THE EFFECT OF VESICULAR – ARBUSCULAR MYCORRHIZA ON GROWTH, YIELD AND INCIDENCE OF DISEASES ON TOMATO (*Lycopersicon esculentum* Mill.) IN ZAMBIA

BY

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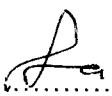
DECLARATION

I, Linen Mulala Masuwa hereby declare that this dissertation represents my own work and that it has not been previously submitted for a degree at this or any other university.



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APPROVAL

THIS DISSERTATION BY LINEN MULALA MASIWA **IS APPROVED AS FULFILLING THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF** PLANT PATHOLOGY **BY UNIVERSITY OF ZAMBIA.**

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DEDICATION

To my dear husband Harry for his love, patience and endurance throughout the course of this study.

To my children Mweenda, Komana and Chabota for their patience when I was away from them while they needed my love and care.

To my father and mother for bringing me forth and educating me.

God bless you all!

ABSTRACT

Mycorrhizae are obligate symbionts which enhance plant growth and protect plants from certain diseases. The mycorrhizal flora of three soils collected from a virgin land at the University of Zambia School of Agriculture Experimental site, Airport and the State lodge consisted of *Glomus etunicatum, Glomus fasciculatum* and *Acaulospora scrobiculata.* This composite mycorrhizal flora was used to determine the effect of vesicular-arbuscular mycorrhiza (VAM) on the growth, yield and incidence of *Septoria* leaf spot, caused by *Septoria lycopersici,* and *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* on tomato (*Lycopersicon esculentum* Mill.). The experiment was carried out during the 2000/2001 growing season in the greenhouse at the experimental field of the School of Agriculture, at the University of Zambia and in two field experimental sites, one at the School of Agriculture Field Station and the other at Mount Makulu Central Research Station, Chilanga.

The VAM flora was multiplied for a period of 8 weeks by planting surface disinfected maize seeds of the variety MMV 600 in heat sterilized soil. This VAM soil inoculum was mixed in proportions of 3 VAM soil inoculum to 1 sterilized soil (high level), 1 VAM soil inoculum to 1 sterilized soil (medium level) and 1 VAM soil inoculum to 3 sterilized soil (low level). Surface disinfected seeds of the Floradade tomato were planted in these soil mixtures in plastic pots and in seedling beds later to be transplanted to the field for field experiments. A Randomized Complete Block Design (RCBD) with 3 treatments and 1 control was used. Tomato plants were periodically examined to measure plant height, stem diameter, number of leaves, number of days

taken to flowering, number of aborted flowers, dry shoot and root weight and tomato plant yield.

Studies on disease incidence of *Septoria* leaf spot involved spraying 2 ml conidial suspension of *Septoria lycopersici* on each plant at the flowering stage. The 2 ml conidial suspension of *Fusarium oxysporum* f. sp. l*ycopersici* was sprayed at the shoot-root interface when plants were 90 days old from planting. The disease incidence for *Septoria* leaf spot was studied by monitoring disease progress at one week interval for one month and *Fusarium* wilt by weighing the fresh root weight at the end of the experiment.

The analysis of variance (ANOVA) of the measured parameters indicated that VAM enhanced the growth and development of tomato by 53%. The plant yield was increased by 38%. To achieve this tomato crops should be inoculated with suitable VAM fungi before transplanting them to the field. High mycorrhizal levels (3 VAM soil inoculum to 1 sterilized soil) resulted in reduced plant growth during the early stages of fungal establishment. The results showed that 1 part of inoculum to 1 part of sterilized soil (medium mycorrhizal level) was better than high levels.

The results also showed that there was 8% reduction of disease incidence of *Septoria* leaf spot and 10% reduction of *Fusarium* wilt in tomato treated with VAM inoculum. However, the use of high levels of VAM inoculum is not advisable for the control of *Septoria* leaf spot since the fungi deprive the plant of the required photosynthates.

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LIST OF ABBREVIATIONS

ANOVA.....Analysis of variance

CV.....Coefficient of variation

DAP.....Days after planting

d.f.....Degree of freedom

E.....Exponential

FAO.....Food and Agricultural Organization

Fpr.....F probability

LSD.....Least significant difference

M.....Mycorrhiza

m.s.....means of squares

P.....Phosphorus

PDA.....Potato Dextrose Agar

PDI.....Percent Disease Incidence

ppm.....parts per million

RCBD. .....Randomized Complete Block Design

S.....Sterilized soil

s.s.....Sum of squares

TERI.....Tata Energy and Research Institute

UAEF.....University of Zambia Agricultural Experimental field

µl .....Micro litre

VAM. .....Vesicular-arbuscular mycorrhiza

v.r.....Variance ratio

CHAPTER ONE

1.0 INTRODUCTION

Mycorrhiza is the 'fungus root' association of fungus and plant root. They can either be endomycorrhizae classified as *Zygomycetes* or ectomycorrhizae classified as *Basidiomycetes* (Menge, 1981; Sieverding, 1991). Mycorrhizae exhibit extremely broad host ranges. The infective propagules of endomycorrhizae are spores, sporangia and vesicles of previously infected root residues in the soil while ectomycorrhizae produce spores above the ground which are wind disseminated (Stan, 1981). A density of 200-500 propagules per kg of soil is sufficient to promote good plant growth (Gianinazzi *et al.*, 1990) and the best form of inoculum for micropropagation technique is either spores or mycorrhizal infested roots. Vesicular-arbuscular mycorrhizal (VAM) fungi, such as the species of *Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora* form endomycorrhizae (Alexopoulos *et al.*, 1996; Schenck and Perez, 1990; Sieverding, 1991) and are characterized by the production of both vesicles and arbuscules. The majority of VAM fungi belong to the genus *Glomus* (Morton, 1988; Gerdemann and Trappe, 1975).

The colonization of roots by infective VAM fungi increase plant growth and resistance to root pathogens due to morphological changes in the root and root tissues (Dodd and Thomson, 1992; Agrios, 1997; Linderman, 1994). Studies of the kinetics of phosphorus (P) absorbing sites by mycorrhizal and non-mycorrhizal tomato roots suggest that mycorrhizal

roots have more P absorbing sites and these have greater affinity for P than those on control roots (Cress *et al.*, 1979). VAM mycelium grows far beyond the rhizosphere zone of the plant root where diffusion rate of phosphorus is extremely low. According to Powell (1979) the distance that VAM fungi can spread through soil is approximately 0.6 to 3.2 cm per year.

In recent investigations by Dodd and Thomson (1992), additional benefits to the plant following colonization of roots by infective VAM have been demonstrated and include:

i Increased tolerance to water stress

ii Increased resistance to root pathogens such as *Phytophthora, Pythium, Fusarium* and *Meloidogyne.*

iii Increased tolerance to soil pollutants

iv Increased seedling establishment after out planting

v Earlier flowering

vi Increased trace element availability

Tomato (*Lycopersicon esculentum* Mill.) is one of the most common and important vegetables in Zambia. It is eaten raw and its nutritional value is high and is a good source of vitamin C. It is also used for flavouring soups and salads (Rice *et al.*, 1987). For tomatoes to grow well, they need fertile soils with a good moisture retaining capacity and a relatively high level of organic material (Rice *et al.,* 1987). Night temperatures of 18 – 20 °C are considered ideal for most cultivars. High temperatures combined with low relative humidity can seriously affect fruit setting and excessive rainfall increases the spread of foliar diseases

such as early blight and leaf spot. Since VAM are generally present in the roots of host plants, research on mycorrhizae-disease-incidence-interactions has been largely focused on soil-borne pathogens in relation to mineral uptake and nutrition of individual plants. VAM is an economic microbiological resource for increased tomato production as it increases P uptake in low levels of P and other minor elements in the soil (Gerdemann, 1965; Hayman and Mosse, 1971; Tinker, 1975).

Although there is great potential in the use of mycorrhizae as bioprotectants, research in this field, especially in developing countries, still remains poor (Azcon-Aguilar Berea, 1997). Most studies reported from different parts of the world used sterilized media in pot experiments in the green house. There is no information on the function of the mutualism between VAM and plants in improving plant growth, yield and disease control in non-sterile field soils under the Zambian conditions.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Definition of mycorrhiza

The term mycorrhiza literally means 'fungus - root' and refers to symbiotic associations between host plants and fungi that colonize the cortical tissues of roots during periods of active plant growth (Sylvia *et al.*, 1998). This is a mutual association which benefits both organisms. It is characterized by the movement of plant-produced carbon (photosynthates) to the fungus (Kendrick, 1992) and nutrients acquired by the fungus to the plant. The mycorrhizal associations with plants are also described as a physiologically well-balanced reciprocal parasitism by Hacskaylo (1972).

2.2 Types of mycorrhiza

According to Harley and Smith (1983) mycorrhizae may be either ectomycorrhizae or endomycorrhizae. In the former the fungus forms a network of hyphae around the root and grows into the air spaces between the cells of roots whereas in endomycorrhizae the fungus is inter- and intracellular and forms specific fungal structures. Endomycorrhizae form finely branched haustoria-like structures in plant cells called arbuscules, which represent the most intensive connection between the fungus and the host plant and are involved in nutrient

transfer from fungus to the host plant (Stan, 1981) and vice versa. They also form apical or intercalary large swollen food-storing hyphal swellings called vesicles (Sieverding, 1991). Most vesicular-arbuscular mycorrhizal (VAM) fungi are of the endomycorrhizal type.

