Seedling quality, plant growth and fruit yield and quality of tomato (*Solanum lycopersicum* L.) in response to *Trichoderma harzianum* and arbuscular mycorrhizal fungi

By

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DECLARATION

I, Bombiti Nzanza, hereby declare that the thesis, which I hereby submit for the degree of Doctor of Philosophy at the University of Pretoria, is my own work and has never been submitted by myself at any other University. The research work reported is the result of my own investigation, except where acknowledged.

B Nzanza

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LIST OF ACRONYMS AND ABBREVIATIONS

AA	Antioxidant activity
AMF	Arbuscular mycorrhizae fungi
An	*Ascorphollum nodosum*
AOAC	Association of official analytical chemists
B	Boron
BCA	Biological control agent
C	Carbon
ca.	Approximately
Ca	Calcium
CEC	Cation exchange capacity
CFUs	Colony forming units
Cu	Copper
DAG	Days after germination
DGGE	Denaturing Gradient Gel Electrophoresis
DI	Disease index
ELF	Extra large fruit
Em	*Ecklonia maxima*
FAO	Food and Agricultural Organization
Fe	Iron
FM	Fresh mass
GI	Germination index
GUS	b-glucuronidase
IAA	Indole acetic acid
ICO-AES	Inductively Coupled Plasma-Atomic Emission Spectroscopy
ICP	Inductive Coupled Plasma
ITS	Internal Transcribed Spacer
K	Potassium
LC	Lycopene content
LF	Large fruit
M	Mycorrhizae
MF	Medium fruit
Mg	Magnesium
MGT	Mean germination time
Mn	Manganese
Mo	Molybdenum
MYC	Myecocytomatosis viral oncogene
MYP	Marketable yield per plant
N	Nitrogen
Na	Sodium
NaCl	Sodium Chloride
NaOCl	Sodium hypochlorite

NFP	Number of fruit per plant
P	Phosphorus
PAUP	Phylogenetic Analysis Using Parsimony
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PGPR	Plant growth-promoting rhizobacteria
RDNA	Ribosomal DNA
RI	Retention indices
RMC	Reduced mycorrhizal colonisation
RRNA	Ribosomal RNA
S	Sulphur
SF	Small fruit
SG	Speed advantage
SG	Germination speed
Si	Silicon
Spp.	Species
SWE	Seaweed extracts
T	*Trichoderma*
TBR	Bisection reconnection
TSS	Total soluble solids
TTV	Total treatment variation
TYP	Total yield per plant
USEPA	United State Environmental Protection Agency
VC	Vitamin C
Vd	*Verticillium dahliae*
Wp	Water primed
Zn	Zinc

SEEDLING QUALITY, PLANT GROWTH, FRUIT YIELD AND QUALITY OF TOMATO (*SOLANUM LYCOPERSICUM* L.) IN RESPONSE TO *TRICHODERMA HARZIANUM* AND ARBUSCULAR MYCORRHIZAL FUNGI

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ABSTRACT

Existing evidence suggested that nursery inoculation with *Trichoderma harzianum* and arbuscular mycorrhizal fungi (AMF) could reduce deleterious effects of biotic and abiotic stresses and improve seedling quality, fruit yield and quality of tomato (*Solanum lycopersicum* L.). However, studies of their combined inoculation on seedling growth, fruit yield and quality of tomato plants are not well-documented. Experiments were carried out to investigate the combined effect of *T. harzianum* and AMF on tomato crop performance under various conditions. When combined with a *T. harzianum* and AMF mixture, seaweed extract from

Ecklonia maxiama inhibited AMF root colonisation of tomato seedlings. Treating seedlings with a mixture of *T. harzianum* and AMF reduced the incidence of *Verticillium* wilt in tomato grown in a nethouse at early season, with negligible effect on fruit yield. Further investigations were initiated to find out whether *T. harzianum* and AMF were efficient when applied as a mixture or alone, at different inoculation times. Co-inoculation with *T. harzianum* and AMF (*Glomus mosseae*) improved seedling growth and development, except when both fungi were simultaneously applied two weeks after sowing. When the seedlings were allowed to grow up until full harvest in a greenhouse, both fungal inoculants increased total yield and marketable yield, but these increases were not significant. Furthermore, inoculation with AMF increased the percentage of extra-large fruit. Field experiments conducted under commercial tomato production confirmed greenhouse studies. Inoculation of tomato with *T. harzianum* and AMF, either alone or in combination increased early fruit yield (four first harvesting weeks). Throughout the studies, percentage AMF root colonisation in seedlings and plants remained low, despite nursery inoculation. Field experiments investigated the effects of AMF-inoculated transplants combined with biochar-amended soils on AMF root colonisation and their resultant effects on overall crop performance and microbial community structure. Biochar had no effect on AMF root colonisation, and also when combined with AMF, it had no influence on tomato productivity. Interestingly, biochar altered the fungal community while AMF might have influenced the bacterial community such as plant-growth promoting rhizobacteria, which are associated with improved plant growth, nutrient uptake and disease control in the rhizosphere. These benefits could contribute to improved yield and fruit quality. In conclusion, although the results were variable, there was a clear indication that *T. harzianum* and AMF can play an important role in tomato production.

CHAPTER 1

GENERAL INTRODUCTION

1.1 RATIONALE

Traditionally, tomato farmers relied on pesticides and fertilisers to obtain optimum yields, with undesirable effects to the ecosystem and human health. Increased fertiliser and pesticide input costs, incidence of pests and diseases, soil degradation and environmental concerns, with consequent legislation, prompted farmers to adopt alternative farming systems. For instance, nematicide fumigants destroyed all forms of life in the soil (Carson, 1962), resulting into crop yields declining despite increased fertiliser and pesticide inputs. In that context, worldwide, farmers had to reduce fertiliser and pesticide dependence, while maintaining or improving crop yields (Clark *et al*., 1999). Among the strategies used, pre-sowing treatments with microbial inoculants such as arbuscular mycorrhizal fungi (AMF), *Trichoderma*, seaweed extract (SWE) or even silicon (Si), should be considered as alternatives to synthetic pesticides and fertilisers.

Tomato (*Solanum lycopersicum* L.) is a popular vegetable with a worldwide production of 141 million tons in 2009, of which half a million ton was produced in South Africa (FAO, 2011). In Limpopo Province, of the eight leading vegetables produced, tomato leads in terms of tonnage and income generation (StatsSA, 2009). Tomato fruit consumption either as fresh fruit or in processed form is higher than that of any other fruit or vegetable (Gómez-Romero *et al*., 2010). The fruit, which is an excellent source of health-promoting compounds, has been linked to the reduction of some cardiovascular diseases in humans (Toor & Savage, 2005), probably due to

key antioxidants such as carotenoids, vitamins and phenolic compounds (Gómez-Romero *et al*., 2010). Consequently, the demand for this fruit is increasing while alternative but sustainable production technologies are necessary.

Generally, treating seeds prior to sowing increases the germination rate (Kaya *et al*., 2006), enhances uniformity of germination, improves seedling vigour and reduces the disease pressures in the field (Badek *et al*., 2006). Inoculating tomato seedlings with AMF enhanced plant resistance and/or tolerance to biotic and abiotic stresses (Smith & Read, 1997), improved plant growth, increased total yield and mineral uptake (Al-Karaki *et al*., 2001) and enhanced fruit quality and nutritional value of tomato (Martin, 2007). *Trichoderma* is well-known for its mycoparistic effects in limiting growth and activity of plant pathogens as well as inducing defense resistance in plants (Yedidia *et al*., 1999; Howell, 2003; Bal & Altintas, 2006). Certain studies with *Trichoderma* inoculation have shown improved plant growth, mineral nutrient uptake and yield in some vegetables (Baker, 1989; Inbar *et al*., 1994; Poldma *et al*., 2002; Bal & Altintas, 2006).

Bio-stimulants such as SWE also have the potential to improve crop yield. Generally, treating seedlings prior to transplanting with SWE containing *Ecklonia maxima* improved growth rates and crop yields, while suppressing pest infections (Featonby-Smith & Van Staden, 1983; Crouch, 1990). Furthermore, the effects of Si on alleviation of crop stress and controlling pests and diseases are also widely reported in literature (Fawe *et al.*, 1998; Ghanmi *et al.*, 2004; Hammerschmidt, 2005; Rémus-Borel *et al.*, 2005). Tomato belongs to the group of the so-called "Si excluders" and the benefits gained from this plant nutrient on tomato production are less in

comparison to that for the group of "Si accumulators" such as rice (Hein, 2005). Integrating Si with biological materials such as AMF and SWE is an avenue still un-explored in the quest of improved tomato production.

Seaweed extracts contain a mixture of growth promoting compounds, which can inhibit seedling growth at higher dosages (Sivasankari *et al*., 2006), whereas the uptake of Si is limited on tomato as this is considered as Si excluders (Hein, 2005). Attempts of applying *Trichoderma* and AMF in combination revealed that these two fungal inoculants may interact on each other in certain crops (McAllister *et al*., 1994). The interactions were synergistic, antagonistic or neutral, depending on the strains, species and application time (Fracchia *et al*., 1998). Although studies had shown that *Trichoderma* and AMF, each had a profound impact on plant productivity (Windham *et al*., 1989; Yedida *et al*., 1999; Al-Karaki, 2006; Kaya *et al*., 2009), their combined effect on yield and fruit quality of tomato is not well documented. Similarly, the interaction between SWE and AMF or Si is not documented. The application of all these materials on tomato seedling production would probably have considerable benefits. Prior to widespread application, a careful investigation on their interactions under various environmental conditions need to be ascertained. The following research questions were raised:

1. Seaweed extracts are used for improved seedling growth and development, but do contain hormones, which can inhibit plant growth. What are the appropriate SWE rates? Could SWE be applied simultaneously with AMF, or Si? Could pre-sowing treatments with AMF, Si or SWE enhance plant growth and alleviate biotic stress caused by *Verticillium* wilt?

2. Preliminary studies suggested that inoculating seedlings with AMF was the best pre-sowing treatment when compared to SWE or silicon. Could AMF (*Glomus mosseae*) be inoculated with *T. harzianum* without reducing the effectiveness of each fungal inoculant alone? If so, what would be the best time for co-inoculation? Could the interactions affect seedling growth and development?

3. Previous reports suggested that *T. harzianum* and *G. mosseae*, when applied separately could improve plant growth and development. Increased yield and quality by AMF has been reported. Could co-inoculation with *T. harzianum* and AMF (*G. mosseae*) improve yield and fruit quality of tomato under greenhouse conditions?

4. Could co-inoculation of *T. harzianum* and AMF (*G. mosseae*) improve yield and fruit quality of tomato under field conditions?

5. Studies have shown that AMF counts remain low in tomato production. Possible causes were microbial competition in the rhizosphere and fertiliser application. According to Warnock (2007), biochar, a by-product of pyrolysis, could serve as refuge for AMF against fungal grazers and therefore increase AMF root colonisation. Could simultaneous application of biochar and AMF improve yield and fruit quality of tomato under field conditions?

1.2 OBJECTIVES

The objectives of this study were:

1. To investigate the effect of seed priming with SWE, nursery inoculation with AMF and *T. harzianum* mixture, and silicon amendment on seedling growth and development of tomato. This study further examines the influence of pre-treatment of seedlings on growth, yield and disease incidence of tomato infested with *Verticillium dahliae*.

2. To investigate the interactive effects of nursery inoculation with *T. harzianum* and AMF on growth and development of tomato seedlings under greenhouse conditions.

3. To determine the effects of nursery inoculation with *T. harzianum* and AMF on fruit yield, fruit quality and nutrient content of tomato fruit produced under greenhouse conditions.

4. To determine the effects of nursery inoculation with *T. harzianum* and AMF on fungal root colonisation, plant growth, fruit yield and quality of tomato produced under field conditions.

5. To investigate the effects of AMF-inoculated transplants and biochar-amended soil on mycorrhizal root colonisation, nutrient content, plant growth and yield of field-grown tomato and to assess their resultant effects on the microbial community in the rhizosphere.

1.3 RESEARCH APPROACH AND THESIS OUTLINE

A general introduction outlining the scope of the study is first presented (Chapter 1). This is followed by a review of literature on the effect of *T. harzianum* and AMF, with emphasis on tomato plants. Each fungal inoculant is reviewed with regard to taxonomy, benefits and effects, crop productivity and disease control (Chapter 2).

Various trials were conducted to investigate the combined effects of *Trichoderma* spp. and AMF on tomato productivity. The general approach included conducting trials in a growth chamber, seedling trays, greenhouse and field with *T. harzianum*, AMF, SWE, Si and biochar using tomato as test crop. Preliminary studies were conducted to determine the optimum dosages for priming. Two seaweed extracts were compared in a growth chamber study, whereas different seed pre-treatments were investigated under greenhouse conditions. Finally, pot trials were conducted in an unheated greenhouse to determine the responses of tomato plants to seed treatment, infested with *V. dahliae* (Chapter 3).

Seedling trials, with *T. harzianum* and AMF (*G. mosseae*) applied at different times, were conducted under greenhouse conditions to determine the best co-inoculation time (Chapter 4).

Pot trials were conducted with different *T. harzianum* and AMF (*G. mosseae*) combinations under greenhouse conditions to determine the effect of these fungal inoculants on growth, yield and quality of tomato (Chapter 5).

Field experiments were conducted at ZZ2 commercial farms using four of the nine combinations used in Chapter 5. Experiments were meant to investigate the practicability of using the two fungal inoculants under commercial tomato production systems (Chapter 6).

Pot and field experiments were conducted at ZZ2 commercial farms to investigate whether biochar could enhance AMF root colonisation. Also, the study investigated the impact of AMF when combined with biochar on root colonisation, tomato yield and microbial community (Chapter 7).

Finally, significant findings were summarised and recommendations for future studies were made (Chapter 8).

CHAPTER 2

LITERATURE REVIEW

2.1 ARBUSCULAR MYCORRHIZAL FUNGI

2.1.1 Taxonomy and benefits

Mycorrhizae are the most common symbiotic species on earth, with arbuscular mycorrhizae fungi (AMF) being the most frequent type of importance for agriculture (Andrade *et al*., 2009). The fungus, which is characterised by highly branched haustorium-like fungal structures within root cortical cells, belongs to the phylum Glomeromycota (Schüßler *et al*., 2001; Hause & Fester, 2005). The Glomeromycota is divided into eight families and ten genera. *Glomus* is the largest genus with more than 70 morphospecies (Redecker & Raab, 2006). The AMF are obligate symbionts and their associations with hosts are mutually beneficial. The fungus provides hosts with mineral nutrients via fungal mycelia in exchange for photosynthetic carbohydrates (Tahat *et al*., 2008). Arbuscular mycorrhizal fungi improve the mineral nutrient uptake of P (Smith & Read, 1997) and immobile nutrients such as Cu and Zn (Marschner & Dell, 1994) by increasing the surface area of roots and exploring soil by extraradical hyphae beyond the root hair and P-depletion zone (Borowicz, 2001). The AMF can also benefit plants by stimulating growth regulating substances, increasing photosynthesis, improving osmotic adjustment under drought stress, increasing resistance to pests and tolerance to environmental stresses such as drought and salinity, while improving soil properties (Bethlenfalvay *et al*., 1988; Al-Karaki, 2006).

Arbuscular mycorrhizal fungi form symbiosis with more than 80% of all terrestrial plant species. Generally, only a few plant families do not form mycorrhizal symbiosis and these include: Brassicaceae, Caryophyllaceae, Cyperaceae, Juncaceae, Chenopodiaceae and Amaranthaceae (Cardoso & Kuyper, 2006). However, each of these families has some representatives that are usually colonised by AMF (Cardoso & Kuyper, 2006). Even between crop species or cultivar within the same species that form symbiosis with AMF, the extent of mycorrhizal colonisation differs (Sjöberg, 2005).

2.1.2 Mycorrhizal root colonisation as affected by AMF inoculation

Reports on the effect of nursery inoculation on the percentage of mycorrhizal root colonisation are contradictory. Martin (2007) found a low mycorrhizal colonisation (< 10%) with inoculated tomato seedlings, whereas Karagiannidis *et al*. (2002) obtained nearly 50% root colonisation. Generally, high mycorrhizal infection is hardly observed in tomato seedling production. Chandanie *et al.* (2009) argued that even a low level of colonisation (< 13%) before transplanting should be considered adequate for successful establishment as these fungi would spread rapidly to new roots after transplanting (Bierman & Linderman, 1983). Following nursery mycorrhizal inoculation, Latef and Chaoxing (2011) found more than 50% mycorrhizal root infection in greenhouse produced tomato.

Under field production, farmers face the challenge of low levels of root colonisation. Cavagnaro and Martin (2010) conducted a field survey which included the majority of processing tomato farms in southeastern Australia. More than 75% of the farms had less than 4% mycorrhizal

colonisation of the roots. In almost 40% of the cases, mycorrhizal root colonisation was completely absent. Soil fumigation was put forward as the main limiting factor. Unfortunately, data on the natural veld area was not available. In California, the colonisation of tomato roots by AMF is typically in the range of 7–37% for fresh market organic tomato farms, (Cavagnaro *et al*., 2006). Soil disturbance and cultural practices negatively affect the performance of mycorrhiza in the field, the level of native mycorrhiza in these studies might well be too low or nil to permit any symbiosis. Low AMF colonisation in field production has also been attributed to (i) the use of inappropriate strains, (ii) relatively high available soil P (Strzemska, 1975) and (iii) microbial competition in the rhizosphere.

Biochar, a derivative of carbon biomass, can increase mycorrhizal root colonisation and/or provide refuge for AMF against fungal and bacterial grazers (Warnock *et al*., 2007). Farmers can also overcome low AMF field colonisation by inoculating seedlings with AMF prior to planting. Inoculating plants with AMF at sowing and before transplanting has proven to be more efficient or at least successful, in producing AMF colonisation (Martin, 2007). According to Barea *et al*. (1993) and Chang (1994), nursery inoculation with AMF presents multiple advantages, which included: (i) enhanced seedling growth, (ii) reduced phosphate requirements, (iii) increased survival rate and development of micropropagated plantlets, (iv) increased resistance to fungal root pathogens, (v) increased tolerance to abiotic stresses, (vi) earlier flowering and fruiting, (vii) increased crop uniformity, (viii) improved rooting of cuttings and (ix) increased fruit production (Azcón-Aguilar & Barea, 1997). However, the challenge with inoculation was that there was little information to indicate which AMF species would be most effective on which crop species, including competition of the introduced AMF with indigenous AMF (Gosling *et al*., 2006).

2.1.3 Effect of nursery inoculation with AMF on plant growth

The main purpose of nursery inoculation with AMF is to produce higher quality seedlings for improved performance in the field (Gianinazzi *et al*., 2001). Studying the effect of AMF inoculation on the performance of tomato seedlings in vermiculite, Oseni *et al*. (2010) found that nursery inoculation with AMF did not increase tomato seedling growth. But, AMF-treated seedlings showed higher dry biomass and root:shoot ratio. According to Sylvia (1989), for nursery production, established root mycorrhizal colonisation should be the goal rather than to improved seedling growth.

Studies with AMF in crop production have mainly focused on the potential of AMF to alleviate stress. A study was conducted to determine if AMF inoculation of transplants could alleviate effects of salt stress on growth and yield of tomato when irrigated with saline water (Al-Karaki, 2006). In that study, inoculation with AMF increased dry shoot and dry root mass under both saline and nonsaline conditions. In addition, AMF-treated plants had higher shoot P, Cu, Fe and Zn contents than non AMF-treated plants, regardless of the salinity status. Under salinity, yield increase due to AMF inoculation was up to 60%, whereas with nonsaline water a 29% yield increase was recorded. In a separate salinity study, Kaya *et al*. (2009) demonstrated that mycorrhizal inoculation enhanced growth and fruit yield in pepper plants by reducing leaf Na^+ and increasing membrane stability and concentrations of essential inorganic nutrients such as P, K and N. Mycorrhizal inoculation did not improve pepper fruit yield of non-stressed pepper plants. However, inoculation with AMF improved fruit yield by 20% and 35%, when plants were stressed with low (50 mM NaCl) and high salinity (100 mM NaCl), respectively. Subramanian *et*

al. (2006) exposed AMF-inoculated tomato seedlings to varying intensities of drought stress under field conditions. Arbuscular mycorrhizal fungi inoculation improved drought tolerance of tomato plants as a secondary consequence of enhanced nutritional status of the host plant, especially in terms of N and P. Regardless of drought intensity, AMF inoculation enhanced tomato fruit production although it was more pronounced under severe drought conditions than in mild-drought or well-watered conditions. In fact, data showed that mycorrhizal colonisation increased tomato fruit production by 25%, 23% and 16% under severe, moderate and mild drought stress conditions, respectively. Interestingly, AMF also increased the yield of non-stressed plants by about 12%.

