2 g

Agar 20 g

Distilled water 1000 ml

NOTE: Pieces of sterile filter paper may be placed on the agar. Recommended for the cultivation of *Fusarium*, but also for poorly sporulating Deuteromycetes

Prepartion of Minimal glucose Agar (MGA)

Glucose-6-phosphate; 1.0M- 2ml

$MgCl_2$/KCL; 0.4M $MgCl_2$, 1.65M KCl- 10ml

Sodium phosphate buffer; 0.2M, pH7.4- 100ml

Preparation of CMD

Corn Meal Infusion from (Solids) 2.0 g

Agar 15.0 g

Distilled water 1000ml