2.3 Occurrence and survival

Endomycorrhizae constitute the most common and important association and are widely distributed. VAM fungi exist in soil as thick-walled chlamydospores, or as vegetative propagules in roots that germinate in the rhizosphere. According to Agrios (1997) endomycorrhizae are mostly produced by *Zygomycetes* of the genus *Glomus* (67 species) and *Acaulospora* (22 species). These types of mycorrhizae are found under natural conditions in free association with almost all-tropical and subtropical agronomic crops (Sieverding, 1991). It has been suggested by Law and Lewis (1983) that up to 25,000 VAM-trophic plant species have the potential to form VAM though these plants differ in the rate of root colonization by VAM. Tropical crop plants such as cassava, sweet potato, cowpea, soybean, maize, sorghum, barley, upland rice, sugarcane, tobacco, cotton, cocoa, rubber, tea, oil palms, tropical pasture grasses and legumes are often heavily colonized by VAM fungi while other crops like wheat, beans, coffee and tomato are only colonized to a moderate extent under natural conditions. Hayman (1981) and Sieverding (1991) demonstrated that VAM fungi are found everywhere and occur in tropical, temperate and arctic soils where soil temperatures of 26 °C to 30 °C and soil water content of 40% to 80% commonly occur. VAM fungi are affected by various agricultural and horticultural practices, particularly fertilizer additions, pesticide application and crop rotation (Pinochet *et al.*, 1998; Azcon-Aguilar Berea, 1997;

Kumaran and Azizah, 1995; Helgason *et al.*, 1998; Daniell *et al.*, 1998). Mycelium is normally disrupted in disturbed soils and this reduces the mycorrhizal colonization of the new roots and therefore phosphorus absorption is reduced (Miller *et al.*, 1992). It has been shown that the density of VAM is lowest in fumigated soils, badly eroded soils and soils with degraded vegetation. Degradation of the vegetation negatively affects the VAM propagule density because VAM fungi depend on photosynthetic assimilates from the host plants (Sieverding, 1991).

2.4 Sources of inoculum

VAM fungi are biotrophs and largely depend on photosynthates of the plant for growth and reproduction, (Harley and Smith, 1983). In recent years 5 genera belonging to Glomales's families *Glomaceace, Acaulosporaceae* and *Gigasporaceae* have been successfully cultured *in vitro* and have been maintained over several successive generations by means of sub-culturing (Declerck *et al.*, 1996; Declerck *et al.*, 1998). The infective structures of fungi used include fungal spores, infected roots and soil based VAM inoculum (Sieverding, 1991).

2.4.1 Spores of VAM fungi as inoculum source

The resting spores of VAM are zygospores or chlamydospores (Agrios, 1997). They are the largest fungal spores known and are full of nutrient reserves. Their individual survival potential is high, which perhaps compensates for their relatively small number as compared to many other fungi (Sieverding, 1991). They can survive and retain infectivity for at least

one year in the soil as infected root fragments, clumps of hyphae or as mycelium for the non-sporulating endophytes (Hayman, 1981).

Spores are important sources of inoculum for the establishment of clean cultures of VAM fungi on host plants because small numbers of spores can be easily isolated from soil substrates and distinguished morphologically for the identification of the endophytes. The use of spores constitute a better mode of inoculation as spores can easily be disinfected by chemicals to obtain inocula free of other microorganisms. Spores as an inoculum source have been used in a few field experiments with agronomic crops (Sieverding, 1991). It is also well known that a single spore (or a few spores) can initiate the mycorrhizal symbiosis (Sieverding, 1991). A density of 200 – 500 propagules per kg of soil is sufficient to promote good plant growth and inoculation should be done at the earliest possible moment in order to use minimum amounts of inoculum, that is inoculating at the sowing or out planting stage (Gianinazzi *et al.,* 1990)

2.4.2 VAM infested roots as inoculum source

A simple way to obtain VAM inoculum is by collecting infested roots from field grown VAM-trophic crops. Sieverding (1991) demonstrated that infested roots contain internal fungal mycelium as well as external mycelium and VAM spores. The root part of plants together with its soil are chopped into small pieces before being used as inoculum. It is well known that the infectiveness of the inoculum source is higher in infected roots than that of spores (Ross and Harper, 1970). New root colonization can take place within 1-2 days of

inoculation with infected roots. Small amounts are enough to obtain good growth responses in nurseries and greenhouse experiments (Sieverding, 1991).

Infected roots when stored at 4 °C in water or moist vermiculite can maintain their infection potential for 2 months (Hung and Sylvia, 1987).

2.4.3 Soil – based VAM inoculum

Soil inoculum contains all VAM structures and as an inoculum source it is most frequently used in greenhouse and field experiments. The technique is reliable, highly efficient and simple to get infective material. The soil inoculum with all VAM structures is thoroughly mixed before being applied and the success of producing good soil inoculum depends on the selection of the VAM-trophic plant and the ambient conditions under which a defined VAM fungus is to be reproduced (Sieverding, 1991).

2.5 Influence of VAM on plant growth

VAM fungi are beneficial to plants in improving their nutrition and growth in phosphorous deficient soils (Harley and Smith, 1983). Arbuscular mycorrhizal fungi improve plant growth, increase hormone production as well as fixation of nitrogen (Hayman, 1986). The extensive growth of mycelium assists in increasing the root's ability for better nutrient uptake and absorption by increasing the absorbing surface of the root system. VAM mycelium on the external roots of plants grows far beyond the rhizosphere zone with

extremely slow diffusion rate of phosphorus and increase the soil volume which is exploited for phosphorus uptake and trace elements like copper, zinc, manganese and boron (Lambert *et al.*, 1979). It has been demonstrated that the external hyphae have a capacity to take up to 80% of phosphorus, 25% of nitrogen, 10% of potassium, 25% of zinc and 60% of copper from soil (Marschner and Dell, 1992). Mycorrhizal plants also accumulate potassium, calcium, copper and manganese in the leaves in higher concentration than non-mycorrhizal plants (Ross and Harper, 1970). Wright *et al.*, (1998) have shown that VAM plants attain consistently higher rates of photosynthesis and exhibit a higher specific leaf area compared to non-mycorrhizal plants, a response which maximizes the area available for carbon dioxide assimilation per unit carbon invested. Mycorrhizae have also been shown to increase seed production in many plant species such as soybean, barley, tomato and oats (Stanley *et al.*, 1993 and Koide *et al.*, 1988). Dodd *et al.*, (1983) have shown an increase in flower bud production in pepper plants by mycorrhizal infection. The improvements observed following mycorrhizal infection is believed to be due to the increased phosphorus uptake.

In climatic stress situation, including adverse soil conditions like soil acidity, alkalinity, salinity and high concentrations of toxic elements of Aluminium (Al), Iron (Fe) and Manganese (Mn), plants with VAM are still able to grow at a faster rate (Sieverding, 1991; Rhodes, 1980) and are able to withstand drought conditions. This is due to the development of an extensive and deeper root system used to efficiently extract water (Baylis, 1972). Furthermore, VAM mycelium appears to be more resistant to abiotic stresses than the root itself. The vascular systems of the plants are increased and feeder roots are kept functional for a long time. VAM fungi have also been shown to increase translocation of water and

have high chlorophyll concentrations due to increased cytokinin levels stimulated by VAM infections (Allen *et al.*, 1981). Plants with mycorrhizal roots also survive transplant shock better on the infertile soils of marginal lands and other areas needing re-vegetation (Kendrick, 1992). The net effect of these changes is a healthier plant that can withstand environmental stresses and also tolerate or reduce the effects of plant diseases (Linderman, 1988).

Sieverding (1991) reported that crop production can be improved when certain amounts of organic or inorganic fertilizers are applied and when certain amendments are combined with biological crop production technologies such as the use of VAM fungi. These biological technologies are able to maintain soil productivity and improve plant growth on a sustainable basis and in this way enhance food production.

2.6 Interaction of plant pathogens and VAM

The presence of VAM is often accompanied by morphological and biochemical changes in plant and probably these changes help the plant to resist pathogen infection. According to Linderman (1994), mycorrhizae can be used against many root-borne diseases as a bioprotectant. However, the effect and the mechanisms involved depend on the conditions of the tests, host plant, edaphic conditions and the species of VAM involved. Biological control of plant diseases may be influenced by VAM as well as by some other mechanisms as outlined below (Linderman, 1994).

2.6.1 Morphological changes in root tissues of VAM colonized plants

The effective VAM association results in the lignification of host plant root cell walls and reduces nematode penetration into the plant tissue (Smith, 1987). Dehne and Schoenbeck (1979a and b) have reported that there is increased lignification of tomato and cucumber (*Cucumis satvus* L.) root cells of the endodermis in VAM colonized plants and that such responses account for reduced *Fusarium* wilt caused by *F. oxysporum* f. sp. *lycopersici.* There is also increased concentrations of anti-fungal chitinase in VAM roots (Dehne *et al.*, 1978). The root tissue becomes more resistant to pathogen attack and the induced resistance is strictly limited to the site of mycorrhizal establishment (Dehne, 1982). The feeder roots of the plants also become resistant to infection by certain soil fungi such as *Phytophthora, Phythium* and *Fusarium* (Agrios, 1997).

2.6.2 Changes in the microbial populations of the mycorrhizosphere

Studies have indicated that disease suppression by VAM involves changes in the microbial populations of the mycorrhizosphere. The work of Caron *et al.*, (1985 and 1986a and b) showed a reduction in *Fusarium* populations in the mycorrhizosphere soil of tomato and a corresponding reduction in root rot in VAM plants relative to non-VAM plants, possibly due to increased antagonism to pathogens in the VAM mycorrhizosphere of the VAM plants. The analysis of the antagonist microbial composition by Foster and Nicolson (1981) showed a range of fungi, bacteria, actinomycetes, algae and cyanobacteria in the mycorrhizosphere.

Thus, if potentially effective microbial antagonists are present and are increased by VAM, disease suppression would occur (Linderman, 1992; Meyer and Linderman, 1986).

2.6.3 Reduction of abiotic stress by avoiding nutrient stress

In reviews of biological control of plant diseases by Baker (1986) and Baker (1987), mycorrhizae are thought to contribute to biological control of plant diseases primarily by means of stress reduction by overcoming conditions of nutritional stress, soil drought, poor soil textures and associated disease predisposing effects. Studies of Javid and Irshad (1999) and Manian *et al.*, (1999) have shown that *Glomus fasciculatum* is an effective biocontrol agent of root-knot diseases caused by nematodes, and if applied, can offer the possibility of management of diseases of tomato plants like *Fusarium* wilt and *Alternaria* leaf spot with VAM. In general mycorrhizal plants suffer less damage and disease incidence is either greatly reduced or pathogen development is considerably inhibited. Improved plant nutrition may enhance plant development and, especially under field conditions, increased plant vigor may lead to disease escape or to higher tolerance towards soil-borne pathogens (Dehne, 1982).