Most of the studies reported in literature referred to pot or seedling trials with little information on open field production. However, one study by Martin (2007) aimed at demonstrating the contribution of AMF to yield and quality of field grown tomato, the reduced mycorrhizal colonisation (rmc) tomato mutant and its progenitor 76R were used as test crops. In the pot trial part of the study, AMF inoculation only affected plant growth and nutrient content from the second harvest onwards. However, in the open field experiment, AMF-inoculated plants had ca. 20% higher total above-ground fresh mass than uninoculated seedlings. Unfortunately, a lack of dry matter production data and comparison made it difficult to evaluate the growth responses of tomato to AMF inoculation. Similarly, Cavagnaro *et al*. (2006) used a tomato mutant rmc and its mycorrhizal wild-type progenitor, 76R MYC+, in an organic tomato farming production system, where AMF-inoculated plants had ca. 9% higher dry shoot mass than the uninoculated plants. However, at harvest there were no differences between the two genotypes in terms of shoot dry mass. Cavagnaro *et al*. (2006) argued that growth differences were likely to occur in the early

symbiosis, when C was allocated to the AMF rather than plant biomass and before the initial ‘‘C investment’’ in the AMF. In addition, AMF colonisation increased shoot N, P and Zn contents by ca. 12%, 74% and 53%, respectively. Conversely, shoot Mg, Mn and C concentrations were significantly lower in mycorrhizal plants. Improvements in nutrition of AMF-treated plants could be attributed to uptake of nutrients via the mycorrhizal pathway and/or to indirect effects brought about by morphological and physiological changes in the roots due to colonisation by AMF (Cavagnaro *et al*., 2008).

2.1.4 Effect of nursery inoculation with AMF on fruit yield and quality

From a practical viewpoint, the most important growth response to AMF inoculation should occur in yield, because it is the major variable by which production efficiency is measured (Martin, 2007). Generally, results on the effect of AMF inoculation on yield improvement had been contradictory or unsatisfactory. Cavagnaro *et al*. (2006) did not find any yield increase with organically produced tomato. Ryan and Angus (2003) studied the role of AMF in nutrition and yield of wheat and field pea in a 2-year crop sequence experiment on red a loam soil in Australia, where high root colonisation did not translate into increased growth or yield of wheat or pea. Ryan and Angus (2003) argued that AMF was unimportant for productivity of the major field crops. Nursery inoculation with AMF increased tomato yield by ca. 40% on a processing tomato farm (Martin, 2007). In another study (Regvar *et al*., 2003), two month old tomato seedlings were inoculated with a mixture of indigenous mycorrhiza and transplanted into pots in a greenhouse and three months later, the plants were transplanted into the field and allowed to grow for a further two months. A 26% increase in yield was observed when using inoculated

seedlings. The methodology followed by Regvar *et al.* (2003) is not the norm since growers in general use young seedlings (3-4 weeks) with inoculation being done during sowing. Increased yield with AMF was previously shown to correlate with P supply or soil P status. In a field experiment, inoculating tomato seedlings with the AMF (*G. fasiculatum*) increased tomato yield by up to 13% (Mohandas, 1987). Li *et al.* (2005) examined the interactive effect of AMF and P supply in wheat, where with low P, AMF plants produced lower grain yield per plant, whereas with higher P, AMF plants produced higher grain yields than uninoculated plants. Similarly, Douds and Reider (2003) observed that inoculating tomato with AMF before transplanting increased yield in high-P containing soils.

Martin (2007) found a 4% decrease in fruit brix despite an increase in fruit P, Zn and Ca contents of AMF-inoculated plants when compared to the uninoculated plants. Martin (2007) argued that the decrease was due to increased demand for carbohydrates by the increased number of fruits in AMF-treated plants. Cavagnaro *et al.* (2006) observed 50% higher fruit Zn content in AMF-treated plants when compared to the control. The uptake of Zn has a profound impact in human health and Cummings and Kovacic (2009) reported that Zn deficiency in humans altered the immune and gastrointestinal systems, blood cell development and thyroid hormone metabolism, as well as the activities of pancreas, liver and brain, and can also increase the risks of diabetes, coronary artery disease and cancer. Mycorrhizal association improved tomato fruit quality by enhancing ascorbic acid content and reducing the acidity (Subramanian *et al.*, 2006). Symbiosis with AMF can also stimulate the synthesis of secondary metabolites such as phenolic acids, anthocyanins, flavonoids, phytosterols, stilbenes, vitamins and carotenoids, which are beneficial for human health (Hooper & Cassidy 2006; Kirby & Keasling 2009; Gianinazzi *et al.*, 2010).

2.1.5 Effect of nursery inoculation with AMF on disease control

Evidence exists that AMF could suppress or reduce the incidence of soil-borne diseases such *Pythium*, *Rhizoctonia*, *Fusarium*, *Phytophthora* and *Verticillium* (Rosendahl, 1985; Slezack *et al*., 1999; Harrier & Watson, 2003; Hause & Fester, 2005). *Verticillium dahliae* can cause serious economic losses to many crops including tomato, and to date, no efficient chemical control has been developed. In the absence of effective long-term cultural control options, there is an increased interest in utilising biological control agents (BCAs) such as AMF to reduce disease inoculum potential (Baker & Paulitz, 1996). Karagiannidis *et al*. (2002) studied the influence of AMF on the incidence of *Verticillium* wilt in tomato and eggplant seedlings grown in pots. Infection by *V. dahliae* reduced plant height and dry shoot mass by 14% and 35%, respectively in tomato. The respective increases in eggplant were 30% and 104%. However, the combination of AMF and *V. dahliae* increased tomato plant height and dry shoot mass by 21% and 24%, respectively, as compared to the control plants. The respective increases in eggplant were 16% and 10%. Similarly, the combination of AMF and *V. dahliae* increased strawberry total plant biomass by ca. 27-48%, whereas a 40% reduction was recorded when *V. dahliae* was applied alone (Tahmatsidou *et al*., 2006). *Verticillium dahliae* reduced the marketable tomato fruit yield by ca. 200%, but when AMF was added, the yield increased by 46% as compared to the control plants. This could be due to induced resistance to *V. dahliae* caused by AMF (Karagiannidis *et al*., 2002). According to Morandi (1996), this resistance is due to the fact that AMF cause an accumulation of phenolics, in particular, phytoalexins and associated flavonoids and isoflavonoids in roots of their host plants.

2.2 *TRICHODERMA*

2.2.1 Taxonomy and benefits

Trichoderma, with an estimated 130 species, is a species-rich genus of micro fungi belonging to the Ascomycota phylum. *Trichoderma* spp. are predominant over wide geographic regions in all climatic zones and can be isolated from nearly every soil, decaying wood, compost or other organic matter (Harman *et al*., 2004; Hoyos-Carvajal *et al*., 2009; De Respinis *et al*., 2010). *Trichoderma* spp. are remarkable for their rapid growth, capability of utilising diverse substrates and resistance to noxious chemicals (Kubicek *et al*., 2003). Some of the species are of economic importance because of their production of enzymes and antibiotics, or use as biocontrol agents (Gams & Bissett, 1998; Sivasithamparam & Ghissalberti, 1998; Kubicek *et al*., 2003). According to Hoyos-Carvajal *et al*. (2009), *Trichoderma* spp. can form intimate associations with plant roots, providing an endemic level of biological control or stimulating plant growth by producing soluble forms of mineral nutrients and growth-promoting metabolites.

Three important characteristics that some strains of *Trichoderma* spp. have been shown to exhibit are the ability to (1) protect seeds and seedlings from organisms that cause damping-off, (2) be rhizosphere competent and protect the subterranean portions of growing plants from attack by pathogens, and (3) enhance plant growth and development (Harman & Taylor, 1990). Most *Trichoderma* isolates rapidly colonise the rhizosphere of seedlings, persist at considerable population levels and remain active for extended periods against plant pathogens (Papavizas, 1985).

2.2.2 *Trichoderma* spp. and plant growth promotion

Trichoderma is no longer considered as a biological control agent (BCA) only but also as plant growth enhancer, which is supported by reports on growth promotion of several species of plants treated with *Trichoderma* spp. (Windham *et al*., 1989; Björkman *et al*., 1998; Yedidia *et al*., 1999; Brimner & Boland, 2003; Hoyos-Carvajal *et al*., 2009). Enhanced tomato seedling growth with *T. harzianum* was investigated under greenhouse conditions, and at four weeks after sowing, root colonisation of tomato seedlings by *T. harzianum* strains was more than 90% (Ozbay & Newman, 2004). In addition, *T. harzianum* strains T22 and T95 increased shoot length, stem diameter, and fresh and dry shoot mass by 12%, 21% and 11%, respectively. In this study, the isolates had no significant effect on fresh or dry root mass. Also, the mechanism involved in growth promotion by *Trichoderma* spp. was not clearly elucidated. Gravel *et al*. (2007) studied the effect of *T. atroviride* and seven other biological control agents on growth of tomato grown hydroponically. The production or degradation of indole acetic acid (IAA) by *T. atroviride* was investigated as a possible mechanism for plant growth stimulation. *Trichoderma atroviride* synthesised IAA from different feature precursors *in vitro*. The addition of L-tryptophan, tryptamine and tryptophol in the culture medium stimulated the production of IAA by 417%, 718% and 3108%, respectively. The observation supported the theory that microbial IAA could have been involved in growth stimulation. Under greenhouse conditions, the growth of seedlings inoculated with *T. atroviride* increased as the concentration of L-tryptophan increased in the pouches, suggesting that the synthesis of IAA through tryptophan-dependent pathways by *T. atroviride,* affected the growth of the tomato seedlings. Gravel *et al*. (2007) concluded that growth stimulation was the synergic result of numerous modes of action exhibited

by *T. atroviride*, which included a regulation in the concentration of IAA in the rhizosphere and a regulation of the concentration of ethylene within roots.

Increased mineral uptake by *Trichoderma*-inoculated plants has also been suggested as a possible mechanism for plant growth promotion. The potential of *T. harzianum* strain T-203 to induce a growth response in cucumber plants was investigated under field and greenhouse conditions, and at four weeks after treatment initiation, *T. harzianum*-inoculated plants increased cumulative root length, shoot length, leaf area and dry shoot mass by 75%, 45%, 80% and 80%, respectively (Yedidia *et al*., 2000). Similarly, an increase of 90% and 30% in P and Fe concentration respectively, was observed in shoots. In order to characterise the effect of *T. harzianum* during the early stages of root colonisation, experiments were carried out under axenic hydroponic growth (Yedidia *et al*., 2000). Five days after inoculation, *T. harzianum*-inoculated plants increased root length, shoot length, dry root mass and dry shoot mass by ca. 45%, 60%, 24% and 40%, respectively, when compared with controls. Shoot Zn, P and Mn concentrations increased by 25%, 30% and 70%, respectively. The observations suggested that improvement of plant nutritional level might be directly related to a general beneficial growth effect of the root system following *T. harzianum* inoculation.

2.2.3 *Trichoderma* spp. and yield and fruit quality

Reports on the effect of *Trichoderma* on crop yield and quality are scarce, perhaps due to the fact that *Trichoderma* is more often used as BCA than a biofertiliser. Recently, Bal and Altintas (2006) investigated the effect of *T. harzianum* on yield and fruit quality of tomato under

unheated greenhouse conditions, using four dosages of *T. harzianum* (0 g/m^2, 4 g/m^2, 10 g/m^2 and 24 g g/m^2). In this study, increasing dosages of *T. harzianum* did not increase yield, marketable yield, total soluble solids (TSS) or titratable acidity of tomato. Applying *T. harzianum* at 4 g/m^2, 10 g/m^2 and 24 g g/m^2 increased early yield of tomato by 29%, 13% and 16%, respectively. However, at the final harvest, the total yield of *Trichoderma*-treated plants were similar to those of the untreated controls, suggesting that effectiveness of *T. harzianum* in the root zone was reduced during the long tomato growing season. Bal and Altintas (2006) hypothesised that regular application of *T. harzianum* during the growing season would likely increase yield and marketable yield of tomato. Similar yield results were obtained with lettuce (Bal & Altintas, 2008) and onion (Poldma *et al*., 2002; Altintas & Bal, 2008).

2.2.4 *Trichoderma* spp. and biological control of plant diseases

A review of the biology and systematic of the genus *Trichoderma* by Samuels (1996) provided detailed analysis of diseases controlled by *Trichoderma* spp. Some of these diseases include *Rhizoctonia* damping-off in radish (Lifshitz *et al*., 1985), maize and soybean (Kommedahl *et al.,* 1981); cucumber fruit rot caused by *R. solani* (Lewis & Papavizas, 1980); grey-mould on tomato (Migheli *et al.,* 1994), grapes and strawberry (Elad *et al.,* 1995; Harman *et al.,* 1995); take-all disease in wheat (Ghisalberti & Sivasithamparam, 1991) and *Sclerotinia sclerotiorum* in pea (Knudsen & Eschen, 1991). According to Howell (2003), six mechanisms are employed by *Trichoderma* spp. to provide biological control against diseases, which include: (i) mycoparatism and production of antifungal metabolites, (ii) competition and rhizosphere competence, (iii)

enzymes secretion, (iv) induction of defence responses in plants, (v) metabolism of germination stimulants and (vi) adjunct mechanisms such as increased plant growth and resistance to stress.

In tomato production, the most salient biological control activity of *Trichoderma* spp. has been the suppression of damping-off caused by *Pythium* spp. *Pythium* poses serious threats in greenhouse and field production with considerable damage to plants, particularly in the early stages of seedling growth (Blancard *et al.,* 1994; Rachniyom & Jaenaksorn, 2008). Generally, *Pythium*-challenged seedlings are removed from the field as no chemical control is available. *Verticillium* wilt caused by *V. dahliae* is another fungal disease, which can cause considerable yield loss in tomato. The fungus can survive in soils for many years and infect their hosts by entering the vascular system being transported within the conductive xylem (Green, 1981), whereby it interacts with nutrient and water movement upward and downward in the plant. Jabnoun-Khiareddine *et al*. (2009) indicated that *Trichoderma* spp. have the potential to provide disease control against this soil-borne pathogen. Jabnoun-Khiareddine *et al*. (2009) tested three different strains (*T. harzianum, T. virens and T. viride*) with *Verticillium* wilt causal agents in tomato grown in growth chamber and greenhouse conditions. *Trichoderma* spp. reduced the radial growth of all *Verticillium* wilt agents. In the growth chamber, the leaf damage index was reduced by 60% though all *Verticillium*-infected plants showed disease symptoms. Inoculating plants with *T. virens* increased the fresh and root mass by ca. 40%, whereas *T. harzianum* and *T. viride* had no effect. Conversely, in the greenhouse, all *Trichoderma* strains increased fresh root and shoot mass by more than 50% when compared to untreated plants. Jabnoun-Khiareddine *et al*. (2009) postulated that a reduction in mycelial growth was mainly due to the important

competitive potential of the antagonists used and a reduction in the abundance of resting structures of *Verticillium* isolates as compared to the untreated control.

2.3 ARBUSCULAR MYCORRHIZAL FUNGI AND *TRICHODERMA*

2.3.1 Interactions and root colonisation

Interactions between *Trichoderma* spp. and AMF can be antagonistic, synergistic or neutral (Fracchia *et al*., 1998). Calvet *et al*. (1992) observed a stimulatory effect of *Trichoderma spp*. on *G. mosseae in vitro*. Inoculation with *Trichoderma* spp. stimulated the germination of *G. mosseae* and development of AMF mycelium. Calvet *et al*. (1992) argued that the production of volatile compounds by *Trichoderma* spp. was responsible for the stimulatory effect. Similarly, Chandanine *et al*. (2009) noted that inoculating cucumber seedlings with *G. mosseae* alone increased root colonisation in 6-week old seedlings by ca. 47% after planting. Interestingly, combining *G. mosseae* with *T. harzianum* increased the level of mycorrhizal colonisation by 63% when compared to plants inoculated with *G. mosseae* alone. The population density of *T. harzianum* was initially similar to that of combined *T. harzianum* and *G. mosseae* inoculated plants, when analysed at four weeks after planting. But, three weeks later, dual inoculation reduced the population density of *Trichoderma* by 26% when compared to *T. harzianum* alone.

Green *et al*. (1999) used a compartmented growth system with root-free soil compartments to study the interactions between *G. intraradices* and *T. harzianum* without any interfering effect on the roots. Hyphal ^{33}P transport and b-glucuronidase (GUS) activity were used to monitor

activity of *G. intraradices* and a GUS-transformed strain of *T. harzianum*, respectively. *Glomus intraradices* reduced the population density and GUS activity of *T. harzianum*. Although *T. harzianum* reduced the mycorrhizal root colonisation by *G. intraradices*, it did not affect the hyphal length and density and ^{33}P uptake of *G. intraradices*. However, Masadeh *et al*. (2004) did not observe any negative interactions between the two species with regard to AMF root colonisation or population development of *T. viride* in the rhizosphere.

Interactions between *Trichoderma* and AMF are species specific. Fracchia *et al*. (1998) investigated the effect of saprophytic fungi on *G. mosseae* spore germination on water agar. *Trichoderma pseudokoningii* and *T. harzianum* increased the production of auxiliary cells by 138% and 131%, respectively. The period of auxiliary cell formation was shortened by four days when compared to the controls. None of the species had any effect on percent germination of *G. mosseae* spores. Under greenhouse conditions, *T. pseudokoningii* increased the percentage of mycorrhizal root colonisation in 4-week-old soybean plants, whereas *T. harzianum* had no effect. *Glomus mosseae* increased the population of *T. pseudokoningii* by 41% but decreased the population of *T. harzianum* by 17%. Fracchia *et al*. (1998) argued that interactions between AMF and saprophytic fungi might differ between species of the same genus. Similarly, Vázquez *et al*. (2000) studied the effect of *Trichoderma* upon mycorrhizal colonisation in maize plants inoculated with *G. mosseae*, *G. deserticola* and indigenous isolates of AMF. Four enzyme activities (phosphatase, chitinase, esterase and trehalase) were used as an index to detect changes in the microbial functioning in soil. *Trichoderma* increased phosphatase activity in the rhizosphere of *G. deserticola and G. mosseae*-colonised plants by 188% and 121%, respectively, but decreased it by 89% in the rhizosphere of plants inoculated with indigenous AMF. Generally,

chitinase activity in the rhizosphere of mycorrhizal plants was higher when compared with the control. Inoculation with *Trichoderma* increased this activity in the rhizosphere plants inoculated with indigenous AMF by 121%, but not in *G. deserticola and G. mosseae*. Similarly, esterase activity was higher in the rhizosphere of *G. mosseae*-treated plants when compared to the control plants. However, in *G. mosseae*-colonised rhizosphere, this activity was suppressed by the application of *Trichoderma*, which did not affect the trehalase activity. In addition, *Trichoderma* did not influence the percentage of mycorrhizal root colonisation. These authors argued that root colonisation ability depended on the AMF species used, indigenous AMF being the least infective and *G. deserticola* being the most infective.

The interactions between *T. pseudokoningii* strains and *G. mosseae* were studied *in vitro* and in a greenhouse, with the strains of *T. pseudokoningii* and the volatile compounds produced by these strains inhibiting the percentage germination of *G. mosseae* spores *in vitro* (Martinez *et al*., 2004). Likely, a direct interaction between the two fungi occurred before the establishment of the symbiotic phase of *G. mosseae*. In greenhouse experiments, except for *T. pseudokoningii* 2212, interactions had no effect on the mycorrhizal root colonisation of soybean, while *G. mosseae* also did not influence the number of colony forming units (CFUs) of *T. pseudokoningii*, suggesting that the effect of the saprophytic fungi on AMF development is strain specific (Martinez *et al*., 2004).

The interactions between *Trichoderma* and AMF depend on the inoculation time of each fungus. McAllister *et al*. (1994) studied the interactions between *T. koningii* and *G. mosseae in vitro* and in the rhizosphere of maize plants. The percentage germination of *G. mosseae* spore initially

decreased in the presence of *T. koningii*; but two days later, the mycelia of these two fungi were intermingled; with no hyperparasitism of the *T. koningii* hyphae on *G. mosseae* hyphae. In maize, the percentage of the mycorrhizal root colonisation significantly decreased by 88% when *T. koningii was* inoculated simultaneously with *G. mosseae*. Inoculating *T. koningii* 2 weeks after *G. mosseae* only decreased it by 19%. Similarly, a 21% decrease in the population of *Trichoderma* was observed when both fungi were applied at the same time, with late application of *T. koningii* significantly decreasing colonisation by 70%.