VAM plants suffer less from the disease than plants without mycorrhiza. The disease incidence and virulence of pathogen are decreased in plants with VAM than without VAM plants. It should be noted that higher disease incidence in the symbiotic system in general, does not affect genetical resistance to plant pathogens because genetically resistant plants

remain resistant whereby susceptibility may be modified by the symbiosis (Schoenbeck and Dehne, 1981).

Garcia-Garrido and Ocampo (1989) showed that tomato plants with VAM inoculated with VAM and *Pseudomonas syringae* pv. *syringae* were not susceptible to the disease while tomato plants without VAM were highly susceptible.

2.7 OBJECTIVES

The objectives of this study were:

1. To extract, characterise and determine the effect of VAM flora of Zambian soils on the growth and yield of tomato in the greenhouse and the field.
2. To determine the effect of VAM on the incidence of diseases caused by *Septoria lycopersici* and *Fusarium oxysporum* f.sp. *lycopersici* on tomato in greenhouse and field conditions.
3. To determine the effect of VAM inoculation on phosphorus content in tomato tissues.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sites of inoculum source

Soil used to isolate available species of mycorrhizae was collected at the beginning of January 2001 from three randomly selected virgin areas in Lusaka. These sites were, east of the Agricultural experimental field station at the School of Agricultural Sciences (site 1), the International Airport (site 2) and site 3 at the State Lodge (Figure 1). Each experimental site was over 20km apart.

3.2 VAM soil samples

The soils were randomly obtained from ten points from the top 10-20cm of the soil within a 10m x 10m area. The samples from the ten sampling points were later thoroughly mixed. The total amount of soil collected from each site was 100kg from which 10kg was used for VAM extraction and 5kg for soil chemical and physical analysis. The remaining soil was used for greenhouse experiments. The different types of plants found within the 10m x 10m sampling area were collected for identification.

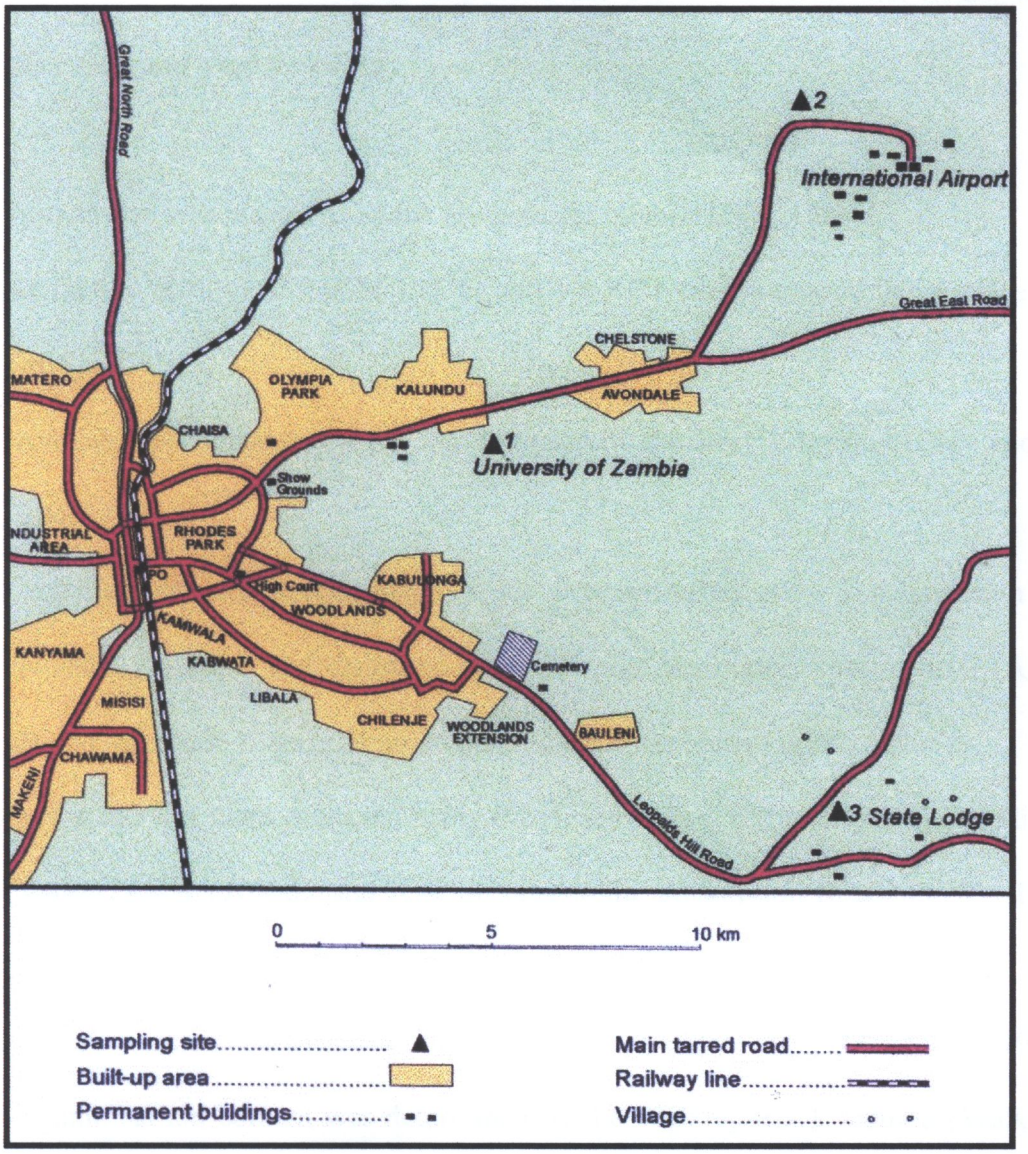


Figure 1. Soil sampling sites for the extraction of vesicular – arbuscular mycorrhiza (VAM)

3.3 Physical and Chemical analysis of soil

The soil samples collected from the three sites and the two experimental fields, were sun dried separately and were tested for:

1. Particle size was measured by the Hydrometer method (Day, 1965).
2. pH (active acidity) in 0.01M $CaCl_2$ and 1N KCl was measured using pH electrode (Michael, 1965).
3. Available phosphorus content was analysed by the Bray 1 method (Bray and Kurtz, 1945)
4. Exchangeable cations (Ca, Mg, and K) were extracted using 1 Ammonium acetate (NH_4OA_c) at pH 7 and measured on an Atomic Absorption Spectrometer (AAS) for Ca and Mg while K was measured on a Flame Photometer (Walter, 1965).
5. Zn, Fe and Mn were extracted using Ethylenediamine Tetraacetic acid Disodium salt (Na_2EDTA) at pH 4.65 and read on an Atomic Absorption Spectrometer (Dirt and Myrna 1984).
6. Total nitrogen content was determined by Macro-Kjeldahl method (Bremner, 1960).
7. Organic carbon content was determined by the Walkley-Black method (Walkley and Black, 1934).

3.4 Phosphorus analysis of tomato plants

The tomato plants collected at the end of the experiment from the greenhouse and the field were separately dried and ground to a powder before 1g of the powder was ashed in an oven at 500°C for 4 hours. The tomato samples replicated three times were then tested for phosphorus levels using official methods of analysis (Dirt and Myrna, 1984; Phiri and Damaseke, 1999).

3.5 Vegetation identification

Plants found within the 10m x 10m area from where soil for VAM propagule extraction were collected, pressed and taken to the University of Zambia Herbarium for identification. Identification of plant species was done using reference books in the Herbarium (Doreen, 1995; Braam and Piet, 1997; Storrs, 1995; Phiri, 1998).

3.6 Isolation of VAM propagules from the soil

The VAM propagules were extracted using the wet sieving and decantation method (Sieverding, 1991; Gerdemann and Nicolson, 1963; Pacioni, 1992; Phillip and Hayman, 1970). Ten kilograms (10 kg) soil was collected from each site and was divided into two equal parts of 5kg each. One 5kg of the soil was put in a bucket containing 10 litres of water and the soil suspension was vigorously stirred with a stirring rod. The coarse sand was allowed to sediment by waiting for about 10 - 20 seconds before passing it through a

1.14mm sieve. The filtrate was shaken and passed through a 0.40mm sieve. The process was repeated to allow for further sedimentation of coarse sand before passing the filtrate through a 0.35mm sieve and then a 0.25mm and finally through a 0.05mm sieve. The VAM propagules (spores/sporangia/sporocarps) were trapped on the 0.05mm sieve. These were washed with distilled water and stored in a refrigerator at 8 °C before examining it microscopically for the identification of VAM fungi.

3.7 VAM propagule density and identification

The collected propagules from each site were put in a beaker in 150 ml of distilled water. The suspension was stirred for 60 seconds using a magnetic stirrer. The 100µl suspension was released in six separate small drops with a micropipette on to a glass slide. A count of spores per droplet was made. The cumulative total of the six droplets was taken. The procedure was repeated 10 times to obtain the VAM propagule density.

During propagule counting, VAM species were also identified using a light Olympus microscope at magnifications of 100x and 400x. Species identification was based on the propagule features and confirmed by using available keys and photographs of VAM from Sieverding (1991) and Morton (1988). Synoptic keys by Trappe (1981) were also used to identify the VAM to genera and species level of zygomycetous mycorrhizal fungi. Species identification was confirmed by Tata Energy and Research Institute, New Delhi, India.

3.8 Production and amplification of soil inoculum

The soil used for inoculum production was collected from the three experimental sites and mixed thoroughly. The soil was sterilized in an oven at 105 °C for 3 hours to eliminate pathogens and exclude indigenous arbuscular endomycorrhizal fungi. It was left to cool and eighteen kilograms (18 kg) of the sterilized soil was transferred to twelve, twenty litre plastic pots in a green house. The maize seeds of the cultivar MMV 600 were surface sterilized in 5% sodium hypochlorite (Jik) for 3 minutes and then rinsed three times in distilled water before planting them in pots containing the sterilized soil.