2.3.2 Plant growth promotion

Vázquez *et al*. (2000) investigated the interactions between *G. mosseae* and *T. harzianum* in maize plants; where *T. harzianum* increased dry shoot mass of *G. mosseae*-uninoculated and *G. mosseae*-inoculated maize plants by 30% and 3.5%, respectively. *Glomus mosseae* increased the dry shoot mass of *Trichoderma*-uninoculated plants by 18%, but decreased the *Trichoderma*-inoculated plants by 14%. Colonisation by *G. mosseae* eliminated the positive effect caused by *Trichoderma* on plant growth, with *T. harzianum* also negatively affecting the positive effect of *G. mosseae* on plant growth. Vázquez *et al*. (2000) suggested that there was a possible interaction between *G. mosseae* and *T. harzianum* in the root and/or complex interactions with other components of the soil microbiota.

Co-inoculation of *G. intraradices* with *T. harzianum* decreased dry shoot mass of cucumber seedlings by ca. 4%, but increased dry root mass by ca. 10%, when compared with control plants. Interestingly, when *T. harzianum* was inoculated alone, dry shoot mass increased by ca.

3%, whereas dry root mass decreased by ca. 23%. Conversely, when *G. intraradices* was applied alone, dry shoot mass decreased by ca. 12% with no effect on dry root mass. The observation suggested that simultaneous inoculation of *G. intraradices* and *T. harzianum* could lower the negative effect of *G. intraradices* on dry shoot mass, while alleviating the negative impact of *T. harzianum* on dry root mass (Green *et al*., 1999).

Strains of *T. pseudokoningii* did not affect dry shoot mass of inoculated soybean with *G. mosseae*, except for *T. pseudokoningii* 2212, which inhibited dry shoot mass by ca. 22% when compared with the *Trichoderma*-uninoculated plants. However, there was a trend of lowering dry shoot mass by all strains, except strain 741A. Strains of *T. pseudokoningii* lowered the dry root mass of *G. mosseae*-uninoculated plants by 7–21%; but increased the variable by 8–23% when plants were inoculated with *G. mosseae* (Martinez *et al*., 2004).

Dual inoculation of *T. harzianum* and *G. mosseae* was evaluated on melons under field conditions using conventional or reduced fertiliser application rates (Martinez-Medina *et al*., 2011). Regardless of fertiliser conditions, *G. mosseae* increased fresh shoot mass by ca. 10%. Inoculating *G. mosseae* and *T. harzianum* increased fresh shoot mass of melons by 21% and 12% under reduced and conventional fertiliser regimes, respectively. Similarly, *T. harzianum* alone increased fresh shoot mass under reduced and conventional fertiliser conditions by 27% and 16%, respectively. However, Martinez-Medina *et al.* (2011) argued that combined inoculation of *T. harzianum* and *G. mosseae* did not result in any additive effect. Although *T. harzianum* alone resulted in the highest shoot mass, this was not significantly different to combined inoculation

with *G. mosseae*. Also, it would have been interesting to compare the dry shoot mass as this could have reduced variability that is inherent in fresh mass used in the cited works.

2.3.3 Disease control

The majority of strategies for biocontrol of soil-borne pathogens rely on single microbial pathogen suppression (Larkin *et al.*, 1998; Roberts *et al.*, 2005). Unfortunately BCA applied alone is not likely to perform consistently against all pathogens of the crop or under diverse rhizosphere and soil environmental conditions. Raupach and Kloepper (1998) and Meyer and Roberts (2002) have reported increased suppression of pathogens by combinations of biocontrol agents. However, the potential interactions among BCAs could reduce their activity. Leeman *et al.* (1996) warned that incompatibility amongst microbes in a biocontrol preparation is an unlikely possibility since biocontrol agents are typically selected on their antagonistic behaviour towards other microbes, but Roberts *et al.* (2005) noticed a reduced performance when using combinations of BCAs relative to individual agents.

According to Datnoff *et al.* (1995), AMF is compatible with other BCAs such as *Trichoderma,* and as a result can be used in combinations providing levels of control superior to any of the agents used alone. The assertion was noticed after observing the changes that AMF caused in the rhizosphere which eventually increased the activity of other BCAs. Studying the interactions between AMF and *Trichoderma* in field-grown tomato conditions, Datnoff *et al.* (1995) suggested that the combination of these two BCAs were consistently more effective than either

agent applied alone. Results were in agreement with those of Linderman (1988), who observed that AMF and *Trichoderma* function in tandem in the biological control of root diseases.

In addition to the antagonism among BCAs, many other factors could reduce the effectiveness of the agents. Pozo *et al*. (1999) reported that the ability of AMF symbiosis to enhance resistance or tolerance in roots against soil-borne pathogens is not similar for different AMF species and needed to be ascertained for each particular combination of AMF, host plant genotype, pathogen and environmental conditions. For effective control, inoculation of AMF should generally take place prior to exposure to the pathogen, although there are few exceptions known (Caron *et al*., 1986; St-Arnaud *et al.,* 1997). *Glomus mosseae* was effective in reducing disease severity when inoculated prior to the pathogen but not when inoculated simultaneously with the pathogen (Chandanie *et al.,* 2009).

CHAPTER 3

GROWTH, YIELD AND *VERTICILLIUM* WILT INCIDENCE OF TOMATO (*SOLANUM LYCOPERSICUM* L.) AS INFLUENCED BY DIFFERENT PRE-SOWING TREATMENTS

3.1 ABSTRACT

The influence of seaweed extract (SWE), silicon and arbuscular mycorrhizal fungi (AMF) and *Trichoderma harzianum* mixture as pre-sowing treatments on control of *Verticillium* wilt of tomato was investigated. To determine the optimum dosage for priming, SWE derived from (i) *Ecklonia maxima* (Em) and (ii) *Ascorphollum nodosum* (An) were applied at five different concentrations (10%, 20%, 30%, 40% and 100%) and compared with water-primed (Wp) and un-primed seeds in a growth chamber. Results showed that seed priming reduced radicle growth and fresh mass of tomato seeds. Priming seeds with *E. maxima* extract shortened mean germination time (MGT), increased germination index (GI) and speed advantage (SG) over seeds primed with *A. nodosum*, with Em-10% having the highest GI. Based on these findings, two SWE treatments (i) Em-10% primed or (ii) un-primed seeds (dry) were sown into cell trays filled with (i) peat moss only, or (ii) peat moss amended with silicon (Si), or (iii) pre-inoculated with a *T. harzianum* and AMF mixture (T+M) or (iv) pre-inoculated with a *T. harzianum* and AMF mixture and amended with silicon (T+M+Si). Seedlings were allowed to grow in the greenhouse for four weeks to determine the total plant biomass and mycorrhizal root colonisation. Pre-sowing treatments had no effect on shoot length, root length and dry biomass of tomato

seedlings. Combining *E. maxima* extract with a mixture of *T. harzianum* and AMF (Em-10%+T+M) inhibited root mycorrhizal colonisation of tomato seedlings. In order to evaluate the effect of pre-sowing treatments on *Verticillium* wilt incidence, four pre-treated seedling treatments: (i) a *T. harzianum* and AMF mixture (T+M), (ii) silicon-treated (Si), (iii) *E. maxima* at 10% (Em-10%) and (iv) untreated seedlings were transplanted in pots infested, or not, with *Verticillium dahliae*. At mid-season, 10 weeks after transplanting, the *T. harzianum* and AMF mixture reduced the incidence of *Verticillium* wilt in the nethouse but failed to prevent *V. dahliae* infection. At the end of harvest, 20 weeks after transplanting, all infested plants showed *Verticillium* wilt symptoms. In the absence of the pathogen, *T. harzianum* and AMF slightly increased tomato yield ($P \geq 0.5$). Results of this study suggested that pre-inoculating tomato with fungal mixture of *T. harzianum* and AMF have the potential to reduce the incidence of *Verticillium* wilt but with a negligible yield increase.

Keywords: Arbuscular mycorrhizal fungi, pre-sowing treatments, seaweed extracts, silicon, T*richoderma*, *Verticillium*

3.2 INTRODUCTION

Seed treatment is a common technique employed in horticultural crops. Priming seeds with water or seaweed extract (SWE) is aimed to improve seed germination and uniformity (Olouch & Welbaum, 1996), whereas bioprotectants allow proliferation and colonisation of fungal inoculants in planted seeds. This is to ensure protection of the entire subterranean plant portions after field transplantation (Ahmad & Baker, 1987). Nursery inoculation with fungal inoculants

such as *Trichoderma* and arbuscular mycorrhizal fungi (AMF) is aimed to improve tomato seedling growth, alleviate transplant shock and control the incidence of soil-borne pathogens such as *Verticillium* spp. Silicon (Si) could also be considered as pre-sowing treatment, as this nutrient controlled disease in rice and cucumber.

Conferring to seedlings intrinsic qualities before transplanting to overcome *Verticillium* wilt, while improving tomato yield and quality could be rewarding. *Verticillium dahliae* is a destructive soil-borne vascular wilt fungus causing serious economic losses to a large number of crops (Schnathorst, 1981) and to date no efficient chemical control has been developed. The fungus enters the plant through root tips or wounds on roots (Garber & Houston, 1966) and then moves upward through the xylem (Bubici *et al*., 2006), where it interferes with nutrient and water movement. According to Antonopoulos *et al*. (2008), micro-organisms capable of growing in the rhizosphere could be potential biological control agents (BCAs).

The use of *Trichoderma* spp. and AMF has been increasing worldwide and is a promising alternative for controlling soil-borne diseases in sustainable and organic agriculture (Erdogan & Benlioglu, 2009). Azcón-Aguilar and Barea (1997) reported that AMF can protect plants against soil borne pathogens through its mutualistic relationship with the host plant. Karagiannidis *et al*. (2002) found that AMF enhances the tolerance of tomato plants to *V. dahliae*. In pepper, AMF reduced the negative effect of *V. dahliae* and improved fruit quality and yield of *Verticillium*-inoculated plants (Garmendia *et al*., 2004b). Similarly, *T. harzianum* controlled soil-borne diseases, particularly damping-off in tomato production (Lewis & Lumsden, 2001). Yedidia *et al*. (2000) demonstrated that *Trichoderma* spp. activated defense mechanisms in the form of

glucanases, chitinases, cellulases and peroxidases. Although the application of *Trichoderma* spp. and AMF individually had shown the potential to control soil-borne diseases, Roberts *et al*. (2005) believed that BCAs applied alone are not likely to perform consistently against all pathogens under different rhizospheres and, thus, suggested a combined application of inoculums.

Evidence of Si in enhancing disease resistance in crops is accumulating. Generally, supplying plants with soluble Si increases their resistance to fungal infection (Ghanmi *et al.*, 2004; Rémus-Borel *et al.*, 2005; Hammerschmidt, 2005). Fauteux *et al.* (2005) demonstrated that continuous feeding with Si enhanced the ability of plants to mount defences against powdery mildews. The proposed Si-enhanced mechanisms are (i) the creation of a mechanical barrier to impede fungal penetration (Kim *et al.*, 2002) or (ii) soluble Si acts as a modulator of host resistance to pathogens (Ma & Yamaji, 2006). In roots of cucumber plants being infected and colonised by *Pythium* spp., Si enhanced the activity of chitinases, peroxidases and polyphenoloxydases (Chérif *et al.*, 1994). According to Ma and Yamaji (2006), in order to benefit from Si, plants must be able to acquire the element in high concentrations regardless of whether they are monocots or dicots. However, tomato is called a non-accumulator of Si (Mitani & Ma, 2005) due to its rejective mode of uptake, which tended to exclude Si. In a study conducted by Hein (2005), Si failed to control *P. aphanidermatum* infection in tomato. Previous reports have shown that SWE could reduce diseases and promote plant growth (Lizzi *et al*., 1998; Jayaraj *et al*., 2008). In view of their cytokinin content, SWE might affect the resistance of plants to disease without eliminating the infestation itself (Featonby-Smith & Van Staden, 1983). Also, it can stimulate plant growth (Blunden, 1991; Sivasankari *et al.*, 2006) and improve fruit quality.

As explained above, SWE, AMF, *T. harzianum* or Si have each the potential to reduce disease incidence, improve plant growth or yield of tomato. However, responses of plants to their combined application are not documented. The objective of this study was three-fold: (i) to determine the concentration of SWE for seed priming, (ii) to determine the effect of pre-sowing treatments with SWE, Si, and a mixture of *T. harzianum* and AMF on growth and development of tomato seedlings, and (iii) to investigate the influence of pre-sowing treatments on growth, yield and disease incidence of tomato infected with *V. dahliae*.

3.3 MATERIALS AND METHODS

3.3.1 Determination of SWE concentration for seed priming

Site description

The experiment was conducted in a growth chamber at the Hatfield Experimental Farm, Department of Plant Production and Soil Science, University of Pretoria during 2008/2009. The site is located at 23° 45' S, 28° 16' E, and at 1372 m above sea level. The growth chamber was kept at 25ºC with a 16 h photoperiod.

Experimental design and treatments

Treatments consisted of SWE derived from (i) *E. maxima* (Em) and *A. nodosum* (An), each at five different concentrations *viz.* 10%, 20%, 30%, 40% and 100%; water-primed (Wp) and untreated control (dry), giving twelve treatments (Em-10%, Em-20%, Em-30%, Em-40%, Em-100%, An-10%, An-20%, An-30%, An-40%, An-100%,Wp and dry). The experiment was

repeated three times with ten tomato seeds per Petri dish for each treatment. Tomato cv. Nemo-Netta was used as a test crop.

Tomato seeds were soaked in the solutions containing the two different SWE at different concentrations for 24h as described by Sivasankari *et al.* (2006). Two controls were used, namely, water-primed (Wp) and untreated seeds (dry). After that, seeds were placed on top of filter paper in separate Petri dishes and moistened daily with 10 ml of water. Germination counts were made daily for 14 days. Two millimetre of radicle protrusion was considered to be a germinated seed (Demir *et al.*, 2006). Mean germination time (MGT) was calculated according to the formula of Ellis and Roberts (1981) as under:

$$MGT = \frac{\sum Dn}{\sum n}$$

Where: n is the number of seed, which were germinated on day D.

D is the number of days counted from the beginning of the germination.

The germination index (GI) was measured according to the formula of Association of Seed Analysts (AOSA, 1983) as follows:

$$GI = \frac{Number\ of\ germinated}{Days\ of\ first\ count} + \cdots . + \frac{Number\ of\ germinated\ seeds}{Days\ of\ last\ count}$$

Speed of germination (SG) was measured by determining the ratio of number of germinated seeds after three days over total seeds germinated after 14 days. Seedling length and fresh mass were also recorded.

3.3.2 Effect of pre-sowing treatments on tomato seedling growth and development

Site description

The experiment was conducted under greenhouse conditions at the Hatfield Experimental Farm, University of Pretoria, South Africa, during the 2008/2009 growing season. Details of the study location are presented in this Chapter (Refer to 3.3.1).

Experimental design and treatments

Eight pre-sowing treatments: (i) 10% *E. maxima* extract (EM-10%) (ii) Si-amended peat (Si), (iii) a *T. harzianum* and AMF mixture (T+M), (iv) EM-10%+T+M, (v) EM-10%+Si, (vi) T+M+Si, (vii) EM-10%+T+M+Si, and (viii) the untreated control were arranged in a completely randomised design, with six replications.

Tomato seeds (primed or un-primed) were sown into cell plug trays filled with either untreated peatmoss or with peatmoss thoroughly mixed with a *T. harzianum* and AMF mixture or Si, as per treatment requirement, and covered with vermiculite. Afterwards, the trays were placed into the germination room for three days and then moved into the greenhouse. Seedling emergence was determined every other day. After four weeks, five seedlings were selected at random from each treatment and destructively harvested for growth analysis. Plants were separated into shoots and

roots, measured for length and oven-dried at 50ºC for 70 h for the determination of dry matter. For mycorrhizal colonisation determination, randomly selected root samples (1 cm) were cleared with 10% KOH, rinsed with distilled water, acidified with 2.5% HCl and stained with trypan blue in lactophenol (Phillips & Hayman, 1970). Stained roots were dispersed in Petri dishes with grid lines, examined under Olympus light microscope at X40 magnification and quantified for the presence of AMF

3.3.3 Effect of pre-sowing treatments on growth, yield and *Verticillium* incidence of tomato

Site description

A pot trial was conducted at the experimental farm of ZZ2 Natuurboerdery, Mooketsi, South Africa, during autumn 2009. Plants were grown in an unheated nethouse, with 40% black knitted shade cloth without temperature and humidity control. The site is located at 23°56'61" S, 30°15'83" E, and at 687 m above sea level, in the northern part of South Africa.

Experimental design and treatments

Four-week-old tomato seedlings treated with (i) 10% *E. maxima* extract (EM-10%), (ii) silicon-treated (Si), (iii) a *T. harzianum* and AMF mixture (T+M), or (iv) untreated control were transplanted into 5 L pots filled with a sand coir mixture (ratio 2:1), and pre-inoculated with or without *V. dahliae* (Vd). The resulting treatments were: (i) 10% *E. maxima* extract (Em-10%), (ii) silicon (Si), (iii) a *T. harzianum* and AMF mixture (T+M), (iv) *V. dahliae* (Vd), (v) Em-10%+Vd, (vi) Si+Vd, (vii) T+M+Vd and (viii) untreated control. Plants were spaced 0.4 m between plants in double rows with 1 m between rows. Treatments were arranged in completely

randomised design with six replications. Hoagland modified solution was used for fertigation (Hoagland & Aaron, 1950).

Data collection

Disease assessment

Disease assessment was carried out twice, at 10 and 20 days after transplanting. Disease incidence was non-destructively measured by recording the percentage of plants showing visible symptoms, whereas disease severity was calculated as the sum of wilted, chlorotic and necrotic leaves related to the total leaves per plant, expressed as a percentage (Goicoechea *et al*., 2004). Biological control efficacy was calculated using the following formula: biological control efficacy = ([Disease incidence of control - disease incidence of treatment group]/Disease incidence of control) × 100% (Guo *et al*., 2004).

Plant biomass

Eight weeks after transplanting, three randomly selected plants were collected for the determination of dry matter as previously described in this Chapter.

Yield

Harvesting period started at 10 weeks after transplanting and was carried out for six successive weeks, with two harvests per week. At each harvest, fruit were weighed and total yield determined. The marketable yield was calculated as the total number of fruit per plant (total yield) minus small fruit and unmarketable fruit (defects, disease or physiological disorders).

3.3.4 Sources of extracts, inoculants and Si

Seaweed extract

Commercial SWE was obtained from Afrikelp (Cape Town, South Africa) as liquefied fresh *E. maxima* containing natural auxins, cytokinins and gibberellins, whereas for *A. nodosum*, commercial product Göemar was used. Seeds were soaked into solution containing 40% of Afrikelp or Göemar for 24 h before sowing.

Fungal inoculants

Commercial mycorrhizal and *Trichoderma* inoculum Biocult© containing spores of *G. mosseae*, *G. intraradices* and *T. harzianum* were obtained from Biocult© (Sommerset West, South Africa). The fungal inoculants were thoroughly mixed with peat moss and vermiculite before seed sowing.

Silicon

Silicon in the form of silicic acid was supplied by Plant Bio Regulators Pty (Ltd) (Centurion, South Africa) and thoroughly mixed with peatmoss (0.5%) before seed sowing. Additional Si was not supplied during the course of the study.

3.3.5 *Verticillium* inoculum production

The isolate (*V. dahliae*) was supplied by Agricultural Research Council, Biosystemics Division (Pretoria, South Africa). Individual microsclerotia was produced by incubating sterile vermiculite inoculated with *Verticillium* isolates at room temperature for at least two weeks and

then air-dried for a further two weeks (Sivasankari *et al.*, 2006). The numbers of microsclerotia per gram of vermiculite were estimated by grinding 1 g subsamples in 10 ml of sterile water. One ml of the suspension was transferred to 9 ml 0.1% water agar. The process was repeated to establish a dilution series from 1×10^{-1} to 1×10^{-6}. Thereafter, 100 µl aliquot of each dilution was transferred to 3 PDA plates and incubated for 7 days at 25°C in the light. The number of colony-forming units per gram of vermiculite was determined as follows: cfu/ gram vermiculite = number of colonies on the plate $\times 10^{1+ \text{ (positive value of number of dilution)}}$. Inoculation was performed by adding 3.6×10^{7} conidia to the substrate of each pot (Hoyos *et al.*, 1993).

3.3.6 Data analysis

Data were subjected to analysis of variance using SAS (SAS Institute Inc., Cary, NC, USA. (2002-2003). Relevant ANOVA tables can be found in the Appendix. Mean separation was achieved using Fisher's least significant difference test. Unless stated otherwise, treatments discussed were different at 5% level of probability.