Maize plants were raised (Figure 2) by planting ten (10) seeds per pot on the 26th January 2001 and a composite sample of the inoculum was prepared by mixing 80 ml of the suspension of the mycorrhizal propagules from each site. Baertschi *et al.*, (1982) found that an unidentified mixed mycorrhizal population was considerably more effective in reducing pathogen development than a pure culture of *G. mosseae*. To ensure successful colonization in the roots of maize seedlings and early establishment of VAM in the roots prior to pathogen invasion, 2 ml of the spore suspension was poured on top of each seed before covering them with soil. The pots were watered at a rate of 1 litre water every second day per pot. The roots of growing maize plants were periodically examined for the presence of mycorrhiza. Roots were taken every week from the soil. These roots cut from the maize plants were cleared and stained following the recommended method by Sieverding (1991) and Phillip and Hayman (1970). The colonization of the maize roots started in the 4th week

Figure 2. Maize plants used for Vesicular – arbuscular amplification in a greenhouse.

after planting. This was followed up to the 8th week when colonization was well advanced.

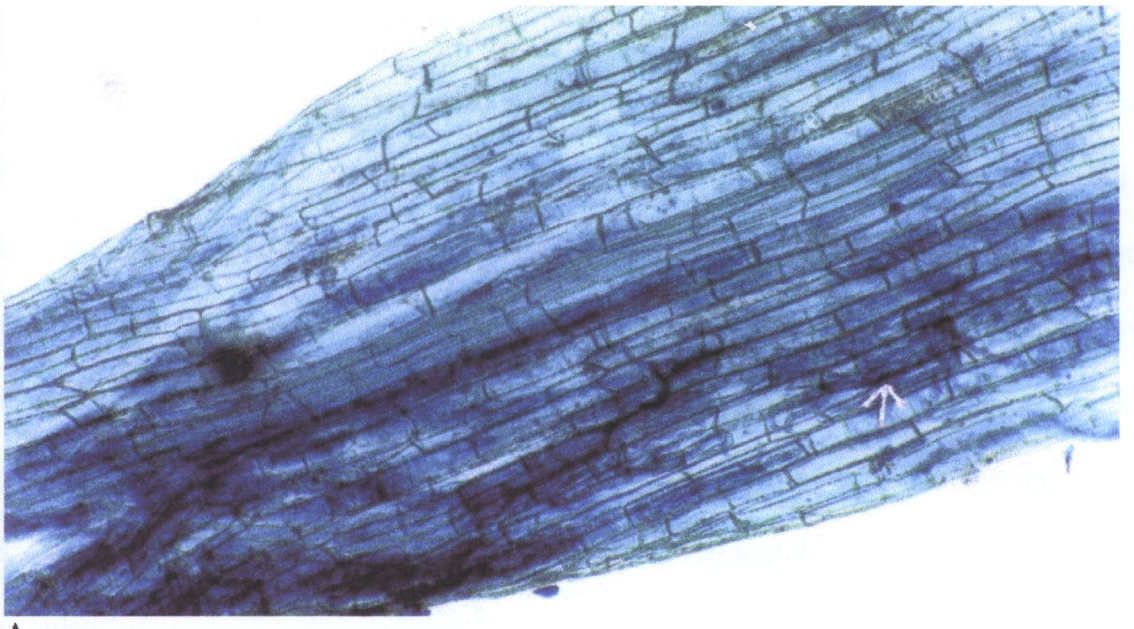
3.9 Determination of the effect of VAM on growth and yield of tomato

The determination of VAM on growth and yield of tomato was carried out in a greenhouse and the field with and without *Fusarium* wilt and *Septoria* leaf spot pathogens of tomato diseases.

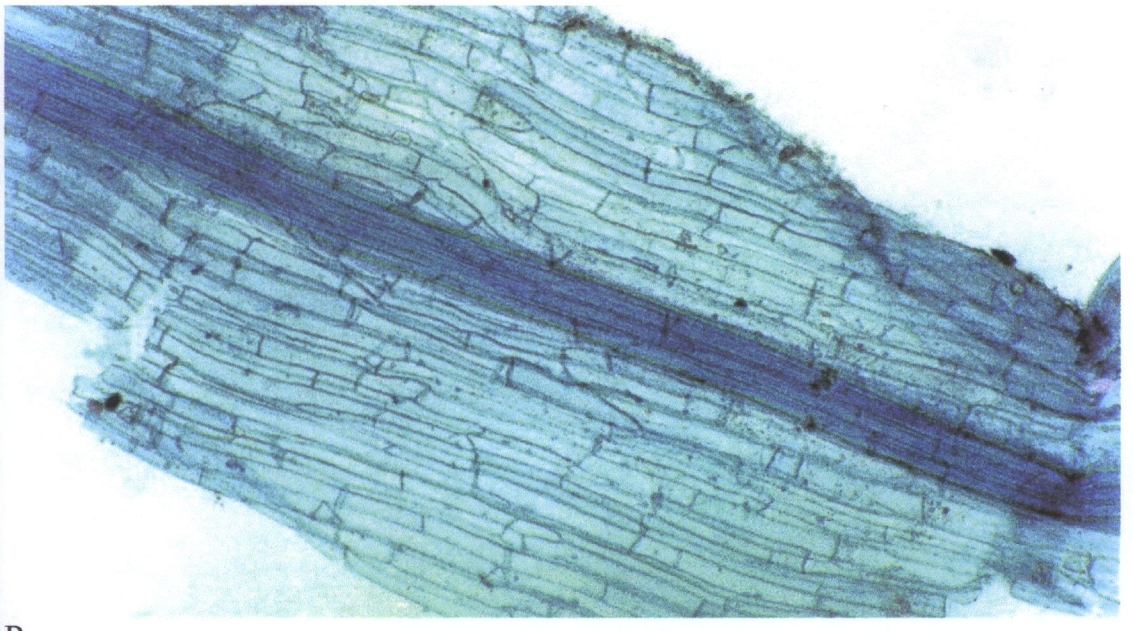
3.9.1 Greenhouse studies:

After mycorrhizal colonization had been confirmed in the 8th week (Figure 3), the shoot part of the plants were removed leaving behind the VAM colonized root system which was chopped up and used as inoculum. The inoculum was mixed with sterilized soil collected from the experimental sites. The experimental design used was a Randomized Complete Block Design (RCBD) with 3 treatments and 1 control, each with three replications. The treatment inoculum was prepared as follows:

Treatment	VAM inoculum + sterilized soil	Ratio
1	3 VAM + 1 soil (high level)	3:1
2	1 VAM + 1 soil (medium level)	1:1
3	1 VAM + 3 soil (low level)	1:3
4	Control (no mycorrhiza)	0



A.



B.

Figure 3. Tomato root tissue colonized by VAM.

A – shows root colonized in areas (arrow) of differentiation and elongation of active "feeder roots".

B – a healthy non-colonized tomato root.

These four treatments were used in the study to determine the effect of VAM on the incidence of *Fusarium* wilt caused by *Fusarium oxysporum* f. sp *lycopersici* and *Septoria* leaf spot caused by *Septoria lycopersici*. A total of 36 nine litre plastic pots were used. For each treatment, a total of 3 pots per block were used, and each treatment was replicated three times. Eight (8) disinfected tomato seeds of cultivar Floradade (from Zamseed) were planted in each pot and later thinned to four (4) plants per pot. The plants were watered with 1 litre of water every second day and daily temperatures of the greenhouse were recorded. Growth and development of tomato plants was observed and the following parameters were recorded:

i. Plant height
ii. Stem diameter
iii. Number of leaves per plant
iv. Number of days to flowering
v. Number of aborted flowers
vi. Root dry weight
vii. Shoot dry weight
viii. Fresh fruit weight

3.9.2 Field studies:

The field experiments were conducted at two places, the University of Zambia, School of Agriculture Field and Mount Makulu, which were over 20km apart. The fields were approximately 35m x 15m in area, each with 3 blocks and 12 plots per block (Appendix 1).

Each plot was 3m x 1.8m in area and had 4 lines of tomato plants of cultivar Floradade (60 cm apart) with 5 plants per line at 30 cm spacing. The space between blocks was 2m. The tomato nursery beds were prepared in the greenhouse by planting surface disinfected tomato seeds in the soil containing VAM inoculum. The seedlings were transplanted to the field plots after VAM root colonization (Figure 3). In the nursery bed, seeds were also sown in sterilized soil for control plots. The mycorrhizal and non-mycorrhizal plants were transplanted to the field plots 42 days after sowing of seeds. All plants were of similar size and in the same development stage. Growth of tomato plants was observed and same plant parameters as in greenhouse were recorded.

The first measurements for plant height, stem diameter and number of leaves per plant were taken 14 days after transplanting. Plant height measurements were taken using a 30 cm ruler and numbers of leaves were counted after every 14 days for a period of 10 weeks. Stem diameter measurements were carried out with a vernier callipers 42 days after transplanting and were recorded every 28 days for 5 weeks. The stem diameter measurements were for the part of the tomato plant where the first leaf developed. The root and shoot dry weights were taken at the end of the experiment.

The fruit yield per treatment was calculated by first harvesting all ripe tomatoes from each plot every after 7 days for 4 weeks. These were later weighed. This was done when the tomato plants were 115 days old.

3.10 Determination of the effect of VAM on the incidence of diseases

Two diseases of tomato were studied in a greenhouse and the field. These included a foliar disease *Septoria* leaf spot caused by *Septoria lycopersici* and a soil borne disease *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *lycopersici.* The isolation of the pathogens was done by using Potato-dextrose agar (PDA) as the culture media (Onkar and James, 1985). The diseased tissues collected from diseased tomato plants were surface disinfected in 5% sodium hypochlorite (Jik) to ensure the removal from the tissues of undesirable organisms. The disinfected pieces were then transferred to petri dishes containing PDA. These were incubated at 25 – 30 °C for a few days whilst observing the fungal colony of *Septoria lycopersici* and *Fusarium oxysporum* f.sp *lycopersici* grow out from infected tissue. The pathogens were observed microscopically and identified using the manual for identification of fungi (Funder, 1961).

3.10.1 *Septoria* leaf spot inoculation

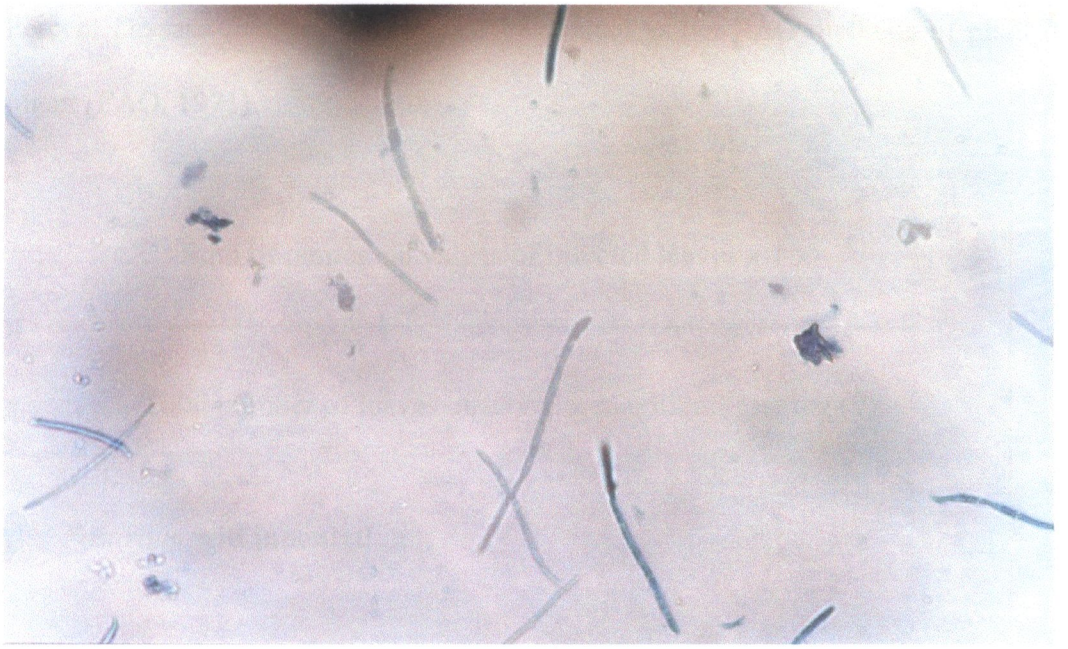
The greenhouse and field grown tomato plants containing VAM were inoculated with *Septoria* leaf spot by spraying 2 ml conidial suspension, containing 3.98 x 10^4 conidia of *Septoria lycopersici* (Figure 4a) on the leaves of each plant. This was done at the flowering stage when plants were 90 days old. The environment was made favourable for disease development (conidia germination and penetration) by keeping high moisture levels in the greenhouse. The plots in the field were flooded with water before spraying plants with conidia. The plants were watered using drip irrigation everyday for three days following

inoculation. Disease inoculation, both in the greenhouse and in the field, was done late in the afternoon when temperatures were relatively cooler. The temperatures were 19.5 °C in the greenhouse and 13.5 °C in the field (Appendices II and III).

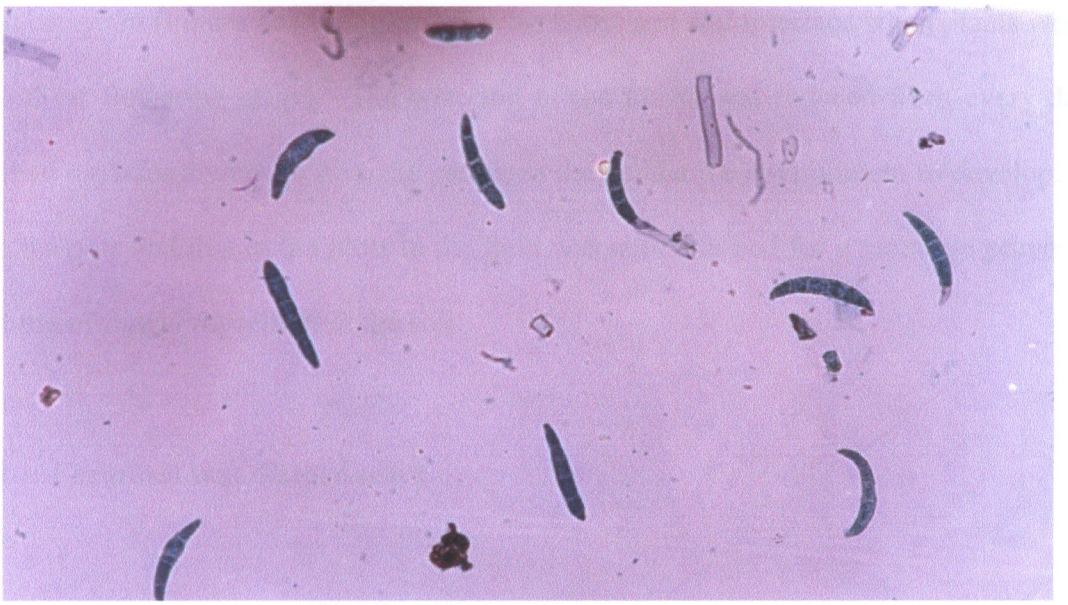
3.10.2 Septoria leaf spot disease score

The disease incidence on the plants was scored using a 0 to 9 scale with 0 representing no disease and 9 representing the highest disease incidence. The number of infected leaves per plant were counted and recorded every 7 days for a period of 5 weeks using the following method of assessment.

No. of leaves infected	Grade
No leaves infected	= 0 grade
1 leaf infected	= 1st grade
2 leaves infected	= 2nd grade
3 leaves infected	= 3rd grade
4 leaves infected	= 4th grade
5 leaves infected	= 5th grade
6 leaves infected	= 6th grade
7 leaves infected	= 7th grade
8 leaves infected	= 8th grade
9 leaves or more infected	= 9th grade



a



b

Figure 4. Diagnostics features of tomato pathogens.

a. conidia of *Septoria lycopersici*, the cause of *Septoria* leaf spot disease of tomato.

b. macroconidia of *Fusarium oxysporum* f. sp. *lycopersici* the cause of *Fusarium* wilt of tomato.

The Percent Disease Incidence (PDI) was calculated as per FAO methods of plant disease assessment (FAO, 1971).

$$PDI = \frac{\text{Sum of numerical values of infected leaves} \times 100}{\text{Total number of leaves observed} \times \text{maximum grading (9)}}$$

3.10.3 *Fusarium* wilt inoculation

The 2 ml conidial suspension containing 4.08×10^4 of macroconidia of *Fusarium oxysporum* f. sp. *lycopersici* (Figure 4b) was sprayed at the shoot and soil interface when plants were 90 days old (at flowering stage). The watering of the plants was reduced from every day to every two days for a week to give the pathogen the conducive environment to develop. The soil in the pots and that in the plots in the field was not disturbed for a month to prevent the disturbing of fungal mycelium in the soil.

3.10.4 *Fusarium* wilt disease score

The disease incidence on the plants was scored using a 0 to 5 scale with 0 representing no disease and 5 representing high disease incidence. The fresh roots of the plants were weighed and the weight was used to score the disease.

Weight of roots	Grade
41 – 50g	= 0 grade
31 – 40g	= 1st grade
21 – 30g	= 2nd grade
11 – 20g	= 3rd grade
1 – 10g	= 4th grade
< 1g	= 5th grade

Therefore,

$$PDI = \frac{\text{Sum of numerical values of fresh root weight x 100}}{\text{Total number of plants measured x maximum grading (5)}}$$

3.10.5 Data analysis

Genstat statistical package was used to analyse data. Data were subjected to analysis of variance (ANOVA) and means were separated using the Least Significance Difference (LSD) test (Gomez and Gomez, 1984; Thomas and Hill, 1978; Wayne 1987).

CHAPTER FOUR

4.0 RESULTS

4.1 Soil Analysis for physical and chemical properties

The soil collected from the University of Zambia Agriculture Experimental Field (UAEF), State Lodge and Mount Makulu had a sandy-clay-loam texture while the soil from the Airport had a sandy loam texture. The soils from the three sites from where VAM inoculum was extracted (Figure 1) were acidic but those from the two experimental fields (where tomato seedlings were transplanted) were alkaline. All the soil samples had low levels of nitrogen, carbon and exchangeable cations. The levels of phosphorus and iron were relatively high in the soils from Agriculture Experimental Field while Mount Makulu soils had slightly high levels of calcium and manganese (Table 1a and b).

Table 1. (a) Physical and chemical properties of soil from sampling sites.

Site Sample	Soil Texture	pH	N %	P ppm	K %	Ca %	Mg %	Zn ppm	Fe ppm	Mn ppm	C %
UAEF	Sandy-clay-loam	5.5	.04	8	0.29	2.5	1.1	2.0	50	105	0.55
Airport	Sandy-loam	4.5	.01	4	0.10	0.4	0.2	4.0	37	21	0.11
State lodge	Sandy-clay-loam	4.7	.03	4	0.22	1.1	0.8	5.0	23	38	0.40

Table1. (b) Physical and chemical properties of soil from experimental fields

Field sample	Soil Texture	pH	N %	P ppm	K %	Ca %	Mg %	Zn ppm	Fe ppm	Mn ppm	C %
UAEF	Sandy-clay-loam	7.4	.05	73	0.19	6.7	1.4	5.0	103	1.41	0.65
Mount Makulu	Sandy-clay-loam	7.6	.05	18	0.40	30.3	2.3	4.0	66	172	0.74

4.2 Vegetation composition of sampling area

The vegetation of the sampling sites mainly composed of herbaceous plants of the families *Poaceae, Asteraceae* and *Cyperaceae.* There were also woody plants of the families *Fabaceae, Combretaceae, Ochnaceae, Euphorbiaceae, Bignoniaceae* and *Burseraceae.* The common plants collected from the three sites were *Brachystegia boehmii*, *Commiphora mollis*, *Indigofera hilaris*, *Combretum psidioides*, *Ochna leptoclada* and *Bidens pilosa.*

4.3 VAM propagule composition and density

The number of mycorrhizal spores extracted from east of the Agricultural experimental field station at the School of Agricultural Sciences (site 1), International airport (site 2) and State lodge (site 3) per 100g of soil were 8, 099, 6, 116 and 4, 518 spores respectively (Table 2). The identification revealed that there were two species found in site 1 and site 2 and these were *Glomus etunicatum* and *G. fasciculatum.* In addition to the two species of *Glomus* identified in site 1 and 2, site 3 also had *Acaulospora scrobiculata.* The predominant species was *G. etunicatum* which accounted for about 88.92%, 93.96% and 83.47% of the total spores observed in sites 1, 2 and 3 respectively. *G. fasciculatum* accounted for about 11.08%, 6.04% and 13.71% respectively. The proportion of *A. scrobiculata* found in the soils of site 3 was marginal and accounted for only 2.82% of the total spores observed. *Glomus* species like *G. fasciculatum* are prevalent in acidic soils while *A. scrobiculata* is prevalent in alkaline soils (Hayman, 1981). Sites 1, 2 and 3 had acidic soils (Table 1a).