3.4 RESULTS

3.4.1 Determination of SWE concentration for seed priming

Priming tomato seeds with Em reduced mean germination time (MGT) when compared to the untreated seeds (Table 3.1). With exception of Em-20%, Em-primed seeds had lower MGT as compared to water-primed seeds. In contrast, all An-primed seeds significantly increased the MGT. An-primed seeds had a MGT of more than 7 days after germination (DAG), with An-100

showing the highest mean value (11 DAG). Similarly, An-primed seeds had lower germination index (GI) ranging from 4 (An-40%) to 8 (An-10%), whereas the GI of Em-primed seeds were similar to both water-primed (23) and untreated dry seeds (20) (Table 3.1). The highest GI was obtained with Em-10% (24), followed by Em-30% and Em-40%, with a GI of 23.5. Results of this study also showed that Em-priming was more advantageous for germination than was An-priming. The highest germination speed (SG) was found with Em-30% and Em-40%, but this was not significantly different to other Em-primed treatments, except for Em-20% (Table 3.1). Water-priming, which was similar to Em-primed treatments, germinated faster than the untreated seeds (0.53).

3.4.2 Effect of pre-sowing treatments on tomato seedling growth and development

Seed priming reduced seedling fresh mass and radicle length (Table 3.1). With the exception of An-10%, An-priming reduced fresh mass by more than 150%, with the highest reduction obtained with An-100% (351%). Em-priming also reduced fresh mass by between 22% (Em-30) and 45% (Em-40). Water-priming reduced seed fresh mass by ca. 25%. With regards to radicle length, An-100% resulted in a 2900% reduction when compared to untreated seeds. With the exception of AN-10%, An-priming reduced radicle length by more than 290%. Among the Em-priming dilutions, Em-30% had the highest reduction (50%). The lowest reduction (11%) was found with Em-20%, followed by water-priming (15%).

Table 3.1 Effect of seaweed extract types and ratios on the germination, fresh mass and radicle length of tomato seedlings in a growth chamber

Treatment	Germination			Fresh mass	Length
	MGT	GI	SG	(g seed^{-1})	cm
Dry (control)	1.74b	20.1b	0.53c	0.150a	13.00a
Water-primed	0.78b	22.7ab	0.90ab	0.120ab	11.30ab
Em-10%	0.71b	24.1a	0.93ab	0.120ab	10.30bc
Em-20%	1.06b	22.1ab	0.83b	0.137ab	11.70ab
Em-30%	0.68b	23.5a	1.00a	0.123ab	8.67c
Em-40%	0.50b	23.5a	1.00a	0.103bc	9.50bc
Em-100%	0.58b	22.6ab	0.93ab	0.113ab	10.33bc
An-10%	9.52a	8.23c	0.00d	0.107ab	8.67c
An-20%	9.93a	7.23cd	0.03d	0.057d	2.67de
An-30%	8.77a	6.60cd	0.00d	0.060cd	3.00d
An-40%	7.83a	4.03d	0.000d	0.053d	3.33d
An-100%	10.90a	5.23d	0.000d	0.033d	0.43e

Means followed by the same letter in a column were not significantly different ($P \leq 0.05$) according to Fisher's LSD test

MGT = Mean germination time; GI = Germination index; SG = Speed advantage

Em-10%; Em-20%; Em-30%; Em-40%; Em-100% are SWE derived from *E. maxima* at 10%, 20%, 30%, 40% and 100%, respectively.

An-10%; An -20%; An -30%; An -40%; An -100% are SWE derived from *A. nodosum* at 10%, 20%, 30%, 40% and 100%, respectively

Seedlings pre-inoculated with a *T. harzianum* and AMF mixture (T+M) resulted in mycorrhizal root colonisation (Figure 3.1) when applied alone or in combination with Si (Si+T+M).

However, when the fungal mixture was combined with *E. maxima* extract (Em-10%+T+M), or with *E. maxima* extract and Si (Em-10%+Si+T+M), no mycorrhizal root colonisation was observed. Treatments had no effect on growth parameters and dry matter production of tomato (Table 3.2).

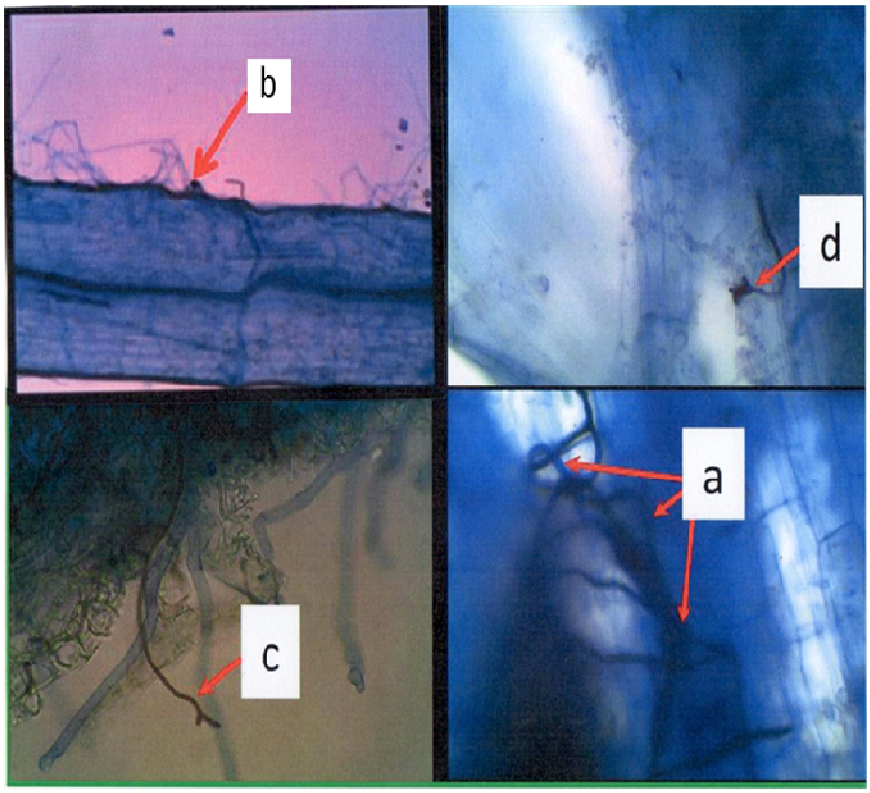

Figure 3.1 Illustration of mycorrhiza structure (a) intracellular mycelia, (b) spores, (c) extracellular mycelia and (d) appresoria confirming the presence of mycorrhizal colonisation in tomato roots inoculated with the mixture of *Trichoderma harzianum* and arbuscular mycorrhizal fungi

Table 3.2 Effect of nursery application of seaweed extracts, silicon and fungal inoculants on various plant growth parameters and mycorrhizal root colonisation of tomato seedlings

Treatments	Shoot length* (cm)	Root length* (cm)	Dry root* (g plant^{-1})	Dry shoot* (g plant^{-1})	Biomass* (g plant^{-1})	Mycorrhiza (Presence)
Control	10.98	11.53	0.19	0.86	1.05	-
Em-10%	11.57	11.63	0.21	0.84	1.05	-
T+M	11.28	11.95	0.19	0.88	1.07	+
Si	11.20	11.65	0.19	0.84	1.03	-
Em-10%+T+M	10.92	11.70	0.19	0.88	1.06	-
Em-10%+Si	10.98	11.02	0.20	0.87	1.06	-
Si+T+M	10.90	11.92	0.19	0.91	2.10	+
Em-10%+Si+T+M	11.10	11.51	0.18	0.89	1.07	-

***** No significant difference ($P \leq 0.05$) according to Fisher's LSD test

Em-10% = *E. maxima* at 10%; T+M = *T. harzianum* and AMF; Si = Silicon

3.4.3 Effect of pre-sowing treatments on growth, yield and *Verticillium* incidence of tomato

At mid-season, 10 weeks after transplanting, T+M (10.8%) had the lowest disease index (DI) followed by Em-10% (18.7%) (Table 3.3). But at the end of the season (20 weeks after transplanting), DI was similar in all the treatments. Similarly, a *T. harzianum* and AMF mixture had a positive effect on *Verticillium* wilt incidence. Treatment T+M+Vd reduced the disease incidence by ca. 42% when compared to the control (Vd). Application of Si also reduced the disease incidence by ca.17% though this was not statistically different to the control. At the end of the experiment, all treatments showed *Verticillium* wilt symptoms with similar disease incidence.

Table 3.3 Effect of nursery treated seedlings with seaweed extracts, silicon and fungal inoculants on the *Verticillium* wilt incidence on tomato

Treatment	Disease Index (%) At 10 weeks	Disease Incidence (%) At 10 weeks	Biocontrol Efficiency (%) At 10 weeks	Disease Index (%) * At 20 weeks	Disease Incidence (%)* At 20 weeks
T+M+Vd	10.8b	58.3b	41.7	53.1	100
Em-10%+Vd	18.7ab	100a	0	59.2	100
Si+Vd	23.4a	83.3ab	16.7	51.6	100
Vd	21.3a	100a	0	60.6	100

Means followed by the same letter in a column were not significantly different ($P \leq 0.05$) according to Fisher's LSD test

* No significant difference ($P \leq 0.05$) according to Fisher's LSD test

T+M = *T. harzianum* and AMF; Vd = *V. dahliae*; Em-10% = *E. maxima* at 10%; Si = Silicon

Inoculating tomato seedlings with *Verticillium* reduced marketable yield of tomato when compared to the un-inoculated treatments (Table 3.4). However, there were no significant differences among non-*Verticillium*-infested treatments. Transplanting seedlings pre-inoculated with a *T. harzianum* and AMF mixture into *V. dahliae* infested pots (T+M+Vd) resulted in the lowest yield reduction (28.2%), followed by Si+Vd (33.4%) and Em+Vd (37%). On the other hand, when the disease was not present, T+M as well as Em-10% slightly increased marketable yield of tomato, though this was not statistically different to other treatments. Dry shoot and root mass did not differ significantly, despite *Verticillium* inoculation (Table 3.4).

Table 3.4 Effect of nursery treated seedlings with SWE, silicon and fungal inoculants on marketable yield and dry matter production of tomato

Treatment	Yield (kg plant^{-1})	Dry shoot mass* (g plant^{-1})	Dry root mass* (g plant^{-1})	Plant biomass* (g plant^{-1})
T+M+Vd	3.962b	71.64	15.30	86.94
Em-10%+Vd	3.708b	71.34	15.49	86.83
Si+Vd	3.807b	72.15	15.86	88.01
Vd	3.699b	71.61	14.88	86.49
T+M	5.361a	77.54	15.85	93.39
Em-10%	5.122a	80.56	15.61	96.17
Si	4.935a	79.60	14.73	94.33
Control	5.078a	77.34	15.23	92.57

Means followed by the same letter in a column were not significantly different ($P \leq 0.05$) according to Fisher's LSD test

* No significant difference ($P \leq 0.05$) according to Fisher's LSD test

T+M = *T. harzianum* and AMF; Vd = *V. dahliae*; Em-10% = *E. maxima* at 10%; Si = Silicon

3.5 DISCUSSION

3.5.1 Determination of SWE concentration for seed priming

Treating seeds prior to sowing increases the germination rate (Kaya *et al*., 2006) and uniformity of germination improves seedling vigour and reduces the disease pressure in the field (Badek *et al*., 2006). In this study, a preliminary bioassay was conducted with two types of SWE to determine the type and ratio of seaweed extract to be mixed with fungal inoculants and/or silicon. Results showed that SWE derived from *E. maxima* shortened the mean germination time (MGT) and increased the germination index (GI) and speed advantage (SG) over *A. nodosum*

extract. Reduced MGT and increased GI and SG have been reported in *Brassica* (Thornton & Powell, 1992), in mustard (Srinivasan *et al*., 1999), in watermelon (Demir & Mavi, 2004) and in cauliflower (Kaya *et al*., 2006).

The discrepancies in findings between the two SWEs used in the present study were probably due to the concentration of each product rather than their efficacy. Seaweed extracts contain growth promoting hormones (IAA and IBA), cytokinins, trace elements, vitamins and amino acids (Challen & Hemingway, 1965) and depending on the ratio, this could promote or inhibit seed germination. For instance, Sivasankari *et al*. (2006) found that *Vigna sinensis* seeds soaked with lower concentrations of SWE showed higher rates of germination, whereas higher concentrations of the extracts inhibited germination. The findings suggested that *A. nodosum* might have been too concentrated and require further dilution before seed treatment. Conversely, this study showed that seed priming reduced radicle growth and fresh mass of tomato seedlings. In contrast, Demir and Mavi (2004) observed an increased seedling mass and hypocotyl length of watermelon seeds.

3.5.2 Effect of pre-sowing treatments on tomato seedling growth and development

Treating seeds prior to sowing with *E. maxima* extract, or mixing the growing medium with Si and/or *T. harzianum* and AMF did not show any differences on growth and dry matter production of four week-old tomato seedlings. However, combining *T. harzianum* and AMF with *E. maxima* extract inhibited mycorrhizal root colonisation of tomato seedlings. Generally, SWE

have the potential to exhibit antifungal properties, which might have affected the mycorrhizal infection during sowing.

3.5.3 Effect of pre-sowing treatments on growth, yield and *Verticillium* incidence of tomato

In the nethouse trial, total plant biomass of healthy and *Verticillium*-infested plants were similar in the mid-season, whereas Garmendia *et al*. (2004) reported that generally, *V. dahliae* decreases the shoot biomass partly due to defoliation. This was corroborated by Karagiannidis *et al*. (2002) who observed a reduction of dry mass following *Verticillium* inoculation in eggplant. The same authors found that inoculation with AMF significantly increased dry shoot mass. In this study, treatments did not show differences probably due to low disease index observed. Results further showed that a *T. harzianum* and AMF mixture delayed the incidence of *Verticillium* wilt in the mid-season probably due to the ability of these fungal inoculants to control soil-borne pathogens. The AMF-treated plants are able to overcome pathogen attacks when compared to untreated plants (Azcón-Aguilar & Barea, 1997). Similarly, *T. harzianum* has been found to inhibit *Verticillium* mycelial growth *in vitro* and reduce its sclerogenesis intensity (Regragui & Lahlou, 2005). In cotton, Hanson (2000) found that seed treatment with *Trichoderma* strains could reduce symptoms of *Verticillium* wilt. In the same line, combined application of *T. harzianum* and AMF enhanced disease protection against *Rhizoctonia* damping-off in cucumber (Chandanie *et al*., 2009). Acquired resistance was probably due to the pre-activation of phenolic compounds, particularly phytoalexins and associated flavonoids and isoflavonoids, in tomato roots by AMF (Karagiannidis *et al*., 2002) or by blocking root entry points and thus influencing pathogen inoculum in the rhizosphere through antibiosis (Tahmatsidou *et al*., 2006).

Peat-amended Si reduced the incidence of *Verticillium* wilt at mid-season, with compelling evidence that Si might provide a mechanical barrier against pests and pathogens (Belanger *et al*., 1995) or elicit biochemical defense reactions, including the accumulation of lignin, phenolic compounds, and pathogenesis-related proteins in the infected plants (Chérif *et al*., 1992, Epstein, 1999; Qin & Tian, 2005). Silicon was observed to enhance activity of chitinases, peroxidases and polyphenoloxydases in roots of cucumber plants infected by *Pythium* (Chérif *et al*., 1994; Ma & Yamaji, 2006). Neither Si nor a *T. harzianum* and AMF mixture controlled *Verticillium* wilt throughout the growing season as all plants were affected during the final harvest. This is in agreement with Garmendia *et al*. (2004b), who observed disease similarity in non-mycorrhizal and mycorrhizal *Verticillium*-inoculated plants at the end of the experiment. Findings of this study support the view that biological control is mainly a means to reduce disease incidence rather than to eradicate it.

This study showed that seed treatment or growing medium amendment could not prevent *V. dahliae* in reducing tomato yield. The non-response of tomato plants to a *T. harzianum* and AMF mixture could also be due to the interactions between these two fungal inoculants, reducing their efficacy. Increased yield of *Verticillium*-challenged plants by AMF has been reported in pepper (Garmendia *et al*., 2004a). Inoculation with AMF increased yield of tomato (Utkhede, 2006). Similarly, *T. harzianum* has been reported to increase yield of cucumber, bell pepper and strawberry (Altintas & Bal, 2005; Bal & Altintas, 2006).

3.5.4 Conclusions

Results of this study clearly demonstrated the potential of a *T. harzianum* and AMF mixture to reduce the incidence of *Verticillium* wilt while slightly increasing marketable yield although not significantly so. Further, it shows that seed priming with SWE derived from *E. maxima* inhibited mycorrhizal root colonisation of tomato seedlings. Findings of this study suggest that *T. harzianum* and AMF could be considered as the ideal pre-sowing treatments on tomato when compared to *E. maxima* or Si. However, further investigation is needed to find out whether *T. harzianum* and AMF used in the mixture are efficient in improving seedling quality of tomato used alone or in combination.

CHAPTER 4[1]

TOMATO (*SOLANUM LYCOPERSICUM* L.) SEEDLING GROWTH AND DEVELOPMENT AS INFLUENCED BY *TRICHODERMA HARZIANUM* AND ARBUSCULAR MYCORRHIZAL FUNGI

4.1 ABSTRACT

Recent trends in soil microbiology suggest that certain soil microbes have a positive effect on seedling growth and development. A study was conducted to investigate the interactive effect of the plant-growth promoting fungi *Trichoderma harzianum* and arbuscular mycorrhizal fungi (AMF) in growth and development of tomato (*Solanun lycopersicum* L.) seedlings grown under greenhouse conditions. A 3 × 3 factorial experiment was laid out in a completely randomised design with six replications. At harvest (42 days after planting), when compared with the control, *T. harzianum* and/or AMF-treated plants improved shoot length, root length, dry shoot mass and dry root mass. Inoculation with AMF increased shoot N, P and S content of tomato seedlings, whereas pre-sowing with *T. harzianum* alone increased the shoot N. Generally, shoot Zn and Mn content were affected by both fungi, with the best results obtained when AMF was applied two weeks after *T. harzianum.* The percentage of roots colonised by AMF was less than 15% regardless of the time when *T. harzianum* was applied. However, the percentage of roots

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colonised by *T. harzianum* was greater than 90% at all times. In conclusion, this study suggested that AMF and *T. harzianum* have the potential to improve tomato seedling growth and development.

Keywords: Essential mineral nutrients, mycorrhiza, plant-growth promoting fungi, seedling quality, *Solanum lycopersicum*

4.2 INTRODUCTION

The need to produce quality tomato seedlings, capable of withstanding adverse abiotic and biotic stresses after transplanting and improve mineral nutrient uptake, inspired producers to consider a combined application with *Trichoderma harzianum* and arbuscular mycorrhizal fungi (AMF) in the nursery. Nursery inoculation of tomato with AMF resulted in stronger and superior quality seedlings (Gianinazzi *et al*., 2001), higher crop uniformity (Waterer & Coltman, 1988), better mineral nutrient uptake (Bethlenfalvay *et al*., 1988; Marschner & Dell, 1994; Chandanie *et al*., 2009), improved tolerance to soil-borne diseases (Pozo & Azcón-Aguilar, 2007), and both reduced stress and increased yields (Lovato *et al*., 1996; Chandanie *et al*., 2009). Similarly, *T. harzianum* enhanced plant growth and development (Harman & Taylor, 1990; Samuels, 2006; Liu *et al*., 2008), and provided protection against soil-borne pathogens that cause damping-off in tomato seedlings (Harman & Taylor, 1990).

The symbiosis between *T. harzianum* and AMF is widely reported in literature (Raupach & Kloepper, 1998; Meyer & Roberts, 2002). *Trichoderma* spp. have both antagonistic (Camporota,

1985; Wyss *et al*., 1992; McAllister *et al*., 1994) and stimulating effects on AMF (Calvet *et al*., 1992; McAllister *et al*., 1994) and vice versa. Antagonistic modes of action of *Trichoderma* include competition, mycoparasitism and production of antifungal metabolites (Lorito *et al.,* 1993; Stefanova *et al.,* 1999). Also, the species have a high reproductive capacity estimated at 12 h for spore germination (Woo *et al*., 2005; Liu *et al*., 2008). In spite of the increasing interest in the interactions between *T. harzianum* and AMF, information about these interactions in tomato seedlings production is scarce (McAllister *et al*., 1994; Fracchia *et al*., 1998). The objective of this study was to investigate the interactive effects of nursery inoculation with *T. harzianum* and AMF on growth and development of tomato seedlings under greenhouse conditions.

4.3 MATERIALS AND METHODS

4.3.1 Site description

The experiment was conducted under greenhouse conditions at the Hatfield Experimental Farm, University of Pretoria. Details of the study location are presented in Chapter 3 (Refer to 3.3.2)

4.3.2 Experimental design and treatments

The nine treatment combinations, *viz*. T_0M_0 (untreated/control), T_0M_1 (treated with AMF only, before sowing), T_0M_2 (treated with AMF only, 2 weeks after sowing), T_1M_0 (treated with *T. harzianum* only, before sowing), T_1M_1 (treated with both fungi before sowing), T_1M_2 (treated with *T. harzianum* before and AMF two weeks after sowing), T_2M_0 (treated with *T. harzianum*

only, 2 weeks after sowing), T_2M_1 (treated with *T. harzianum* at 2 weeks after sowing and AMF before sowing) and T_2M_2 (treated with both fungi 2 weeks after sowing), were arranged in a completely randomised design with six replications.

Commercial mycorrhizal inoculum Biocult© containing spores of *Glomus mossae*, was obtained from Biocult Ltd. (Sommerset West, South Africa). Commercial *Trichoderma* inoculum T-GRO containing spores of *T. harzianum* isolate DB 103 (1×10^9 colony forming units g^{-1}, as a wettable powder) was obtained from Dagutat Biolab (Johannesburg, South Africa). The microbial inoculants were thoroughly mixed with peat moss and vermiculite before applying them into the pasteurised sand:coir (seedling trays) or peat moss (PVC pipe) mixtures used for seedling production. The microbial inoculants were introduced either before sowing the seed or before transplanting the seedlings (two weeks later).

Seeds of tomato cv. Nemo-Netta were sown into cell plug trays filled with a pasteurised sand and coir mixture at a 50:50 (v/v) ratio. Trays were transferred to the germination room for three days and then moved to the greenhouse. Two weeks after sowing, seedlings were transplanted into a 30-cm long PVC pipe (diameter: 3.5 cm) filled with peatmoss and supported by a cylinder base (Figure 4.1). Plants were fertilised twice weekly with half strength modified Hoagland's solution (Spomer *et al*., 1997) and watered daily.

Figure 4.1 Tomato seedlings growing into PVC pipe supported by a cylinder base

4.3.3 Data collection

At harvest, six weeks after initiating the treatment, six randomly selected plants per treatment were collected and roots were separated from shoots. Shoot length and root length were recorded.

The percentage of AMF colonisation was determined using the grid-line intersect method (Brundrett *et al.*, 1996), which consists of recording the presence or absence of colonisation at each intersection of grid-line and roots. Details of root preparation, staining and clearing of root samples are described in Chapter 3. Root colonisation by *T. harzianum* was determined following the procedure described by Datnoff *et al.* (1995). Root pieces of 1 cm in length, washed and disinfected with 5% NaOCl, were plated on acidified potato dextrose agar or water agar amended with 100 µg streptomycin sulphate. Percentage root infection was determined by

counting the number of root pieces containing at least one colony of *T. harzianum* per root segment per plate, then dividing by the total number of root pieces and multiplying by 100.

Details of the determination of dry matter are presented in Chapter 3. Dried shoots and roots were each ground in a Wiley mill (Arthur H. Thomas, Philadelphia, PA) to pass through 1 mm sieve. One gram sample was digested in sulphuric acid at 410^{o}C and N determined by an auto analyser. Other essential nutrient elements were digested with a 2:1 nitric/perchloric acid mixture at 230^{o}C and nutrient elements (P, K, Ca, Mg, S, Mn, Zn, Cu and Mo) determined by inductive coupled plasma (ICP) spectrophotometry.

4.3.4 Data analysis

The analysis of data was done as described in Chapter 3. In addition, the degrees of freedom and their associated sum of squares were partitioned to provide the total treatment variation for different sources of variation (Little, 1981). Relevant ANOVA tables can be found in the Appendix.

4.4 RESULTS

4.4.1 Root colonisation by fungi

The *T. harzianum* × AMF interaction effect on root colonisation by either fungus was not significant for either growing season (Table 4.1). Seedlings inoculated with *T. harzianum* solely

had more than 90% root colonisation, whereas roots of sole AMF-treated seedlings had less than 15% colonisation six weeks after sowing. Using partitioning of the degree of freedom and their associated sum of squares (Little, 1981), *T. harzianum* contributed 99% to total treatment variation (TTV) in the percentage *Trichoderma* root colonisation. Similarly, the main source of variation in the percentage of mycorrhiza root colonisation was AMF, which accounted for over 96% of the TTV (Table 4.1).

Table 4.1 Partitioning of the treatment sum of squares (SS) derived from the ANOVA for the root colonisation of 6-week old tomato seedlings as influenced *Trichoderma harzianum* and arbuscular mycorrhizal fungi

Source of variance	DF	% Mycorrhiza		*% Trichoderma*		% Mycorrhiza		*% Trichoderma*	
		SS	%	SS	%	SS	%	SS	%
		2008 growing season				**2009 growing season**			
T. harzianum (T)	2	19.7	2.1ns	107215	99.7*	4.6	0.2ns	98415	99.8*
AMF (M)	2	902.48	96.1*	15	0.0ns	2013.4	99.3*	104	0.1ns
T×M	4	16.74	1.8ns	296	0.3ns	10.52	0.5ns	74	0.1ns
Total	53	938.92		107526		2028.5		98593	

ns, * are levels of significance (not significant, and P <0.001 respectively according to LSD test)

While treating seedlings with AMF during transplanting (M_2) resulted in a significant lower AMF colonisation as compared to the treatment at seeding (M_1) in both years, it did not have a significant impact on the *T. harzianum* colonisation (Table 4.2). Application of *T. harzianum* at sowing (T_1) or transplanting (T_2) had no significant impact on the colonisation of either AMF or *T. harzianum* in either year.

Table 4.2 Percentage root colonisation of 6-week old tomato seedlings as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi

Response variable	Mycorrhiza (%)	*Trichoderma* (%)	Mycorrhiza (%)	*Trichoderma* (%)
	2008 growing season		**2009 growing season**	
T (*T. harzianum*)				
T_0	5.83a	0.00b	8.50a	0.00b
T_1	5.94a	96.7a	8.61a	91.1a
T_2	4.61a	92.2a	8.72a	90.0a
M (AMF)				
M_0	0.00c	62.2a	0.00c	60.0a
M_1	9.83a	63.3a	14.56a	60.0a
M_2	6.56b	62.2a	10.50b	66.7a

Means followed by the same letter in a column are not significantly different ($P \leq 0.05$) according to Fisher's LSD test
T_0 = no *T. harzianum* applied; T_1 = *T. harzianum* before sowing; T_2 = *T. harzianum* two weeks after sowing; M_0 = no AMF applied; M_1= AMF before sowing; M_2= AM two weeks after sowing

4.4.2 Growth variables

This analysis revealed a significant interactive effect of *T. harzianum* and AMF for shoot and root length, which only explained half of the total variability in both seasons (Table 4.3). *Trichoderma harzianum* contributed ca. 41% of the TTV in the mean shoot length for both seasons. This treatment also explained 21% and 29% of the TTV in mean root length in 2008 and 2009 growing seasons, respectively. In 2008, AMF contributed 29% of the TTV in mean root length but only 15% during the second growing season. The TTV of this treatment in mean shoot length in both seasons were < 14%.

During the first season, inoculating both fungi at sowing (T_1M_1) increased the shoot and root length by 40 and 30%, respectively, as compared to the control plants (Table 4.4). The highest

shoot length was obtained with late *T. harzianum* inoculation (T_2M_0). In 2009, the highest shoot and root lengths were recorded with T_1M_1 and T_2M_0, respectively, whereas the lowest counts were obtained in the untreated plants (T_0M_0). In both seasons, all the microbial inoculated seedlings, except for late microbial inoculations (T_2M_2), increased shoot and root lengths when compared with the control.

Table 4.3 Partitioning of the treatment sum of squares (SS) derived from ANOVA for the plant growth variables of 6-week old tomato seedlings as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi

Source of variance	DF	Shoot length		Root length		Dry shoot mass		Dry root mass	
		SS	%	SS	%	SS	%	SS	%
		2008 growing season							
T. harzianum (T)	2	455.39	41.2***	185.27	20.5***	92.01	45.0***	6.95	56.6***
AMF (M)	2	87.66	7.9***	260.34	28.8***	32.63	15.9*	0.87	7.1ns
T×M	4	561	50.8***	459.21	50.8***	79.99	39.1**	4.45	36.3*
Total	53	1104.05		904.82		204.62		12.27	
		SS	%	SS	%	SS	%	SS	%
		2009 growing season							
T. harzianum (T)	2	145.67	40.1***	135.38	29.3*	37.39	81.1***	1.14	78.7*
AMF (M)	2	50.65	13.9ns	70.27	15.2ns	2.11	4.6ns	0.04	2.6ns
T×M	4	167.34	46.0**	256.99	55.5*	6.59	14.3ns	0.27	18.8ns
Total	53	363.65		462.64		46.09		1.44	

ns, *,**,*** are levels of significance (not significant, P <0.05, P <0.01, P <0.001 respectively according to LSD test)

4.4.3 Biomass production

There was a significant *T. harzianum* × AMF effect for the dry shoot and root mass during the first growing season, which accounted for ca. 40% of the TTV (Table 4.3). The major source of variability was due to *T. harzianum*, which contributed nearly 50% of the TTV. Interestingly, in 2009, *T. harzianum* accounted for ca. 80% of the TTV with small contributions from AMF and *T. harzianum* and AMF interactions. During the first season, compared to the control plants, the combined inoculation of *T. harzianum* and AMF before sowing (T_1M_1) resulted in 35% higher dry shoot mass, whereas inoculating both fungi simultaneously 2 weeks after sowing (T_2M_2) resulted only in 13% increase (Table 4.4). The highest increase (52%) in dry shoot mass was obtained with T_1M_0, while all microbial inoculants increased dry shoot mass. Dry root mass was increased (up to 37%) when *T. harzianum* was inoculated before plant and AMF two weeks later (T_1M_2). However, a negative interaction between *T. harzianum* and AMF was observed when both fungi were applied 2 weeks after sowing (T_2M_2), resulting in the lowest dry root mass in 2008. During the second season, irrespective of the AMF treatment, inoculating *T. harzianum* before sowing increased the dry mass of the shoots and roots by 19% and 11%, respectively, whereas dry shoot and root mass in plants inoculated with *T. harzianum* 2 weeks later, did not differ from those of the control. The only exception was in terms of shoot dry mass in the absence of AMF (T_2M_0).

Table 4.4 Plant growth variables of 6-week old tomato seedlings as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi

Treatment	Shoot length (cm)			Root length (cm)			Dry shoot mass (g plant^{-1})			Dry root mass (g plant^{-1})		
	M_0	M_1	M_2	M_0	M_1	M_2	M_0	M_1	M_2	M_0	M_1	M_2
2008 growing season												
T_0	16.73f	25.12c	21.40e	22.38e	33.74a	29.28bc	6.00d	8.80bc	6.93cd	1.89b	2.46ab	1.94b
T_1	27.34b	28.11ab	28.56a	26.63d	34.23a	32.66a	12.50a	9.17bc	9.36bc	2.91a	2.83a	2.98a
T_2	29.16a	23.08d	17.15f	29.86b	26.92cd	23.21e	10.31ab	6.91cd	6.89cd	2.89a	1.91b	1.84b
2009 growing season												
T_0	20.25d	26.52ab	25.15bc	21.82c	28.88ab	26.33bc	8.24*	9.33*	8.71*	2.47*	2.67*	2.50*
T_1	27.07ab	29.30a	27.31ab	27.80ab	29.68ab	30.10ab	10.58	10.70	10.80	2.79	2.90	2.92
T_2	27.47ab	25.30bc	22.66cd	31.75a	30.00ab	24.82bc	9.75	9.46	8.54	2.69	2.54	2.51

Means followed by the same letter within column and row are not significantly different ($P \leq 0.05$) according to Fisher's LSD test

* No significant differences

T_0 = no *T. harzianum* applied; T_1 = *T. harzianum* before sowing; T_2 = *T. harzianum* two weeks after sowing; M_0 = no AMF applied; M_1= AMF before sowing; M_2= AM two weeks after sowing

4.4.4 Shoot chemical analysis

Neither *T. harzianum* nor AMF affected essential nutrient element content such as K, Ca, Cu, Mg or Mo (Table 4.5). There was a significant *T. harzianum* × AMF interaction term for the shoot Mn and Zn content, whereas P and S were only affected by AMF. Analysis demonstrated that the mean shoot N content of seedlings was affected by both the main effects of *T. harzianum* and AMF but not their interactions.

Table 4.5 Results of ANOVA (P values) executed for the shoot mineral nutrient content for the 2008 growing season

Response variable	N	P	K	Ca	Mg	S	Mn	Zn	Cu	Mo
T (df = 2)	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
M (df = 2)	***	*	ns	ns	ns	**	*	*	ns	ns
T×M (df =4)	ns	ns	ns	ns	ns	ns	*	*	ns	ns

ns, *,**,*** are levels of significance (not significant, $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively according to LSD test)
T = *T. harzianum*; M = AMF

Inoculating with *T. harzianum* before sowing (T_1) increased the N shoot content by 6%, whereas later inoculation (T_2) gave similar results to the uninoculated plants (T_0) (Table 4.6). On the other hand, when compared with the control (M_0), inoculating AMF before (M_1) or 2 weeks after sowing (M_2) increased the shoot N content by 9 and 10%, respectively. Inoculating AMF before (M_1) or after sowing (M_2) increased the shoot P content of tomato seedlings by ca. 18 and 16%, respectively. Shoot S increased by 15% when AMF was inoculated before sowing (M_1), whereas later inoculation (M_2) had no effect on the content of this nutrient element.

Table 4.6 Macronutrient shoot contents of 6-week old tomato seedlings as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi

Response variable	N (%)	P (%)	K* (%)	Ca* (%)	Mg* (%)	S (%)
T (*T. harzianum*)						
T_0	4.42b	0.62a	2.97	4.19	1.06	1.63a
T_1	4.72a	0.63a	2.75	4.17	1.03	1.56a
T_2	4.45b	0.60a	2.72	4.48	1.13	1.77a
M (AMF)						
M_0	4.23b	0.54b	2.80	4.00	1.05	1.57b
M_1	4.65a	0.66a	2.86	4.47	1.13	1.83a
M_2	4.71a	0.64a	2.77	4.37	1.05	1.56b

Means followed by the same letter in a column are not significantly different ($P \leq 0.05$) according to Fisher's LSD test

*No significant difference ($P \leq 0.05$) according to Fisher's LSD test

T_0 = no *T. harzianum* applied; T_1 = *T. harzianum* before sowing; T_2 = *T. harzianum* two weeks after sowing; M_0 = no AMF applied; M_1 = AMF before sowing; M_2 = AM two weeks after sowing

Inoculating *T. harzianum* and AMF before (T_1M_1) or after (T_2M_2) sowing, resulted in 18 and 9% increase in shoot Mn content, respectively (Table 4.7). However, the highest Mn shoot content increase (33%) was obtained with a combination of early *T. harzianum* and late AMF application (T_1M_2). Similarly, for Zn shoot content, the highest increase (34%) was recorded with T_1M_2, while T_1M_1 and T_2M_2 also resulted in an increase of about 13% and 10%, respectively.

Table 4.7 Micronutrient shoot contents of 6-week old tomato seedlings as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi

Treatment	Mn (ppm)			Zn (ppm)			Mo *(ppm)			Cu* (ppm)		
	M_0	M_1	M_2	M_0	M_1	M_2	M_0	M_1	M_2	M_0	M_1	M_2
T_0	19.67d	27.67ab	26.00abc	24.67d	35.00ab	32.67abc	14.86	16.18	14.80	10.28	11.67	10.18
T_1	22.67cd	24.00bcd	29.33a	28.33cd	30.33bcd	37.33a	14.26	14.39	13.72	11.74	12.47	10.45
T_2	23.00cd	22.33cd	21.67cd	29.33bcd	27.67cd	27.33cd	14.20	12.64	14.97	12.60	14.74	8.75

Means followed by the same letter within column and row are not significantly different ($P \leq 0.05$) according to Fisher's LSD test

*No significant difference ($P \leq 0.05$) according to Fisher's LSD test

T_0 = no *T. harzianum* applied; T_1 = *T. harzianum* before sowing; T_2 = *T. harzianum* two weeks after sowing; M_0 = no AMF applied; M_1= AMF before sowing; M_2= AM two weeks after sowing

4.5 DISCUSSION

Nursery inoculation of tomato with *T. harzianum* and AMF improved most of the growth variables of tomato seedlings, increased nutrient element uptake and permitted microbial root colonisation. Uninoculated plants showed no *Trichoderma* or AMF colonisation, indicating that these fungi were not indigenous to the specific growth media. The low mycorrhizal colonisation (< 15%) observed was in agreement with Chandanie *et al.* (2009), who argued that the 13% level of colonisation with AMF observed before transplanting in the field should be considered adequate for successful establishment of mycorrhizal seedlings. According to Bierman and Linderman (1983), less than 13% root colonisation should not be a concern as these fungi would spread rapidly to new roots after transplanting. On the other hand, the higher *Trichoderma* root colonisation could be due to its high reproductive capacity as stated by Woo *et al*., (2005). Results in this study showed that low mycorrhizal and high *Trichoderma* root colonisations were due to the ability of these fungi to colonise roots rather than the interactions on each other. This is not in agreement with McGovern *et al.* (1992) who reported antagonistic effect of *Trichoderma* on AMF in tomato. Chandanie *et al*. (2009) observed a decreased *T. harzianum* growth due to AMF inoculation in cucumber (*Cucumis sativus*). However, Green *et al.* (1999) found a mutually inhibitory interaction between *T. harzianum* and the external mycelia of an AMF, *Glomus intraradices.* Apparently, the interactions between *Trichoderma* and AMF are species and host plant specific (Rousseau *et al*., 1996; Fracchia *et al*., 1998; Green *et al*., 1999).

Trichoderma harzianum and AMF, either inoculated alone or in combination increased the root and shoot length of tomato. Generally, improved plant growth had been observed with

Trichoderma (Duffy *et al*., 1997; Ozbay & Newman, 2004) and AMF inoculations (Tahat *et al*., 2008). Improved plant growth observed in these experiments might be due to increased solubility of insoluble plant nutrients by *Trichoderma* spp. (Kaya *et al*., 2009) or enhanced immobile nutrient elements uptake by AMF (Bethlenfalvay *et al*., 1988; Marschner & Dell, 1994; Chandanie *et al*., 2009).

Results of this study demonstrated the beneficial effect of nursery inoculation with *T. harzianum* and/or AMF on dry matter production of tomato seedlings. This is in agreement with Ozbay and Newman (2004), who observed an increase in dry shoot mass due to *Trichoderma* inoculation, whereas Tahat *et al.* (2008) observed the same trend with AMF. Chandanie *et al*. (2009) demonstrated that the combined inoculation of AMF with *Trichoderma* synergistically increased the dry shoot mass when compared with inoculation of *Trichoderma* and AMF alone. McAllister *et al.* (1994) reported a decrease in dry shoot mass when *Trichoderma* was inoculated before or at the same time with AMF. In this study, both fungi either applied alone or in combination, improved the plant growth, except when simultaneously applied 2 weeks after sowing. The negative interaction when combined inoculation is applied at later date could be due to competition for nutrients or space.

In this study, the nursery microbial inoculation had no effect on K, Ca or Mg shoot content, which is in agreement with Karagiannidis *et al*. (2002), who did not find any positive effect of mycorrhiza on shoot K and Ca content. Increased K and Mg content have been reported in wheat inoculated with AMF (Tarafdar & Marschner, 1995), whereas *Trichoderma* spp. did not increase the shoot Ca, K and Mg content in tomato seedlings grown in hydroponics (Yedidia *et al*., 2000).

Nevertheless, these findings demonstrated the beneficial effect of AMF inoculation on shoot N, P and S in tomato seedlings. Increased N uptake due to AMF inoculation has been reported by Thomson *et al*. (1996) and Karagiannidis *et al*. (2002). Similarly, the increased shoot P content following AMF inoculation is in agreement with other observations (Nurlaeny *et al*., 1996; Yedidia *et al*., 2000; Al-Karaki, 2006), whereas Inbar *et al*. (1994) did not observe any positive effects. With regards to shoot S content, late inoculation was not different to the uninoculated plants, suggesting that early application is advisable for increased S uptake. Increased S content of plants with mycorrhiza has been reported previously (Rhodes & Gerdemann, 1978).

Shoot Zn and Mn increased probably due to an increased availability of these nutrient elements due to *Trichoderma* and AMF inoculation (Kaya *et al*., 2009). However, this is in disagreement with a reduced concentration of Mn and Zn on leaves of AMF-infected maize plants (Weissenhorn *et al*., 1995). Other micronutrients such as Cu and Mo were unaffected by the nursery microbial inoculation possibly due to their low concentration in the growing medium.