Table 2. VAM propagule density and its species composition in the soils from the three experimental sites.

Site	Species	Composition per 100g soil(%)	Spore type	Colour	Shape	Size	Composite Wall Thick-ness
UNZA	*Glomus etunicatum*	88.92	Chlamydospore	Yellow to brown	Globose	80-120µm	8-10 µm
	G.fasciculatum	11.08	Chlamydospore	Pale yellow	Globose to subglobose	100-120 µm x 80-100 µm	7-9 µm
Airport	*G.etunicatum*	96.93	Chlamydospore	Yellow to brown	Globose	80-120 µm	8-10 µm
	G. fasciculatum	6.04	Chlamydospore	Pale yellow	Globose to subglobose	100-120 µm x 80-100 µm	7-9 µm
State Lodge	*G. etunicatum*	83.47	Chlamydospore	Yellow to brown	Globose	80-120 µm	8-10 µm
	G. fasciculatum	13.71	Chamydospore	Pale yellow	Globose to subglobose	100-120 µm 80-100 µm	7-9 µm
	Acaulospora scrobiculata	2.82	Zygospore	Hyaline to yellow	Globose	120-140 µm	6-8 µm

4.4 Analysis for phosphorus content in tomato plants

Tomato samples from the greenhouse showed higher levels of phosphorus of 0.35 ppm of phosphorus in mycorrhizal plants with a ratio of 1 VAM to 1 soil which is the medium mychorrhizal level (Table 3). The mycorrhizal plants with a ratio of 3 VAM to 1 soil (high mychorrhizal level) had 0.34 ppm of phosphorus while the ratio of 1 VAM to 3 soil (low mychorrhizal level) had 0.28 ppm of phosphorus. The lowest value of phosphorus of 0.20 ppm of phosphorus was recorded in non-mycorrhizal plants. The trend was the same in the tomato samples from the Agriculture Experimental Field and Mount Makulu. The tomato samples from Mount Makulu had phosphorus levels higher in 1 VAM to 1 soil (medium mychorrhizal level) with 0.49 ppm of phosphorus followed by 3 VAM to 1 soil (high mychorrhizal level) with 0.46 ppm of phosphorus and lastly 1 VAM to 3 soil (low mychorrhizal level) with 0.33 ppm of phosphorus. The treatment values for phosphorus content were higher than the non-mycorrhizal plants, which contained 0.29 ppm of phosphorus. In case of samples from Agriculture Experimental Field, mycorrhizal plants 1 VAM to 1 soil (medium mychorrhizal level) had the highest level of phosphorus of 0.40 ppm compared to 3 VAM to 1 soil (high mychorrhizal level) with 0.39 ppm and 1 VAM to 3 soil (low mychorrhizal level) with 0.36 ppm of phosphorus. Non-mycorrhizal plants had the lowest value of 0.27 ppm of phosphorus (Table 3).

Table 3. Phosphorus (P) levels of tomato (*Lycopersicon esculentum* Mill.) plants grown in the greenhouse and field in parts per million (ppm).

Inoculum concentration	GREENHOUSE	FIELD	
		Agriculture Expt. Field	Mount Makulu
1 (3M : 1S)	0.34	0.39	0.46
2 (1M : 1S)	0.35	0.40	0.49
3 (1M : 3S)	0.28	0.36	0.33
4 (Control)	0.20	0.27	0.29

4.5 Effect of VAM on tomato growth and development

It can be noted from Table 4 that tomato plants inoculated with VAM were two times taller than non-mycorrhizal plants. The stem diameter of tomato plants with mycorrhiza was four times wider than the non-mycorrhizal plants. The treatment with 1 VAM inoculum to 1 sterilized soil (medium mycorrhizal level) had the highest value. Plants with mycorrhiza had 8 times more leaves, took less number of days to flowering and only fewer number of flowers were aborted. The findings show that the treatment of 1 VAM inoculum to 3 sterilized soil (low mychorrhizal level) had the second highest value to the control (no mycorrhiza). Both shoot dry weight and root dry weights of tomato plants with mycorrhiza were twice more than those for plants without mycorrhiza in the greenhouse (Table 5). The

shoot dry weight of plants at Mount Makulu and the Agricultural Experimental Field at UNZA were three times more than the control plants. The differences between the shoot dry weight at the school of Agriculture Experimental Field and Mount Makulu was 5g whilst the greenhouse values were 35g less than the field weights. Plants with VAM had a tomato fruit yield of 2500kg/ha more than plants without VAM (Table 4).

VAM containing tomato plants with *Fusarium* wilt and *Septoria* leaf spot disease showed greater plant height and stem diameter than non-mycorrhizal plants with the disease. Non-mycorrhizal plants with *Fusarium* wilt had the lowest root dry weight value and also the stem diameter. There were many aborted flowers in non-mycorrhizal plants with *Septoria* leaf spot and their tomato fruit yield was also the lowest.

Table 4: Effect of VAM on growth and yield of tomato plants (*Lycopersicon esculentum* Mill.) grown in a greenhouse and field

Treatments Inoculum concentrations)	Plant Height (mm)	Stem diameter (mm)	Number of leaves	Aborted flowers	Days to flowering	Yield Kh/ha
1. High Level	291.40^{b}	7.572^{b}	16^{b}	17^{a}	89^{b}	4798^{b}
2. Medium Level	323.70^{bc}	7.777^{b}	18^{c}	15^{a}	84^{a}	5783^{b}
3. Low Level	305.44^{b}	7.362^{b}	14^{b}	18^{c}	83^{a}	5044^{b}
4. No Mycorrhiza	183.12^{a}	3.810^{a}	9^{a}	24^{b}	104^{c}	2186^{a}
Mean	**130.96**	**6.130**	**14**	**19**	**90**	**4452.75**
LSD (0.05)	**4.134**	**0.4501**	**1.5178**	**3.154**	**2.195**	**1840.3**
CV%	**5.5**	**13.1**	**35.1**	**42.5**	**5.5**	**47.7**

Means followed by the same letter are not significantly different from each other at 5% level of probability according to Scheffe;s (Thomas and Hill, 1978) Least Significance Difference (LSD) test.

Table 5: **Effect of VAM on shoot dry weight and root dry weight of Tomato (*Lycopersicon esculentum* Mill.) grown in the green house and the field**

Treatments (Inoculum Concentrations)	**Shoot dry weight (g)**			**Root dry weight (g)**		
	Green House	Mount Makulu	UNZA	Green House	Mount Makulu	UNZA
1. High level	10.81^{b}	47.37^{b}	42.85^{b}	1.30^{b}	2.13^{b}	2.02^{b}
2. Medium Level	11.43^{b}	48.98^{b}	46.08^{b}	1.37^{b}	2.39^{b}	2.17^{b}
3. Low Level	12.12^{b}	48.18^{b}	45.53^{b}	1.43^{b}	2.25^{b}	2.10^{b}
4. No Mycorrhiza	5.70^{a}	12.50^{a}	9.96^{a}	0.61^{a}	0.81^{a}	0.84^{a}
Mean	**10.12**	**39.26**	**36.11**	**1.18**	**1.90**	**1.78**
LSD (0.05)	**2.063**	**7.146**	**4.125**	**0.124**	**0.248**	**0.330**
CV%	**16.4**			**17.7**		

Means followed by the same letter are not significantly different from each other at 5% of probability according to Scheffe's (Thomas and Hill, 1978) Least Significance Difference (LSD) test.

4.6 Disease incidence on tomato plants

4.6.1 *Septoria* leaf spot

The symptoms of *Septoria* leaf spot (Figure 5) developed 7 days after inoculation. The percent disease incidence in the greenhouse was lower than in the field. The non-mycorrhizal plants had higher disease incidence than the mycorrhizal plants (Table 6). Spread of disease to uninoculated non-mycorrhizal plants was faster than to the mycorrhizal plants. However, treatment of 1 VAM inoculum to 3 sterilized soil (low mycorrhizal level) had a slightly higher disease incidence than the other treatments of 3 VAM inoculum to 1 sterilized soil (high mycorrhizal level) and 1 VAM inoculum to 1 sterilized soil (medium mycorrhizal level). The low level of inoculum had 1% disease incidence more than the high level while the difference between the high level and medium level was 0.6%. The non-mycorrhizal plants had the highest percent disease incidence of 2.8% (Table 6).

4.6.2 *Fusarium* wilt

The percent disease incidence (PDI) of *Fusarium* wilt was higher in non-mycorrhizal plants (Table 7). The PDI among the mycorrhiza of treatments 3 VAM inoculum to 1 sterilized soil (high mycorrhizal level), 1 VAM inoculum to 1 sterilized soil (medium mycorrhizal level) and 1 VAM inoculum to 3 sterilized soil (low mycorrhizal level) showed that it was lowest in the treatment which had the highest level of mycorrhiza and highest in the control which had no mycorrhiza. The PDI in non-mycorrhizal plants was 10% more than in

mycorrhizal plants. The mycorrhizal plants with the highest inoculum level had 5% disease incidence lower than the other treatments with low inoculum level and medium inoculum level.

Table 6. Effect of VAM on the incidence of *Septoria* leaf spot on tomato (*Lycopersicon esculentum* Mill.) grown in a greenhouse and the field.

Treatments (Inoculum concentrations)	***Septoria* percent Disease Incidence (Plant age of 160 days)**		
	Greenhouse	**Mount Makulu**	**UNZA**
Septoria + high level	1.624[a]	3.887[a]	3.287[a]
Septoria + medium level	1.375[a]	2.937[b]	2.451[a]
Septoria + low level	2.311[b]	4.089[b]	3.538[a]
Septoria + no mycorrhiza	2.820[c]	5.050[c]	4.661[b]
Mean	**2.033**	**3.991**	**3.484**
LSD (0.05)	**0.410**	**0.552**	**0.930**
CV %	**29.9**		

Means followed by the same letter are not significantly different from each other at 5% level of probability according to Scheffe's (Thomas and Hill), 1978) Least Significance Difference (LSD) test.