In conclusion, results showed that nursery inoculation of tomato with *T. harzianum* and/or AMF improved growth and development of tomato seedlings. *Trichoderma harzianum* and AMF synergistically improved most of the growth variables in tomato seedlings. A negative *T. harzianum* × AMF interaction was only observed 2 weeks after sowing, probably due to competition for nutrient elements and/or sites for infection. In contrast to *T. harzianum*, which had little effect on essential nutrient elements, AMF inoculation affected the nutrient uptake of key elements such as N, P, S, Zn, and Mn. Although the mycoparasistic effect of *Trichoderma* spp. is well known, results of this study demonstrated that, this plant-growth promoting fungi can

successfully be inoculated with AMF for improved seedling health and development of tomato production.

CHAPTER 5[1]

YIELD AND NUTRIENT CONTENT OF GREENHOUSE PRODUCED TOMATO (*SOLANUM LYCOPERSICUM* L.) AS INFLUENCED BY *TRICHODERMA HARZIANUM* AND *GLOMUS MOSSEAE* INOCULATION

5.1 ABSTRACT

Recent trends in soil microbiology suggest that fungal inoculants such as *Trichoderma harzianum* or arbuscular mycorrhizal fungi (AMF) have the potential to improve yield and fruit quality of crops. The purpose of this study was to investigate the effect of inoculating tomato (*Solanum lycopersicum* L.) with *T. harzianum* and the AMF (*Glomus mosseae*) on yield and nutrient content of tomato fruit. A factorial experiment (3 × 3) with three application timings for each of *T. harzianum* and AMF, namely uninoculated control, inoculated before sowing and two weeks after sowing, giving nine treatment combinations was conducted in a greenhouse. Both fungal inoculants increased total yield and marketable yield of tomato, but these increases were not statistically significant ($P > 0.05$). Inoculating tomato with AMF before sowing significantly increased the percentage of extra-large fruit, while inoculation with *T. harzianum* two weeks after sowing lowered the Ca and Mg contents of tomato fruit. *Trichoderma harzianum* and AMF

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inoculation increased the lycopene content, but did not affect the antioxidant activity and vitamin C of the tomato fruit. Results of this study suggested that *T. harzianum* and AMF have the potential to improve yield and quality of tomatoes produced in a greenhouse.

Keywords: *Glomus mosseae*, mycorrhizae, nutrient uptake, phytochemical content, *Solanum lycopersicum*, *Trichoderma harzianum*

5.2 INTRODUCTION

Tomato is the second-most important vegetable in the world after potato (Dorais *et al*., 2008), with a worldwide production of 129 million tons in 2008 (FAO, 2010). It is an excellent source of health-promoting compounds due to the balanced mixture of antioxidants including vitamins C and E, lycopene, beta-carotene, lutein and flavonoids (Dorais *et al*., 2008), amino acids, proteins, fatty acids and carbohydrates (Hauffman & Bruce, 2002; Heeb, 2005). Tomato is also rich in macronutrients, especially K (Wilcox *et al*., 2003; Odriozola-Serrano *et al*., 2009), P, Mg and Ca (Suárez *et al*., 2008) and contains high amounts of trace elements such as Fe, Mn, Zn, and Cu (Ahmed *et al*., 2011). Nutritional studies have suggested that regular consumption of fruits and vegetables, including tomatoes, can play an important role in preventing cancer and cardiovascular diseases in humans (Heber, 2000; Rao & Agarwal, 2000; Toor & Savage, 2005).

Since tomato fruit plays an important role in human health (Chapagain & Wiesman, 2004), strategies for increasing fruit production and quality are of great interest to producers (Gruda, 2005; Flores *et al*., 2010). Compelling evidence in literature suggest that mineral nutrients can

affect the antioxidant content of tomato fruit and overall tomato fruit quality. For instance, increased Ca levels in soil solution can increase the Ca content in tomato fruit, but decrease carotene content and lycopene levels (Paiva *et al*., 1998). Adequate Ca supply is essential for fruit firmness and extended shelf life (Cooper & Bangerth, 1976). Increasing K increases carotenoid concentration, particularly the lycopenes (Trudel & Ozbun, 1971). According to Mozafar (1994), beta-carotene content in fruit increases with increasing levels of K, Mg, Mn, B, Cu and Zn, whereas Lester (2006) reported that ascorbic acid increased with increasing levels of K, Mn, B, Cu and Zn. Phosphorus may also increase the fruit concentration of phytochemicals such as ascorbic acid, flavonoids and lycopene (Dorais *et al.,* 2008). The need for producing high quality food, while mitigating deleterious environmental impact (Mader *et al*., 2002) makes the use of biofertilisers a preferred alternative and feasible production practice in contrast to the use of inorganic fertilisers (Mena-Violante & Olade-Portugal, 2007).

Indications are that *T. harzianum* can improve the solubility of soil micronutrients, such as Zn, Cu, Fe, Mn (Kaya *et al*., 2009) whereas arbuscular mycorrhizal fungi (AMF) enhance the uptake of N, P and K (Cardoso & Kuyper, 2006). However, information regarding their combined effects on the phytochemical content, nutrient content and yield of tomato is inconsistent (Gosling *et al*., 2006), inadequate (Dumas *et al*., 2003) or simply lacking. Considering that the effects of soil microbial populations on the yield and quality of crops can be considerable (Bourn & Prescott, 2002; Dorais *et al*., 2008), there is a need to investigate the effects of microbial inoculants such as *T. harzianum* and AMF on tomato production. The effects of combined inoculation of *T. harzianum* and AMF in improvement of nutrient availability and uptake by tomato plants and the resultant improvement in yield and fruit quality of tomato are not

documented. The objective of this study was therefore to determine the effects of nursery inoculation with *T. harzianum* and AMF on fruit yield, fruit quality and nutrient content of tomato fruit produced under greenhouse conditions.

5.3 MATERIALS AND METHODS

5.3.1 Site description

The experiment was conducted under greenhouse conditions at the Hatfield Experimental Farm, University of Pretoria. Details of the study location are presented in Chapter 3 (Refer to 3.3.2).

5.3.2 Experimental design and treatments

The experimental design and treatments are described in Chapter 4, with exception that seedlings were transplanted into 5 L pot filled with a sand plus coir mixture (ratio 2:1) two weeks after sowing. Plant pots were spaced at 0.4 m between plants in a double row with 1 m between rows. Modified Hoagland's solution was used for fertigation.

5.3.3 Data collection

Harvest

Harvesting was done as described in Chapter 3, but for ten successive weeks. At mid-harvest, twenty fruit per replicate of colour stage six, using tomato colour chart standard (Kleur-stadia tomaten, Holland), were used for fruit quality analysis. Fruit were divided into two groups as representative samples for the two fruit quality analysis procedures with the first group being used for the determination of the macro-elements, whereas the second group was used for the analysis of antioxidant activity, vitamin C and lycopene contents.

Yield and fruit size distribution

Details of yield and marketable yield determination are described in Chapter 3. Fruit diameter was measured with a digital caliper (Starreett, 727 Series, Athol, Massachusetts, USA) and divided into four categories, using a scale by Jones (1999): extra-large (> 67 mm), large (54–67 mm), medium (47–54 mm) and small (< 47 mm).

Fruit mineral and phytochemical contents

Total P, K, Ca and Mg were determined by microwave digestion followed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (USEPA, 1986). Phytochemical content in fruit analysis was performed at Limpopo Agro-food Technology Station, Polokwane, South Africa. Lycopene content was extracted from tomatoes with a hexane-acetone-ethanol (2:1:1) mixture using methods of Sharma and Le Maguer's (1996) and Toor's *et al*. (2006). Vitamin C content was measured by a Metrohm 670 titroprocessor (Metrohm Herisau,

Switzerland) using the method of the Association of Official Analytical Chemists (AOAC, 1990; Toor *et al*., 2006). Antioxidant activity was estimated by the Trolox Equivalent Antioxidant Activity method (Miller & Rice-Evans, 1997).

5.3.4 Data analysis

The analysis of data is described in Chapter 3. Relevant ANOVA tables can be found in the Appendix.

5.4 RESULTS

5.4.1 Yield and fruit size distribution

Main treatments and their interaction had no significant effect on the number of fruit (NFP), marketable yield (MYP) and total yield (TYP) of tomato per plant, or percentage of large (LF) and small fruit (SF) (Table 5.1). However, AMF inoculation had a significant effect on the production of extra-large (ELF) and medium fruit (MF).

Both fungal inoculants increased the yield and marketable yield of tomato as compared to the untreated plants ($P > 0.05$) (Table 5.2). Mean comparison showed that the highest total yield (8.16 kg plant^{-1}) and marketable yield (79.8%) were achieved with the combined inoculation of *T. harzianum* and AMF before seeding (T_1M_1).

Table 5.1 Results of ANOVA (p values) executed for the yield and yield components of tomato plants

Treatment	NFP	TYP	MYP	ELF	LF	MF	SF
T (*T. harzianum*)	ns	ns	ns	ns	ns	ns	ns
M (AMF)	ns	ns	ns	*	ns	**	ns
T × M	ns	ns	ns	ns	ns	ns	ns

ns, *,** are levels of significance (not significant, $P \leq 0.05$, $P \leq 0.01$, respectively according to LSD test)

NFP = Number of fruit/plant; TYP = Total yield/plant; MYP = Marketable yield/plant

ELF = Extra-large fruit; LF = Large-fruit; MF = Medium fruit; SF = Small fruit

Table 5.2 Number of fruit, yield and marketable yield of tomato as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi

Treatment	Number of fruit* (plant^{-1})			Total yield* (kg plant^{-1})			Marketable yield* (%)		
	M_0	M_1	M_2	M_0	M_1	M_2	M_0	M_1	M_2
T_0	138.2	147.3	143.0	7.19	7.59	7.47	75.4	76.4	76.4
T_1	139.6	148.1	145.8	7.34	8.16	8.02	75.2	79.8	77.4
T_2	137.7	140.3	140.1	7.42	8.00	7.74	76.0	79.4	77.5

*No significant difference ($P \leq 0.05$) according to Fisher's LSD test

T_0 = no *T. harzianum* applied; T_1 = *T. harzianum* before sowing; T_2 = *T. harzianum* two weeks after sowing; M_0 = no AMF applied; M_1 = AMF before sowing; M_2 = AM two weeks after sowing

Regardless of *T. harzianum* application, inoculating with AMF before sowing (M_1) increased the percentage of extra-large fruit by about 8% as compared to the uninoculated plants (M_0), but were similar to those inoculated with AMF two weeks after sowing (M_2) (Table 5.3). In terms of medium fruit, inoculating AMF before (M_1) or two weeks after sowing (M_2) decreased the percentage of class-3 fruit by about 23.6 and 15.5%, respectively, when compared with uninoculated plants (M_0).

5.4.2 Tomato fruit mineral content

Trichoderma harzianum inoculation had a significant effect on Ca and Mg fruit contents, while K in fruit was only affected by AMF (Table 5.4). Inoculating *T. harzianum* two weeks after sowing (T_2) decreased the fruit contents of Ca and Mg by about 21% and 10%, respectively, when compared to the uninoculated plants (T_0) (Table 5.5).

Table 5.3 Fruit size of tomato as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi

Response variable	Extra-large fruit (%)	Large fruit (%)	Medium fruit* (%)	Small fruit* (%)
T (*T. harzianum*)				
T_0	45.39a	29.49	14.14a	10.86
T_1	45.00a	30.15	14.73a	10.22
T_2	44.58a	32.36	13.15a	9.91
M (AMF)				
M_0	42.43b	31.37	16.09a	10.14
M_1	46.54a	30.94	12.28b	10.23
M_2	46.54a	29.69	13.64b	10.63

Means followed by the same letter in a column are not significantly different ($P \leq 0.05$) according to Fisher's LSD test

*No significant difference ($P \leq 0.05$) according to Fisher's LSD test

T_0 = no *T. harzianum* applied; T_1 = *T. harzianum* before sowing; T_2 = *T. harzianum* two weeks after sowing; M_0 = no AMF applied; M_1 = AMF before sowing; M_2 = AM two weeks after sowing

Table 5.4 Results of ANOVA (p values) executed for the chemical and phytochemical contents

Treatment	P	K	Ca	Mg	AA	VC	LC
T (*T. harzianum*)	ns	ns	*	*	ns	ns	*
M (AMF)	ns	**	ns	ns	ns	ns	*
T × M	ns	ns	ns	ns	ns	ns	*

ns, * are levels of significance (not significant, $P \leq 0.05$, $P \leq 0.01$, respectively according to LSD test)

AA = Antioxidant activity; VC =Vitamin C; LC = Lycopene content

Table 5.5 Chemical fruit contents of tomato as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi

Response variable	P* (mg/100 g FM)	K* (mg/100 g FM)	Ca (mg/100 g FM)	Mg (mg/100 g FM)
T (*T. harzianum*)				
T_0	0.350a	185.7a	12.67b	14.22ab
T_1	0.368a	157.6a	11.89ab	15.33a
T_2	0.340a	162.8a	10.44b	12.89b
M (AMF)				
M_0	0.337a	184.7a	10.44a	14.67a
M_1	0.389a	180.0b	12.56a	14.67a
M_2	0.332a	141.3c	12.00a	13.11a

Means followed by the same letter in a column are not significantly different ($P \leq 0.05$) according to Fisher's LSD test

*No significant difference ($P \leq 0.05$) according to Fisher's LSD test

T_0= no *T. harzianum* applied; T_1= *T. harzianum* before sowing; T_2= *T. harzianum* two weeks after sowing; M_0= no AMF applied; M_1= AMF before sowing; M_2= AM two weeks after sowing

FM: fresh mass

5.4.3 Phytochemical analysis

Lycopene content was the only phytochemical that was significantly affected by main and interactive effects of *T. harzianum* and AMF applications (Table 5.4). Inoculating *T. harzianum* and AMF before sowing (T_1M_1) increased the lycopene content by about ca. 14% as compared to the uninoculated plants (T_0M_0), which in turn was higher (10%) than when both fungi were applied two weeks after sowing (T_2M_2) (Table 5.6). The highest lycopene content (17.9 mg/100 g FM) was obtained with the combined application of *T. harzianum* and AMF, when simultaneously inoculated before sowing (T_1M_1), whereas the lowest count (9.5 mg/100 g FM) was obtained with late AMF, application in the absence of *T. harzianum* (T_0M_2). Vitamin C content and antioxidant activity of tomato fruit were not affected by inoculation.

Table 5.6 Phytochemical fruit content of tomato as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi

Treatment	Lycopene (mg/100 g FM)			Vitamin C* (mg/100 g FM)			Antioxidant activity* (mg Trolox/l)		
	M_0	M_1	M_2	M_0	M_1	M_2	M_0	M_1	M_2
T_0	15.47ab	17.84a	9.45c	20.33	23.33	24.67	5.09	5.06	5.10
T_1	16.05ab	17.96a	16.65ab	25.00	26.33	28.00	5.08	5.02	5.03
T_2	14.75ab	14.07ab	13.86b	22.00	25.33	25.00	5.10	4.98	5.01

Means followed by the same letter within column and row are not significantly different ($P \leq 0.05$) according to Fisher's LSD test

*No significant difference ($P \leq 0.05$) according to Fisher's LSD test

T_0 = no *T. harzianum* applied; T_1 = *T. harzianum* before sowing; T_2 = *T. harzianum* two weeks after sowing; M_0 = no AMF applied; M_1 = AMF before sowing; M_2 = AM two weeks after sowing

FM: fresh mass

5.5 DISCUSSION

In this study, non-significant increases in fruit yield and marketable fruit were observed. Enhanced yield and marketable yield have previously been reported with bacterial inoculants. For instance, inoculating tomato with *Bacillus subtilus* increased yield and marketable yield in tomato (Mena-Violante & Olade-Portugal, 2007). The two authors suggested that facilitating plant nutrition could be the mechanism through which this microbial inoculant enhanced crop yield. Bal and Altintas (2008) reported non-significant increases in lettuce yield with *T. harzianum* inoculation. Similarly, Bal and Altintas (2006) did not observe an increase in fruit yield of tomato. Contrary to Salvioli *et al*. (2008) who reported improved tomato yield following mycorrhizal inoculation, Kaya *et al*. (2009) observed similar effect only when AMF was applied on salt-stressed tomato plants.

Fruit size is an important factor for fresh produce marketing. In this study, AMF significantly increased the percentage of extra-large fruit. Similar results were reported with pepper inoculated with different plant growth-promoting rhizobacteria (PGPR) strains (Vavrina, 1999; Mena-Violante & Olade-Portugal, 2007). The increased fruit size observed during this study could be associated with triggering of molecules or enzymes responsible for modulating tomato fruit cell expansion. In particular, sucrose synthase is thought to play a central role in developing tomato fruit (D'Aoust *et al*., 1999; Carrari & Fernie, 2006), whereas auxins have been suggested to promote fruit cell expansion by causing an increase in cell wall extensibility (Gillaspy *et al*., 1993; Catalá *et al*., 2000). Increased root auxins after mycorrhizal inoculation have been reported for maize (Ludwig-Müller & Güther, 2007).

The role of AMF on the uptake of P is well-documented in the literature. Phosphorus is believed to help increase the number of blossoms during early growth and early fruit set (Zobel, 1966; Sainju *et al*., 2003), thus, increasing tomato fruit yield (Sainju *et al*., 2003). During the course of this experiment, the fruit P content in all the treatments was similar. In the present study, fertiliser was applied thus AMF inoculation would not necessarily increase P content. Contrary to P content of the fruit, K content in fruit was lowered when AMF was applied two weeks after sowing, probably due to assimilated competition for carbon. The findings of this study showed that *T. harzianum* might have a detrimental effect on the uptake of Ca and Mg as both nutrients were significantly reduced in fruit when *T. harzianum* was applied two weeks after sowing. The low transport of these nutrients to the fruit could be due to ion interactions in the root zone (Shear, 1975; Schimanski, 1981).

Although information on the effect of microbial inoculants on phytochemical content of tomato fruit is scarce, results in this study clearly demonstrated that *T. harzianum* and AMF can play a minor role in their accumulation in tomato fruit. The antioxidant activity of tomato fruit, which depends on genetic and environmental factors and varies over the ripening stage (Hart & Scott, 1995; Javanmardi & Kubota, 2006), was not affected by AMF and *T. harzianum*. Similarly, vitamin C content in fruit remained unchanged regardless of *T. harzianum* or AMF inoculation. Lycopene was increased by both *T. harzianum* and AMF inoculation, which was in agreement with Ulrichs *et al*. (2008), who found an increased lycopene content in tomato fruit due to AMF inoculation. A plausible explanation could be that lycopene, which develops rapidly in fruit in darker conditions such as those protected by crop foliage (Soto-Zamora *et al*., 2005; Javanmardi

& Kubota, 2006) has increased due to the higher plant biomass (data not shown) of fungi-inoculated plants observed during the trial.

In conclusion, AMF and *T. harzianum* have negligible influences on yield of tomato. The slight increase in yield as well as in the percentage of extra-large fruit, suggest that these fungal inoculants likely have biofertilizer effects on tomato production. Nutrient and phytochemical contents varied depending on the inoculation time. Generally, combined inoculation with *T. harzianum* and AMF during sowing increased the fruit lycopene content, while late inoculation with *T. harzianum* lowered the fruit Ca and Mg contents of tomato fruit. The findings of this study suggest an early inoculation with *T. harzianum* and AMF for improved tomato fruit quality. Further investigation would however be required to find out if this mixture can improve tomato crop performance under field conditions.

CHAPTER 6[1]

RESPONSE OF TOMATO (*SOLANUM LYCOPERSICUM* L.) TO NURSERY INOCULATION WITH *TRICHODERMA HARZIANUM* AND ARBUSCULAR MYCORRHIZAL FUNGI UNDER FIELD CONDITIONS

6.1 ABSTRACT

The effect of nursery inoculation of tomato (*Solanum lycopersicum* L.) with *Trichoderma harzianum* and arbuscular mycorrhizal fungi (*Glomus mosseae*) on fungal root colonisation, plant growth, yield and quality of field grown tomato was investigated. The four treatments included *T. harzianum*, AMF, *T. harzianum* + AMF, and uninoculated control. At mid-harvest, 84 days after transplanting, no interactive effects of the fungi on the external mycelium growth were observed. Inoculation with AMF alone or in combination with *T. harzianum* increased dry shoot mass by 35% and 30%, respectively, during the first season, and by 30% and 21%, respectively, during the second growing season. *Trichoderma harzianum* increased the percentage of large fruit by 76% in 2008–2009, whereas AMF increased the percentage of extra-large fruit by 44% in 2009–2010. Inoculated tomato seedlings with *T. harzianum* and/or AMF significantly increased early yield of tomato, by 10%, 65% and 70%, respectively, during 2008–2009, and by 27%, 36% and 37%, respectively during the 2009–2010 growing season.