Figure 5. Symptoms of *Septoria* leaf spot on leaves of tomato (*Lycopersicon esculentum* Mill.) from one of the experimental fields.

Table 7. Effect of VAM on *Fusarium* wilt on mycorrhizal and non-mycorrhizal tomato plants (*Lycopersicon esculentum*) grown in a greenhouse and the field.

Treatments (Inoculum concentrations)	*Fusarium* Disease Incidence		
	Greenhouse	**Mount Makulu**	**UNZA**
Fusarium + high level	20.00^{a}	20.00^{a}	11.11^{a}
Fusarium + medium level	20.00^{a}	26.67^{b}	22.22^{b}
Fusarium + low level	24.45^{b}	26.67^{b}	20.00^{b}
Fusarium + no mycorrhiza	31.11^{c}	33.33^{c}	26.67^{c}
Mean	**23.89**	**26.67**	**20.00**
LSD (0.05)	**3.142**	**1.814**	**0.907**
CV %	**13.6**		

Means followed by the same letter are not significantly different from each other at 5% level of probability according to Scheffe 's (Thomas and Hill, 1978) Least Significance Difference (LSD) test.

Control (no mycorrhiza) | 1M:3S (low level) | 1M:1S (medium level) | 3M:1S (high level)

Figure 6. Symptoms of *Fusarium* wilt on tomato (*Lycopersicon esculentum* Mill.) grown in the greenhouse.

CHAPTER FIVE

5.0 DISCUSSION

The study on the effect of VAM on growth, yield and incidence of diseases on tomato (*Lycopersicon esculentum* Mill.) grown in the greenhouse and field has shown that there were significant differences in growth and development of the mycorrhizal and non-mycorrhizal plants. The mycorrhizal tomato plants grew significantly better than the non-mycorrhizal plants (Table 4). This has been shown from the results obtained on the measured parameters. VAM increased plant height and the plant height increased slowly at the beginning until up to about 56 days after planting. This could have been due to the amount of photosynthates the mycorrhizae were getting from the plant since the mutual association is characterized by the movement of plant produced carbon (photosynthates) to the fungus (Kendrick, 1992; Harley and Smith, 1983).

The other parameters studied such as stem diameter, number of leaves per tomato plant, shoot dry weight, root dry weight and yield also increased in the presence of VAM. These results are similar to those of Sitaramiah and Khanna (1997) on the effect of *Glomus fasciculatum* on growth and chemical composition of maize. Their results revealed that mycorrhizal plants always had better vegetative growth and contained more phosphorus than the non-mycorrhizal plants. The stem diameter of the treatment with 1 VAM inoculum to 1 Sterilized soil (medium mycorrhizal level) had the highest value while the treatment of

Fusarium plus no mycorrhiza, which was characterilized by the wilting of the shoot system, had the lowest value. There were more leaves, which dried up in *Fusarium* treated plants. The results of this study are similar to those of Dodd *et al.*, (1983) on pepper. Non-mycorrhizal plants also produced relatively higher number of aborted flowers per plant than mycorrhizal plants.

Shoot and root dry weights were higher in the mycorrhizal plants than non-mycorrhizal plants. The tomato plants at Mount Makulu had the highest shoot dry weight. The larger differences between the shoot dry weight of plants from the field and plants from the greenhouse was due to the growing of plants in pots in the greenhouse. The non-mycorrhizal plus *Fusarium* had the lowest values for both the shoot dry weight and root dry weight (Table 5) because of the drying of the leaves and, also the rotting of the roots respectively. The differences between the shoot dry weight of plants from UNZA and Mount Makulu plants appear due to the high phosphorus levels (Table 1b) in the soils from UNZA (73 ppm phosphorus) than Mount Makulu (18 ppm phosphorus) which produced a negative effect on the activity of the mycorrhiza. Gerdemann (1965) and Tinker (1975) reported that mycorrhiza works better at low phosphorus levels.

The results on tomato yield indicate that treatment 1 VAM inoculum to 1 sterilized soil (medium mycorrhizal level) gave the highest plant height, stem diameter, number of leaves and produced the highest tomato fruit yield (Table 4).

These results of the present study on the effect of mycorrhiza on growth and yield of tomato are similar to those of Halos and Zorilla (1979) who found that tomato plants inoculated with mycorrhiza, and then grown in sterile soil grew taller and healthier. Mycorrhiza containing plants also flowered earlier and gave higher yield than non-mycorrhizal plants. The mycorrhizal plants were able to absorb more phosphorus than the non-mycorrhizal plants. This is shown by the results in Table 3 on the analysis of phosphorus levels in the tomato plants. In all the three experimental sites (Greenhouse, Agriculture Experimental Field and Mount Makulu) the non-mycorrhizal plants had the lowest values of phosphorus.

The results of the present study correspond to those of Hazarika *et al.,* (1999) on the effect of vesicular-arbuscular mycorrhizal fungi inoculation in growth and nutrient uptake of Blackgram. Their results suggested that inoculation of Blackgram with an efficient VAM fungus *G. fasciculatum* and *G. mosseae* significantly improved plant growth, nutrient uptake and yield. The phosphorus content of mycorrhizal plants at Mount Makulu was higher than at Agriculture Experimental Field (Table 3). The results of Sitaramaiah and Khanna (1997) also showed that mycorrhizal fungi significantly increased the phosphorus level in maize.

The results on the effect of VAM on *Septoria* leaf spot showed that the disease incidence (Table 6) was high in the non-mycorrhizal plants. The next high value of disease incidence was obtained in the treatment 1 VAM inoculum to 3 sterilized soil (low mycorrhizal level) plus *Septoria* which contained low levels of the VAM inoculum. The treatment with medium level of VAM inoculum showed the lowest incidence of *Septoria* leaf spot. Similar results were also obtained by Dehne (1982) on the incidence of powdery mildew in

mycorrhizal and non-mycorrhizal cucumber plants. The increase in susceptibility of the host was due to an enhanced development of the pathogen than to an increased frequency of infection. This influence seems to be correlated with the nutritional aspect of mycorrhiza-disease interactions and higher physiological activities in the whole host plant. It should be noted that higher disease incidence in the symbiotic system, in general does not affect genetically defined resistance to plant pathogens. Genetically resistant plants remain resistant, whereby susceptibility may be modified by the symbiosis. VAM encourages luxuriant plant growth (Table 4 and 5) and higher phosphorus content (Table 3) and therefore control *Septoria* leaf spot. Gangopadhyay and Das (1987) demonstrated that the association of VAM with roots of susceptible rice cultivars Jhona 349 and Karuna encouraged luxuriant plant growth, more phosphorus content and higher grain yield. The above cultivars also developed resistance to stem rot and sheath blight disease.

The lower disease incidence of *Septoria* leaf spot in the greenhouse than the field may be due to the high temperatures (Appendix II) which do not favour the disease development. Septoria fungus is killed at a temperature of 36.5 ^{o}C (Singh, 1987). Spread of *Septoria* leaf spot disease in uninoculated non-mycorrhizal plants (control) was faster than in the mycorrhizal plants. However, the treatment with 1 VAM inoculum to 3 sterilized soil (low mycorrhizal level) had a slightly higher disease incidence than the other treatments. This could be due to the low level of mycorrhiza in the plants.

Incase of *Fusarium* wilt, the percent disease incidence was higher in non-mycorrhiza plus *Fusarium* and lowest in 3 VAM inoculum to 1 sterilized plus *Fusarium* (high mycorrhizal

level) apparently because of the high levels of the inoculum (Table 7) which reduced pathogen colonization of root cells. Bushan (1987) reported similar observations and found that VAM induced suppression of *Fusarium* root rot in mungbean while Gangopadhyay and Das (1987) demonstrated the possibility of controlling soil borne diseases of rice through vesicular – arbuscular mycorrhiza. The *Fusarium* disease incidence at Mount Makulu was relatively higher than at Agriculture Experimental Field at the School of Agriculture (UNZA) and could be explained on the basis of higher soil temperatures prevailing at UNZA than Mount Makulu (Appendices III and IV).

The results of this study indicate that *Glomus* species could increase growth of plants and could also control soil-borne diseases since larger proportion of spores identified from the three sites were *G. etunicatum* and *G. fasciculatum*. Similar observations were made by Sitaramaiah and Khanna (1997) with *G. fasciculatum* on the growth and chemical composition of maize. Utkhede and Smith (1992) showed that mycorrhiza can be used to manage apple replant disease (ARD) characterised by poor growth of young apple trees where apple trees had been grown previously.

Based on the results obtained in this study it may be suggested that VAM were responsible for better growth, higher yield and lower disease incidence in mycorrhizal plants than non-mycorrhizal plants.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

The results of this study demonstrate that mycorrhiza can increase growth and yield and also reduce *Septoria* leaf spot and *Fusarium* wilt of tomatoes.

The mycorrhizal treatment 1 VAM inoculum to 1 sterilized soil (medium mycorrhizal level) performed better in comparison to the other two treatments studied. High mycorrhizal levels of 3 VAM inoculum to 1 sterilized soil did not control *Septoria* leaf spot. However, this treatment was found to be suitable for control of a systemic soil-borne pathogen and increased the root resistance to the entry by *Fusarium oxysporum* f. sp. *Lycopersici.* In most cases the treatment 1 VAM to 3 soil (low mycorrhizal level) was not effective and did not increase plant growth and yield of tomato in comparison to other treatments where high levels of mycorrhiza inoculum were used.

The use of mycorrhizal fungi in crop production as part of Integrated Pest Management (IPM) can be profitably used in the production of food at low cost. Tomato seedlings can be inoculated with mycorrhiza in nursery beds for effective root colonization by VAM. This practice will involve less mycorrhizal inoculum. The technique can be applicable in both the greenhouse and field cultivation of tomato.

The refinement of the technique will include carrying out further studies on the role of specific mycorrhizal species and their effect on specific diseases. The levels of mycorrhizal inoculum needed for successful colonization of roots and the effect of climate on mycorrhiza also require further investigation.

VAM can supplement inorganic and organic fertilisers for improved tomato production and can enhance plant growth and also reduce incidence of *Fusarium* wilt and *Septoria* leaf spot diseases of tomato.