[1] **Publication based on this chapter:**

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In conclusion, results of the study suggested that *T. harzianum* and AMF have the potential to improve growth, early yield and fruit size of field-grown tomato.

Keywords: *Mycorrhiza*, nursery inoculation, *Solanum lycopersicum*, *Trichoderma*

6.2 INTRODUCTION

Conventional tomato growers heavily rely on synthetic fertilisers and pesticides to achieve desirable fruit yield, resulting in soil fertility loss, unbalanced nutrition, nutrient leaching and poor soil quality. Increasing concerns over soil degradation and loss of biodiversity have enthused producers to consider alternative low-input agriculture such as organic farming. In South Africa, some growers make use of *T. harzianum* and arbuscular mycorrhizal fungi (AMF) in the nursery to improve plant growth and to control soil-borne pathogens (Taurayi, 2011).

Trichoderma harzianum is well-studied as a biological control agent, with indisputable results that have demonstrated the influence of *Trichoderma* strains in disease protection (Datnoff *et al*., 1995; Tsahouridou & Thanassoulopoulos, 2002), particularly in controlling damping-off in tomato production (Lewis & Lumsden, 2001). Also, *Trichoderma* strains improved tomato plant growth and development (Chang *et al*., 1986; Gravel *et al*., 2007), but with little evidence of increased yield. However, Bal and Altintas (2008) observed a positive result of *T. harzianum* on lettuce yields, but not on yield and marketable yield of tomato in an unheated greenhouse. Most of the cited studies have concentrated on seedling (Chang *et al*., 1986; Inbar *et al*., 1994;

Tsahouridou & Thanassoulopoulos, 2002) or greenhouse production (Bal & Altintas, 2006; Gravel *et al*., 2007), with little field research. Even so, when field studies were conducted, the focus was on suppression of soil-borne diseases (Datnoff *et al*., 1995; Coskuntuna & Özer, 2008) with little attention to yield.

Many research reports have shown the potential of AMF to enhance mineral nutrient uptake (Smith & Read, 1997), particularly P (Marschner & Dell, 1994), alleviation of stresses such as drought (Nelsen & Safir, 1982; Subramanian *et al*., 2006) and salinity (ZhongQun *et al*., 2007) and the suppression of soil borne diseases (Hooker *et al*., 1994). Subramanian *et al*. (2006) found an improvement in fruit production and drought tolerance of AMF-inoculated tomato plants due to enhanced nutritional status of the plants. Al-Karaki (2006) reported an increase in yield and alleviation of deleterious salt stress following inoculation with AMF. Although Bolan *et al*. (1984) found an increase in mycorrhizal colonisation in subterranean clover with increased P application; the general belief is that AMF performs poorly under optimal soil nutrition conditions (Strzemska, 1975). Due to the symbiotic nature of interactions between AMF and the host, which are based on bidirectional nutrient exchange (Karandashov & Bucher, 2005), it is unclear as to whether under optimum field conditions AMF would benefit the host plant or simply become a parasite. When inoculated simultaneously under greenhouse conditions, *T. harzianum* and AMF had the potential to improve plant growth (Nzanza *et al*., 2011). The interactive effects of *T. harzianum* and AMF under field conditions are not well-documented. The objective of this study was to determine the effects of nursery inoculation with *T. harzianum* and AMF on fungal root colonisation, plant growth, fruit yield and quality of tomato produced under field conditions.

6.3 MATERIALS AND METHODS

6.3.1 Site description

Field trials with drip irrigated tomatoes were conducted during the November-May growing season of 2008–2009 and repeated in 2009–2010 at Vreedsaam farm, ZZ2-Bertie van Zyl, Mooketsi, South Africa. The site is located at 23º 65' 17" S, 30º 06' 89" E, and at 772 m above sea level, in the northern part of South Africa. The mean day/night temperatures were 25ºC/15ºC and 27ºC/15ºC in the first and second growing season, respectively. The rainfall of 451 mm and 354 mm was received during the respective growing seasons.

Soil samples were randomly collected at depth of 0–30 cm using a soil auger (7.5 cm in diameter and 20 cm depth). Composite samples were mixed thoroughly, air-dried and sieved to pass through a 2 mm screen for physico-chemical analysis and mycorrhizal spore counts. Soil pH was determined in a 1:2.5 suspension (soil/water), whereas the Walkley-Black (1934) method was used to determine the total organic carbon. Soil K was determined using the flame photometer, while soil Ca and Mg were determined with an atomic absorption spectrophotometer. Soil available P was extracted with Bray 2 solution and determined with a spectrophotometer. The weight-sieving technique was used for mycorrhizal spore counts (Brundrett *et al*., 1996), while the hydrometer method was adopted for soil texture analysis (Kalra & Maynard, 1991). The soil had a pH (H_2O) of 5.9 with 10 mg kg^{-1} P, 202 mg kg^{-1} K, 194 mg kg^{-1} Mg, 731 mg kg^{-1} Ca, and organic carbon of 1.5%. The mycorrhizal spore propagules on the site were less than one kg^{-1} soil, therefore the soil was not fumigated. Soil at the experimental site comprised of sandy loam

with 80% sand, 14% clay and 6% silt. The field experimental was divided into two portions having similar soil texture and nutrient status, with the first planted in 2008–2009, whereas the second was used during the 2009–2010 growing season.

6.3.2 Experimental design and treatments

Treatments consisted of inoculating the growing media with *T. harzianum* alone, AMF alone, or *T. harzianum* + AMF before sowing, and the uninoculated control. Details of the microbial inoculants and seedling production have previously been described (Chapter 4), with the exception that seedlings were allowed to grow for four weeks into cell plug before transplanting to the open field.

The fields were ploughed and harrowed before constructing 30-cm-high raised beds. Seedlings were transplanted into double rows on the beds, with a spacing of 30 cm between plants and 180 cm between rows (Figure 6.1). Each experimental plot measured 20 m in length × 1.8 m in width (36 m^2). Eight weeks before transplanting plots received an organic amendment in the form of compost (10 m^3 ha^{-1}) made from grass clippings, manure, wood chips, sawdust and a mixture of chicken and cattle manure (4 m^3 ha^{-1}) at 1:1 (v/v), which accounted for 50 kg N ha^{-1}, 37 kg P ha^{-1} and 100 kg K ha^{-1}. During both growing seasons, plots received 200 N kg N ha^{-1} as ammonium sulphate, 23 kg P ha^{-1} as phosphoric acid, 300 kg K ha^{-1} as potassium nitrate, 150 kg Ca ha^{-1} as calcium nitrate and 25 kg Mg ha^{-1} as magnesium sulphate, as side-dressing through drip irrigation. Irrigation was scheduled using evapotranspiration rates of the plants. Standard cultural practices for tomato production were applied. Scouting for pests and diseases with low

economical damage was done throughout the trial. Whiteflies and aphids were controlled by drenching the soil around the plants with Actara® (thiamethoxam 25%) at label rates of 0.03 ml plant^{-1}. Biomectin® (Abamectin 18 g l^{-1}) was applied at the rate of 0.6 l ha^{-1} for the suppression of leafminer, whereas Kocide® 2000 (copper hydroxide) and mancozeb® 800 WP (dithiocarbamate) were used for suppressing early blight (*Alternaria solani*), bacterial spot (*Xanthomonas vesicatoria*) and bacterial speck (*Pseudomonas syringae*). Weeds were removed by hand pulling or hoeing.

Figure 6.1 Illustration of tomato plants transplanted into double rows raised beds in open field

6.3.3 Data collection

Root colonisation and dry matter production

Twelve weeks after transplanting, three randomly selected plants per treatment were collected for the determination of dry matter production as previously described (Chapter 3), with the exception that plants were oven-dried at 65ºC for 48h. The procedures for mycorrhizal and *Trichoderma* root colonisations are presented in Chapter 4.

Yield variables

Harvesting was done as described in Chapter 5. Details of yield and marketable yield determination are presented in Chapter 3.

Fruit quality

Details of the vitamin C content and TSS analysis are presented in Chapter 5.

6.3.4 Data analysis

The analysis of data is described in Chapter 3. Relevant ANOVA tables can be found in the Appendix.

6.4 RESULTS

6.4.1 Mycorrhizal and *Trichoderma* root colonisation

Regardless of the growing season, data showed that non AMF-treated plants had less than 1% mycorrhizal root colonisation, whereas AMF-inoculated plants had a root colonisation of above 20% (Table 6.1). For *T. harzianum*, the uninoculated plants had less than 12% root colonisation, whereas *T. harzianum* inoculated plants had more than 80% root colonisation.

Table 6.1 Dry matter content and root colonisation of field-grown tomato as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi

Treatment	AMF colonisation (%)		*Trichoderma* colonisation (%)		Dry shoot mass (g plant^{-1})		Dry root mass* (g plant^{-1})	
	2008	2009	2008	2009	2008	2009	2008	2009
M	23.60a	20.00a	12.00b	5.20b	30.28a	36.01a	5.01	5.23
T	1.00b	0.80b	92.00a	79.60a	30.80a	29.27b	5.15	5.43
T+M	20.40a	22.20a	94.00a	82.40a	29.10a	33.47a	5.52	5.08
Control	0.80b	1.00b	4.96b	4.80b	22.40b	27.61b	4.96	5.00

Means followed by the same letter in a column were not significantly different ($P \leq 0.05$) according to Fisher's LSD test
*No significant difference ($P \leq 0.05$) according to Fisher's LSD test
T = *T. harzianum*; M = AMF; T+M = combined application of *T. harzianum* and AMF

6.4.2 Shoot and root dry mass

Inoculation with AMF alone or in combination with *T. harzianum* increased dry shoot mass by 35% and 30%, respectively, during the first season, and by 30% and 21% during the second growing season when compared to the uninoculated plants (Table 6.1).

6.4.3 Yield and yield components

The AMF alone or in combination with *T. harzianum* increased early yield of tomato by 70% and 64%, respectively, during the first season, and by 37% and 36%, respectively, during the second growing season (Table 6.2). Fungal inoculants did not increase total fruit yield of tomato. However, a slight increase (16%) in the marketable yield as compared to the control was obtained with combined inoculation of *T. harzianum* and AMF during the second growing season. The number of fruit (both seasons) and marketable fruit per plant (first season) were not affected by any of the treatments.

Table 6.2 Yield and yield components of field-grown tomato as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi

Treatment	Number of fruit* (plant^{-1})		Early yield (kg plant^{-1})		Total yield/plant* (kg plant^{-1})		Marketable yield (kg plant^{-1})	
	2008	2009	2008	2009	2008	2009	2008*	2009
M	149.40	137.20	2.79a	2.30a	8.99	8.68	7.05	6.62ab
T	143.20	131.60	1.80b	2.14a	8.38	8.2	6.23	6.00c
T+M	147.60	141.60	2.70a	2.28a	9.02	8.84	7.18	7.00a
Control	149.40	138.00	1.64b	1.68b	8.07	8.19	6.03	6.02bc

Means followed by the same letter in a column were not significantly different ($P \leq 0.05$) according to Fisher's LSD test
*No significant difference ($P \leq 0.05$) according to Fisher's LSD test
T = *T. harzianum*; M = AMF; T+M = combined application of *T. harzianum* and AMF

6.4.4 Fruit size

Differences among the four treatments with regard to percentage of extra-large fruit during the first growing season were not detected (Table 6.3). However, in the 2009–2010 season, the AMF alone or in combination with *T. harzianum* increased percentage extra-large fruit by 44% and 39%, respectively, while *T. harzianum* increased percentage of large fruit by 76% in 2008–2009. The percentages of extra-large and medium fruit in 2009 and 2010, respectively, were not affected by any of the treatments.

Table 6.3 Fruit size class of field-grown tomato as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi

Treatment	Extra-Large fruit (%)		Large fruit (%)		Medium fruit (%)		Small fruit* (%)	
	2008*	2009	2008	2009	2008	2009*	2008	2009
M	34.82	42.24a	27.70b	22.88c	23.84ab	18.38	13.60	16.50
T	31.22	35.18ab	41.18a	32.12a	15.22b	16.88	12.38	15.82
T+M	39.10	39.08a	30.48b	26.20bc	16.80b	21.54	13.60	13.22
Control	30.30	29.32b	23.30b	28.52ab	31.58a	25.66	14.82	16.40

Means followed by the same letter in a column were not significantly different ($P \leq 0.05$) according to Fisher's LSD test
*No significant difference ($P \leq 0.05$) according to Fisher's LSD test
T = *T. harzianum*; M = AMF; T+M = combined application of *T. harzianum* and AMF

6.4.5 Vitamin C and TSS

Inoculation with AMF increased the vitamin C content of tomato fruit by 15% over the untreated plants in 2008–2009 (Table 6.4). However, in 2009–2010, no significant differences were found amongst any of the treatments. All inoculated tomato plants increased the TSS of tomato fruit, with the mixture *T. harzianum* and AMF (T+M) recording the highest increase (9%) over the untreated plants in 2009–2010. In 2008–2009 no significant differences were found amongst any of the treatments.

Table 6.4 Vitamin C content and TSS of field-grown tomato fruit as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi

Treatment	Vitamin C (%)		TSS (%)	
	2008	2009*	2008*	2009
M	29.20a	25.10	5.40	5.68a
T	27.40ab	26.10	5.32	5.62a
T+M	26.50ab	25.00	5.28	5.72a
Control	23.80b	22.60	4.86	5.26b

Means followed by the same letter in a column were not significantly different ($P \leq 0.05$) according to Fisher's LSD test
*No significant difference ($P \leq 0.05$) according to Fisher's LSD test
T = *T. harzianum*; M = AMF; T+M = combined application of *T. harzianum* and AMF

6.5 DISCUSSION

Inoculation of tomato seedlings in the nursery with *T. harzianum* and AMF, either alone or in combination, promoted plant growth, fruit size and early fruit yield of field-grown tomatoes. Enhanced tomato growth was not translated into increased total yield of tomato. In fact, most of the increased yield associated with AMF was either due to its potential to alleviate stress such as severe drought (Subramanian *et al*., 2006), salinity (Kaya *et al*., 2009) or disease incidence. Kaya *et al*. (2009) demonstrated that AMF increased fruit yield of salt-stressed tomato plants but not that of non-stressed plants, whereas Al-Karaki (2006) observed higher yields in AMF-inoculated plants than in uninoculated plants. Reports on increased tomato yield with *T. harzianum* are rare, although Gravel *et al*. (2007) observed an increase in yield with *T. atroviride* in rockwool. Additionally, increased yields in cucumber, bell pepper and strawberry had been reported with *T. harzianum* (Altintas & Bal, 2005; Altintas & Bal, 2008; Bal & Altintas, 2006; Poldma *et al*., 2002). In this study, although all fungal inoculants induced a negligible increase in yield of tomato, treatment effects were not significant.

Findings of this study also demonstrated the beneficial effect of inoculating seedlings with *T. harzianum* and/or AMF on the earliness of the yield, suggesting that these fungal inoculants have the potential to increase the total yield of tomato. Although data showed that combined inoculation of *T. harzianum* and AMF was more effective than either applied alone, marketable yield increase obtained during the second season was rather due to relatively higher rate of unmarketable yield of the untreated plants than the fungal inoculant's effect.

Trichoderma harzianum increased the percentage large fruit in 2008–2009, while AMF increased the percentage extra-large fruit in 2009–2010 growing season. The increased in fruit size by *T. harzianum* and AMF was probably due their ability to trigger enzymes involved in tomato fruit cell expansion. However, combining *T. harzianum* and AMF had little effect on tomato fruit size, when compared to each fungal inoculant alone. These findings are in agreement with Datnoff *et al*. (1995) who did not find any beneficial effect of dual inoculation of tomato with *T. harzianum* and AMF on extra-large fruit.

Inoculating tomato seedlings with *T. harzianum* and AMF improved the TSS of tomato fruit. Higher sugar content, obtained with both fungal inoculants, specifically those treated with AMF, suggested that carbohydrate partitioning in the plant was not solely restricted to AMF. However, this finding did not confirm previous observations where a decrease in the fruit TSS was observed in AMF-treated plants in processing cultivars (Martin, 2007). Another major finding of this study was the increased vitamin C content of inoculated plants, possibly due to increased sugar accumulation (Massot *et al*., 2010) or enhanced nutrient uptake (Mozafar, 1994). Increased vitamin C content in tomato fruit was previously reported in AMF-treated plants (Subramanian *et al*., 2006). The differences in fruit quality parameters between the 2008–2009 and 2009–2010 trials could be attributed to the seasonal differences in terms of rainfall and temperature.

Uninoculated AMF plants had low mycorrhizal colonisation (< 1%) due to the low indigenous mycorrhizal count prior to planting, whereas the lower root colonisation of AMF-treated plants (about 21%) could be due to chemical input or other variables such as irrigation, timing of fertiliser, or interactions with endemic AMF in the rhizosphere. Chandanie *et al*. (2009) reported

an inhibition of *T. harzianum* around cucumber roots following the application of the AMF (*G. mosseae*), whereas Calvet *et al.* (1992) observed a significant enhancement of AMF growth due to the presence of *T. harzianum in vitro*. In this study, *T. harzianum* had no effect on mycorrhizal root colonisation as the mycorrhizal root colonisation for AMF and combined inoculation treatments were not different. Similarly, AMF did not influence the percentage of *Trichoderma* root colonisation as *T. harzianum*-treated plants; either alone or in combination with AMF, maintained a higher root colonisation than the control but were not different from each other. The findings indicated that *T. harzianum* and AMF had no suppressive effect on the development of external mycelial growth of each other.

Dry shoot mass was improved by inoculation with AMF and *T. harzianum*, either alone or in combination. *Trichoderma harzianum* and AMF have been found to promote growth and plant development of numerous crops (Altomare *et al*., 1999; Gravel *et al*., 2007; Kleifeld & Chet, 1992; Liu *et al*., 2008; Samuels, 2006). Chandanie *et al*. (2009) noted that dual inoculation with *T. harzianum* and AMF synergistically increased the plant dry biomass of cucumber when compared with inoculation of *T. harzianum* alone. Results in this and other studies (Whipps, 1997; Gravel *et al*., 2007) suggested that *T. harzianum* and AMF improve plant growth development of tomato, probably due to the production of stimulatory compounds and/or the improvement of mineral nutrient availability and uptake.

Results in this study demonstrated that *T. harzianum* and AMF have the potential to improve vegetative growth, fruit quality and early fruit yield of field-grown tomato. However, further investigation is necessary in order to establish whether the rate of microbial colonisation could

be translated into increased total yield, as these fungi were able to increase early yield. The study did not detect any antagonistic effect between *T. harzianum* and AMF, suggesting that these fungal inoculants could be used in combination to improve the productivity of the tomato crop.

CHAPTER 7[1]

EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGAL INOCULATION AND BIOCHAR AMENDMENT ON GROWTH AND YIELD OF TOMATO (*SOLANUM LYCOPERSICUM* L.)

7.1 ABSTRACT

A field study was conducted to investigate the interactive effects of inoculation of arbuscular mycorrhizal fungi (AMF) *Glomus mosseae* and soil amendment with biochar on AMF root colonisation, plant growth, fruit yield and nutrient uptake of tomato (*Solanum lycopersicum* L.). A 2 × 2 factorial experiment arranged in a randomised complete block design included two *G. mosseae* treatments (inoculated at sowing or uninoculated) and two biochar levels (5 t ha^{-1} or unamended) with six replications were used. At mid-season, 12 weeks after transplanting, biochar addition did not increase the percentage of AMF root colonisation on tomato plants. Inoculation with *G. mosseae* increased dry shoot mass and total plant biomass by 11% and 9%, respectively, whereas biochar amendment decreased dry root mass by 13%. Similarly, biochar amendment lowered shoot K content by 9% when compared to unamended plants. Generally, inoculation with *G. mosseae* and biochar did not affect shoot Ca, B, Cu, Mn, Na or Zn but lowered shoot P by 26% when compared to uninoculated plants. Inoculation with AMF and

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biochar combined did not affect tomato growth variables, yield or yield components. Microbial community assessment revealed that AMF-treated plants shared specific bacterial species, which they did not share with untreated-AMF plants. Interestingly, when AMF-treated plants were transplanted with biochar, fungal diversity was different to treatments without biochar. Nursery inoculation with AMF had the highest dominant bacteria in the rhizosphere. Tentative identification of Denaturing Gradient Gel Electrophoresis (DGGE) suggested that *Alternaria* spp. were only found in untreated plots, whereas *Penicillium pinophilum* was only restricted to the AMF-treated sample without biochar. In conclusion, combined application of AMF and biochar had no effect on AMF root colonisation and performance of tomato plants, but altered the composition of microbes in the rhizosphere of tomato.