The use of vesicular arbuscular mycorrhiza in crop production is an economical and useful biofertilizer and bioprotectant.

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APPENDICES

Appendix I Experimental layout

A. Treatments

1. 3 VAM + 1soil (High VAM level)
2. 1 VAM + 1 soil (medium VAM level)
3. 1 VAM + 3 soil (low VAM level)
4. Control (no mycorrhiza)
5. *Fusarium* + high VAM level
6. *Fusarium* + medium VAM level
7. *Fusarium* + Low VAM level
8. *Fusarium* with no mycorrhiza
9. *Septoria* + high VAM level
10. *Septoria* + medium VAM level
11. *Septoria* + low VAM level
12. *Septoria* with no mycorrhiza

B. RANDOMIZATION

REPLICATION I	REPLICATION II	REPLICATION III
6	9	8
8	7	10
11	6	12
2	3	4
10	4	1
5	1	7
3	11	5
12	10	9
4	2	11
7	8	3
9	12	6
1	5	2

C. FIELD LAYOUT

301 8	302 10	303 12	304 4	305 1	306 7	307 5	308 9	309 11	310 3	311 6	312 2
212 5	211 12	210 8	209 2	208 10	207 11	206 1	205 4	204 3	203 6	202 7	201 9
101 6	102 8	103 11	104 2	105 10	106 5	107 3	108 12	109 4	110 7	111 9	112 1

Appendix II Maximum and minimum temperatures (^{o}C) recorded from the greenhouse

Month/ Parameter	Feb	Mar	April	May	June	July	Aug	Sept
Min/ (range)	22 -26	16 -39	15-26	12-26	8-23	11-21	10-25	11-26
Min/ (mean)	23	23.6	19.6	21.5	15.4	14.7	19.5	23
Max/ (range)	31-48	32-50	38-46	32-44	28-40	25-38	25-39	29-44
Max/ (mean)	41	39.9	42.5	37.9	33.2	30.2	34	38.7

Appendix III Climatic data for University of Zambia where tomato plants were grown in the field (April 2001 – September 2001).

Month	Rainfall (mm)	Humidity %	Temperature °C		Ground/soil temp. °C	
			min	max	5cm	10cm
April	7.7	74.2	15.3	25.9	26.3	25.1
May	0.0	53.7	12.8	24.0	26.5	24.2
June	0.0	53.5	10.3	22.1	23.3	21.3
July	0.0	50.5	9.9	22.3	23.2	20.9
August	0.0	38.9	13.6	26.6	27.2	24.6
September	0.0	36.7	16.0	29.8	28.9	26.6

Appendix IV Climatic data Mount Makulu where tomato plants were grown in the field (April 2001 – September 2001).

Month	Rainfall (mm)	Humidity %	Temperature °C		Ground/soil temp. °C	
			min	max	5cm	10cm
April	1.8	76.3	16.0	27.7	20.5	20.4
May	0.0	72.8	11.6	25.9	17.2	17.6
June	0.0	69.7	9.2	23.2	13.5	13.6
July	0.0	64.1	8.1	22.1	13.4	14.5
August	0.0	52.6	13.5	28.3	16.6	17.9
September	0.0	50.2	15.7	31.7	20.0	21.3

Appendix V. Analysis of Variance for Plant Height

Same of Variation	d.f	s.s	m.s	v.r	Fpr
Replication	2	1500.09	750.05	6.64	
Rep x DAP					
DAP	9	6792574.36	754730.48	6685.64	<.001*
Error	18	2031.99	112.89	2.62	
Rep x DAP x location					
Location	2	1450538.10	725269.05	1.68E + 04	<.001*
DAP x location	18	270531.04	15029.50	348.35	<.001*
Error	40	1725.79	73.14	0.53	
Rep x DAP x Location x treatment					
Treatment	11	36825.30	33479.57	487.77	<.001*
DAP x treatment	99	470432.38	4751.84	69.23	<.001*
Location x treatment	22	1246.78	56.67	0.83	0.695
DAP x location x treatment	198	11706.29	59.12	0.86	0.897
Error	660	45300.92	68.64		
Total	**1079**	**9415863.03**			
CV (%)	**5.5**				

* Significantly different at 0.05 probability level

Appendix VI. Analysis of Variance of Stem Diameter

Source of Variation	d.f.	s.s	m.s	v.r	Fpr
Replication	2	4.2081	2.1041	12.90	
Rep x DAP					
DAP	4	755.2761	188.8190	1157.46	<.001
Error	8	1.3051	0.1631	0.33	
Rep x DAP x location					
Location	2	393.2106	196.6053	396.01	<.001
DAP x location	8	11.1004	1.3876	2.79	0.030
Error	20	9.9294	0.4965	1.30	
Rep x DAP x location x treatment					
Treatment	11	674.1159	61.2833	159.93	<.001*
DAP x treatment	44	38.4007	0.8727	2.28	<.001*
Location x treatment	22	107.5714	4.8896	12.76	<.001*
DAP x location x treatment	88	31.2954	0.3556	0.93	0.657
Error	330	126.4492	0.3832		
Total	**539**	**2152.8623**			
CV (%)	**13.1**				

* Significantly different at 0.05 probability level

Appendix VII. Analysis of Variance for Number of Leaves

Source of Variation	d.f.	s.s	m.s	v.r	Fpr
Republication	2	63.646	31.823	3.38	
Rep x DAP					
DAP	9	14818.226	1646.470	174.79	<.001*
Error	18	169.557	9.420	0.82	
Rep x DAP x location					
Location	2	538.802	269.401	23.43	<.001*
DAP x location	18	278.457	15.470	1.35	0.212
Error	40	459.463	11.487	1.32	
Rep x DAP x location x treatment					
Treatment	11	2637.730	239.794	27.46	<.001*
DAP x treatment	99	2164.974	21.868	2.50	<.001*
Location x Treatment	22	373.887	16.995	1.95	0.006
DAP x location x treatment	198	1800.409	1800.409	1.04	0.354
Error	660	5764.000	8.733		
Total	**1078**	**29069.154**			
CV (%)	**35.1**				

* Significantly different at 0.05 probability level

Appendix VIII. Analysis of Variance for Number of Days to Flowering

Source of Variation	d.f	s.s	m.s	v.r	Fpr
Replication	2	1802.74	901.37	35.60	
Location	2	14712.57	7356.29	290.50	<.001*
Treatment	11	8517.63	774.33	30.58	<.001*
Location x Treatment	22	793.43	36.06	1.42	0.134
Error	70	1772.59	25.32		
Total	**107**	**27598.96**			
CV (%)	**5.5**				

* Significantly different at 0.05 probability level

Appendix IX. Analysis of Variance for Number of Aborted Flowers

Source of Variation	d.f	s.s	m.s	v.r	Fpr
Replication	2	160.17	80.08	2.53	
Location	2	4628.17	2314.08	73.24	<.001*
Treatment	11	1921.00	174.64	5.53	<.001*
Location x treatment	22	452.50	20.57	0.63	0.870
Error	70	2211.83	31.60		
Total	**107**	**9373.67**			
CV (%)	**32.5**				

* Significantly different at 0.05 probability level

Appendix X. Analysis of Variance for Shoot Dry Weight

Source of Variation	d.f	s.s	m.s	v.r	Fpr
Replication	2	39.74	19.87	1.03	
Location	2	17487.34	8743.67	454.12	<.001*
Treatment	11	12910.58	1173.69	60.96	<.001*
Location x treatment	22	3941.15	179.14	9.30	<.001*
Error	70	1347.79	19.25		
Total	**107**	**35726.61**			
CV (%)	**16.4**				

* Significantly different at 0.05 probability level

Appendix XI. Analysis of Variance for Root Dry Weight

Source of Variation	d.f	s.s	m.s	v.r	Fpr
Replication	2	0.07936	0.03968	0.57	
Location	2	9.87568	4.93784	70.74	<.001*
Treatment	11	28.65830	2.60530	37.33	<.001*
Location x treatment	22	2.35488	0.10704	1.53	0.091
Error	70	4.88584	0.06980		
Total	**107**	**7.23456**			
CV (%)	**17.7**				

*Significantly different at 0.05 probability level

Appendix XII. Analysis of Variance for Yield

Source of Variation	d.f	s.s	m.s	v.r	Fpr
Replication	2	5.745E = 06	2.872E + 06	0.75	
Location	2	6.131E + 08	3.066E + 08	80.01	<.001*
Treatment	11	1.889E + 08	1.718E + 07	4.48	<.001*
Location x treatment	22	1.129E + 08	5.130E + 06	1.34	0.179
Error	70	2.682E + 08	3.831E + 06		
Total	**107**	**1.189E + 09**			
CV (%)	**47.7**				

* Significantly different at 0.05 probability level

Analysis of Variance for *Septoria* leaf spot

Source of Variation	d.f	s.s	m.s	v.r	Fpr
Replication	2	2.2177	1.1088	1.58	
Rep x DAP					
DAP	4	96.1633	24.0408	34.32	<.001*
Error	8	5.6035	0.7004		
Rep x DAP x location				1.78	
Location	2	40.3285	20.1642	51.37	<.001*
DAP x location	8	14.5753	1.8219	4.64	0.003
Error	20	7.8503	0.3925		
Rep x DAP x location x treatment				1.22	
Treatment	11	582.4876	52.9534	165.19	<.001*
DAP x treatment	44	73.5075	1.6706	5.21	<.001*
Location x treatment	22	102.0758	4.6398	14.47	<.001*
DAP x location x treatment	88	17.0105	0.1933	0.60	0.997
Error	330	105.7843	0.3206		
Total	**539**	**1047.6041**			
CV (%)	**29.9**				

* Significantly different at 0.05 probability level

Appendix XIV. Analysis of Variance for *Fusarium* Wilt

Source of Variation	d.f	s.s	m.s	v.r	Fpr
Replication	2	5.754	2.877	0.77	
Location	2	301.894	150.947	40.55	<.001*
Treatment	11	6472.319	588.393	158.07	<.001*
Location x treatment	22	310.264	14.103	3.79	<.001*
Error	70	260.558	3.722		
Total	**107**	**260.558**	**3.722**		
CV (%)	**13.6**				

* Significantly different at 0.05 probability level