Keywords: Arbuscular mycorrhizal fungi, biochar, DGGE, inoculation, microbial community, tomato

7.2 INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are obligatory symbiotic soil fungi which colonise roots of most plants (Douds & Millner, 1999). These fungi form mutualistic relationships with more than 80% of terrestrial plants (Ulrich *et al*., 2002) and provide the host with mineral nutrients in exchange for carbohydrates (Tahat *et al*., 2008). Generally, plants inoculated with AMF are more efficient in nutrient and water acquisition, thus resulting in an improved plant growth (Oseni *et al*., 2010). Colonisation of roots by AMF enhanced crop productivity by enhancing tolerance to various biotic and abiotic stress factors (Al-Garni, 2006; Khaosaad *et al*., 2007; Javaid & Riaz,

2008). In tomato, AMF are widely used to improve plant growth and health (Oseni *et al*., 2010). However, even with AMF nursery inoculation or field application, tomato plants exhibit low root mycorrhizal colonisation. Low AMF colonisation in field-grown plants has been variously attributed to (i) use of unsuitable strains, (ii) relatively high available soil P (iii) cultural practices and (iv) microbial competition in the rhizosphere (Strzemska, 1975; Jasper *et al*., 1989).

Soil amendments, which increase AMF abundance and/or functionality, could be beneficial to plant hosts (Rillig & Mummey, 2006; Warnock *et al*., 2010). Biochar (biomass-derived black carbon) can serve as refuge for AMF hyphae and protect them from fungal grazers (Warnock *et al*., 2007), thus enhancing plant host-fungus symbiosis. Ishii and Kadoya (1994) argued that additions of biochar altered soil physico-chemical characteristics, leading to increased soil nutrient availability and enhanced mycorrhizal root colonisation. Similarly, Saito (1990) observed an increase of more than 300% in mycorrhizal root colonisation in field-grown soybean. According to Lehmann *et al*. (2003), biochar addition can improve plant productivity directly as a result of its nutrient content and release characteristics or indirectly, through improved nutrient retention. Although numerous studies indicated that soil biochar amendments can increase AMF percent root colonisation (Ezawa *et al*., 2002; Yamato *et al*., 2006; Warnock *et al*., 2010), little is known about the resultant effects on the soil microbial community (Glaser, 2007; Steinbeiss *et al*., 2009).

Molecular fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE) of ribosomal DNA (rDNA) fragments amplified from total community DNA have been widely used to evaluate the composition of bacterial and fungal communities (Muyzer & Uitterlinden, 1993).

Most commonly, 16S rRNA genes are used to give an overall indication of the species composition of a sample since they can easily be compared on gene databases (Reynolds & Surridge, 2009).

Since AMF might alter the microbial community in the rhizosphere, while biochar could affect percentage mycorrhizal root colonisation and that both could improve crop performance, there is an increasing interest in understanding their potential synergisms in crop production. The objective of this study was two-fold: (i) to investigate the effects of AMF-inoculated transplants and biochar-amended soil on mycorrhizal root colonisation, nutrient content, plant growth and yield of field-grown tomato and (ii) to assess their resultant effects on microbial community in the rhizosphere.

7.3 MATERIALS AND METHODS

7.3.1 Effect of AMF-inoculated plants and biochar-amended soil on tomato production

Site description

The experiment was conducted under greenhouse conditions at the Hatfield Experimental Farm, University of Pretoria. Details of the study location and duration are presented in Chapter 5, with the exception that this study was conducted in 2010 growing season.

Experimental design and treatments

The four treatment combinations (2 AMF × 2 biochar), M_1B_1 (AMF-inoculated seedlings with biochar-amended soil), M_1B_0 (AMF-inoculated seedlings without biochar), M_0B_1 (uninoculated seedlings with biochar) and M_0B_0 (untreated/control), were arranged in a randomised complete block design with six replicates.

Tomato cv. Nemo-Netta seedlings either pre-inoculated with commercial inoculum Biocult© containing spores of *Glomus mosseae* or uninoculated, were supplied by Hishtill nursery, Mooketsi, South Africa. Pre-inoculated AMF seedlings had less than 15% mycorrhizal root colonisation, whereas uninoculated seedlings had no colonisation. Where applicable, biochar was added to the transplanting hole (30 cm depth) at planting at a rate of 500 g/hole corresponding to 5 t ha^{-1}(Hossain *et al*., 2010).

Cultural methods are presented in Chapter 6.

Biochar production

Biochar was produced at the Natuurboerdery Research Center in Mooketsi, South Africa from *Eucalyptus globolus* trees. The trees were cut down, chipped and pyrolysed in a fixed bed reactor. The pyrolysis temperature was maintained at 450ºC for 1 h. Physical and chemical characteristics of biochar are shown in Table 7.1.

Table 7.1 Chemical and physical characteristics of biochar produced from *Eucalyptus globulus*

Parameters	Biochar	Unit
Total Carbon	338	g kg^{-1}
Total Nitrogen	3.7	g kg^{-1}
pH (H_20)	7.6	
Moisture content	3.5	%
Ash content	3.3	%
Phosphorus-Bray 2	84.7	mg kg^{-1}
Total Sulfur	43	mg kg^{-1}
Total Magnesium	0.7	g kg^{-1}
Total Boron	8.45	mg kg^{-1}
Cation exchangeable capacity	9.3	$mmol_c$ kg^{-1}
Bulk density	560	kg m^{-3}

Data collection

The procedures for root colonisation and shoot chemical analysis are presented in Chapter 4. Harvesting was done as described in Chapter 5. Details of yield and marketable yield determination are presented in Chapter 3, while dry matter determination is presented in Chapter 6.

Data analysis

The analysis of data has been previously described (Chapter 3). Relevant ANOVA tables can be found in the Appendix.

7.3.2 Effect of AMF and biochar amendment on fungal and bacterial populations

Site description and soil sampling

Soil samples were collected from tomato roots in the rhizosphere at the end of the growing season. Plants were pulled out and soils gently removed. Soil samples were kept into a cooler box and sent to the laboratory (Soil microbiology laboratory, Department of Plant Production and Soil Science, University of Pretoria, South Africa) where they were maintained at 4ºC until DNA extraction.

***Microbial community structure: denaturing gradient gel electrophoresis* (DGGE)**

Total DNA was extracted from 0.25 g soil using the Zymo Fast spin soil DNA extraction kit (Inqaba Biotec, Pretoria, South Africa). The DNA concentration was determined by agarose gel electrophoresis. A segment of 16S bacterial rDNA was amplified by means of PCR using primers K (Siciliano *et al*., 2003) and M (Fjellbirkeland *et al*., 2001). Complimentary screening of eukaryotic diversity was carried out on a portion of the internal transcribed spacer (ITS) gene sequence of the DNA by means of PCR using the primer set ITS3 and ITS4 (White *et al*., 1990). The PCR product was subjected to DGGE (Muyzer *et al*., 1993), whereas image analysis was performed using the Gel2K (Norland, 2004). Dominant bands were compared and analysed for population diversity determination.

Band reamplification and sequencing were conducted by Ingaba Biotec (Pretoria, South Africa) for DGGE sequencing. Each sequence was subjected to BLAST analysis on the GenBank database and matching hits were selected for alignment using Clustal X (Thompson *et al*., 1994). Phylogenetic analysis was based on parsimony using PAUP 4.0b8 (Phylogenetic Analysis Using

Parsimony) (Swofford, 2000). Heuristic searches were done with random addition of sequences (1000 replicates), tree bisection-reconnection (TBR), branch swapping, MULPAR-effective and MaxTrees set to auto-increase. Phylogenetic signal in the data sets was assessed by evaluating tree length distributions over randomly generated phylogenetic trees. The consistency (CI) and retention indices (RI) were determined for all data sets. Phylogenetic trees of sequences were rooted with *E. coli* as outgroup to the remaining taxa. Bootstrap analyses were conducted, retaining groups with 70% consistency, to determine confidence in branching points (1000 replicates) for the most parsimonious trees generated.

Data analysis

Bacterial community fingerprints were recorded and digital images were analysed using software based on the Shannon-Weaver index. Numbers of dominant bacterial species per sample were plotted. Dendrograms depicting similarities and differences between communities were generated using Jaccard statistics and a group average across the different types of samples.

7.4 RESULTS

7.4.1 Effect of AMF-inoculated plants and biochar-amended soil on tomato production

Growth parameters and mycorrhizal root colonisation

There was a significant main effect of AMF inoculation on dry shoot mass and total plant biomass (Table 7.2). The main effect of biochar was only significant for dry root mass. The interaction of AMF inoculation × biochar amendment was not significant for any parameter.

Regardless of biochar amendment, AMF inoculation increased the shoot dry mass and total plant biomass by 11% and 9%, respectively. Biochar amendment decreased the root dry mass by 13%. Tomato shoot length and root length were not affected by any treatment. Root colonisation of AMF was 15%, with or without biochar addition, whereas, uninoculated seedlings roots had no mycorrhizal colonisation (Table 7.3).

Table 7.2 Growth variables of tomato as influenced by arbuscular mycorrhizal fungi and biochar

Response variable	Shoot length (cm)	Root length (cm)	Dry shoot mass (g plant $^{-1}$)	Dry root mass (g plant $^{-1}$)	Plant biomass (g plant^{-1})
AMF					
M_0	149.88	59.18	10.60b	2.05	12.65b
M_1	148.60	61.19	11.87a	2.00	13.87a
Biochar					
B_0	150.11	58.37	10.85	2.15a	13.00
B_1	148.37	61.99	11.62	1.90b	13.52
ANOVA					
M	ns	ns	*	ns	*
B	ns	ns	ns	*	ns
M×B	ns	ns	ns	ns	ns

Means followed by the same letter in a column were not significantly different ($P \leq 0.05$) according to Fisher's LSD test

ns, * are levels of significance (not significant, $P \leq 0.05$, respectively according to LSD test)

M_0 = no AMF applied; M_1 = AMF inoculation; B_0 = no biochar amendment; B_1 = biochar amendment

Yield and yield components

The yield and yield components of tomato were not affected significantly by the main effects or AMF inoculation × biochar amendment interaction (Appendix A, Table 7.2). However, the AMF inoculation with (M_1B_1) or without biochar (M_1B_0) increased the total yield by 8% (Table 7.3). Uninoculated seedlings combined with biochar (M_0B_1) decreased both early and total yields of tomato by 9%.

Table 7.3 Percentage of mycorrhiza root colonisation, mean yield and yield components of tomato as influenced by arbuscular mycorrhizal fungi and biochar

Response variable	Marketable fruit* (plant^{-1})	Early yield* (kg plant^{-1})	Total yield* (kg plant^{-1})	Marketable yield* (kg plant^{-1})	Mycorrhiza (%)
M_0B_0	89.91	1.73	7.16	6.10	-
M_0B_1	83.04	1.59	7.04	6.13	-
M_1B_0	92.85	1.82	7.69	6.57	15
M_1B_1	94.78	1.72	7.47	6.45	15

*No significant difference ($P \leq 0.05$) according to Fisher's LSD test

M_0 = no AMF applied; M_1 = AMF inoculation; B_0 = no biochar amendment; B_1 = biochar amendment

Shoot chemical analysis

There was no significant effect of either AMF inoculation or biochar amendment on shoot N, Ca, Na, B, Cu, Mn or Zn contents of tomato plants (Table 7.4). Regardless of the seedlings status, amending soil with biochar (B_1) resulted in 9% decrease in shoot K content of tomato as compared to the control (B_0).

Table 7.4 Shoot nutrients content of tomato as influenced by arbuscular mycorrhizal fungi and biochar

Response variable	K (%)	Ca (%)	N (%)	Na (ppm)	B (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)
AMF inoculation								
M_0	2.73	1.93	4.05	2981.3	28.67	37.42	191.25	127.17
M_1	2.70	2.03	4.10	2966.5	30.08	34.00	255.75	150.67
Biochar addition								
B_0	2.83a	2.04	4.05	2929.9	29.33	37.33	217.50	136.33
B_1	2.60b	1.92	4.10	3017.8	29.42	34.08	229.50	141.50
ANOVA								
M	ns	ns	ns	ns	ns	ns	ns	ns
B	*	ns	ns	ns	ns	ns	ns	ns
M×B	ns	ns	ns	ns	ns	ns	ns	ns

Means followed by the same letter in a column were not significantly different ($P \leq 0.05$) according to Fisher's LSD test

ns, *, are levels of significance (not significant, $P \leq 0.05$, respectively according to LSD test)

M_0 = no AMF applied; M_1 = AMF inoculation; B_0 = no biochar amendment; B_1 = biochar amendment

Growing AMF-inoculated seedlings with (M_1B_1) or without biochar (M_1B_0) resulted in 26% and 29% decreases in shoot P content, respectively (Table 7.5). Similarly, uninoculated seedlings with biochar added (M_0B_1) also showed a decrease of about 32% in shoot P content as compared to the uninoculated seedlings grown without biochar amendment (M_0B_0).

Table 7.5 Phosphorus shoot content of tomato as influenced by arbuscular mycorrhizal fungi and biochar

Parameter	P (mg kg^{-1})			
	Biochar amendment			
AMF inoculation	B_0	R-E (%)	B_1	R-E (%)
M_0	0.45a		0.31b	-32
M_1	0.32b	-29	0.33b	-26

Means followed by the same letter within column and row were not significantly different ($P \leq 0.05$) according to Fisher's LSD test

M_0 = no AMF applied; M_1 = AMF inoculation; B_0 = no biochar amendment; B_1 = biochar amendment

7.4.2 Effect of AMF and biochar amendment on fungal and bacterial populations

DNA extraction and PCR

DNA was successfully extracted from all samples collected. No evidence of RNA or protein contamination was visible either below the lanes or in the wells of the gel, respectively (Figure 7.1).

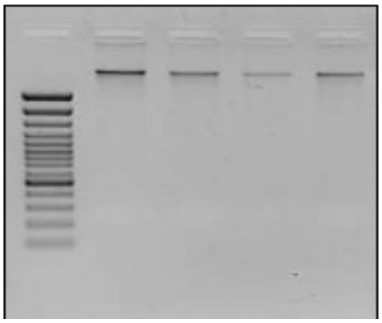

Figure 7.1 Tris-acetate–EDTA (TAE) agarose gel (1.5%) showing high-quality, clean genomic DNA extracted from soil samples

PCR of prokaryotes was successful yielding a ca. 510bp PCR product on a 1.5% TAE agarose gel. The negative control lane (first in row) shows that there was no contamination of the

reaction and that PCR product is thus a true indication of the microbial population being targeted (Figure 7.2).

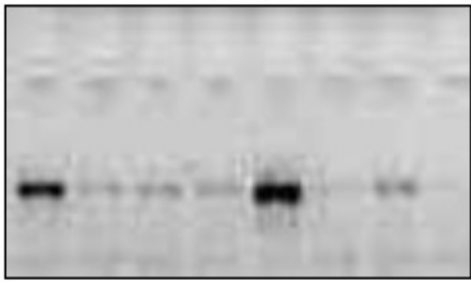

Figure 7.2 Tris-acetate –EDTA (TAE) agarose gel (1.5%) showing 5µl of PCR product from each of the 16S bacterial gene amplifications

DGGE

DGGE yielded gels showing clear multiple banding, forming a fingerprint in each lane (Figure 7. 3). These gel images were loaded into Gel2K (Norland, 2004) and a graphical image of the gels was produced (Figure 7.4) for further species diversity bioinformatics analysis. Dominant species per lane are indicated as dark prominent bands across the lane.

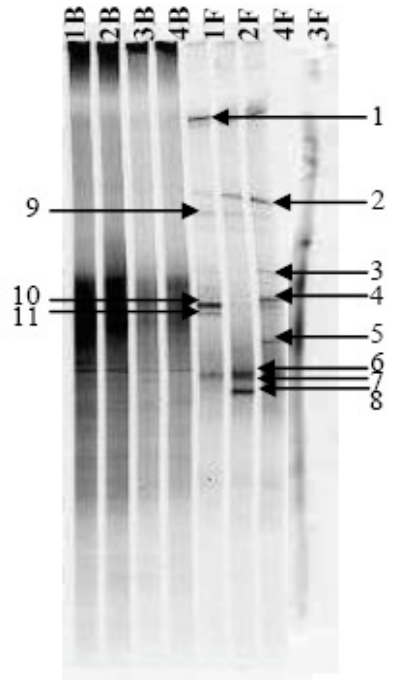

Figure 7.3 Denaturing gradient gel electrophoresis (DGGE) gel showing species diversity of bacteria (B) and fungi (F) from soil samples, run at 40-60% denaturants. PCR product is separated according to base-pair sequence differences to determine community richness and diversity of microorganisms based on these fingerprints

Arrows (1-11) point to bands that were excised for sequencing and tentative fungal identification
Sample 1 = no AMF + Biochar added (M_0B_1); Sample 2 = no AMF + no Biochar added (M_0B_0);
Sample 3 = AMF + Biochar added (M_1B_1); Sample 4 = no AMF + no Biochar added (M_0B_0)
B: Bacterial population; F: Fungal population

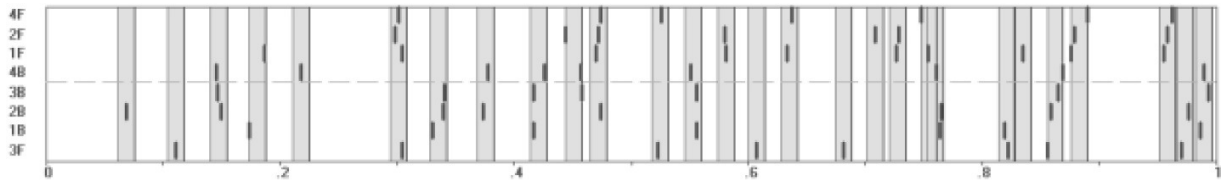

Figure 7.4 Graphic representation of the denaturing gradient gel electrophoresis (DGGE) gel in Figure 3 depicting the band pattern, indicating species diversity within bacterial (B) and fungal (F) populations, produced by each of the samples

Results suggested that soil amended with biochar had the highest dominant fungal species when compared with AMF, AMF and biochar or the untreated plots, whereas AMF alone had the highest number of bacterial species (Figure 7.5).

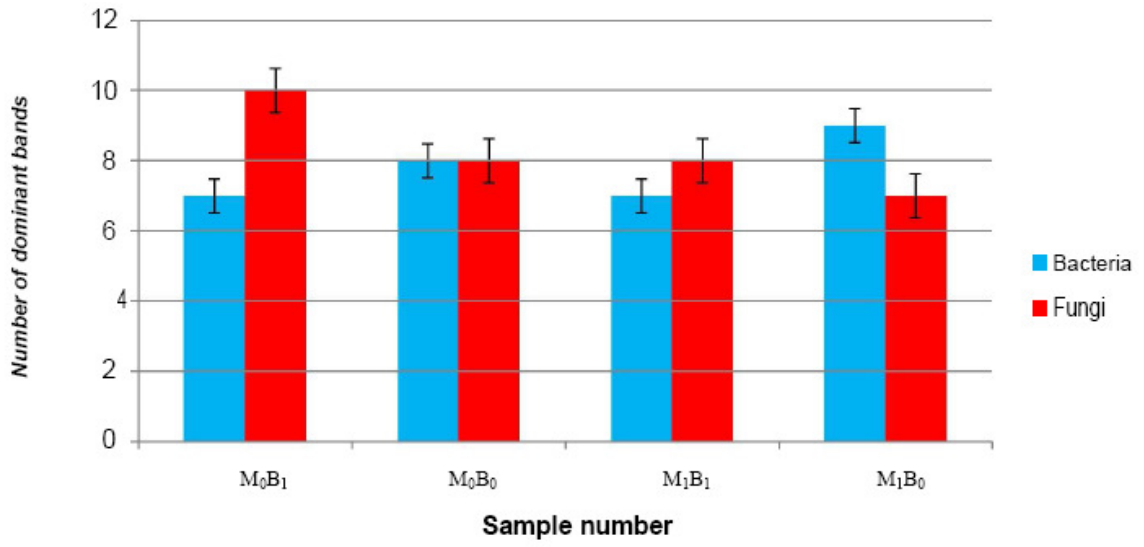

Figure 7.5 Number of dominant bacterial or fungal species per sample visible from denaturing gradient gel electrophoresis (DGGE) band

Patterns (error bars were calculated using standard error across the respective sampling)

M_0B_1 (Sample 1) = no AMF + Biochar added; M_0B_0 (Sample 2) = no AMF + no Biochar added; M_1B_1 (Sample 3) = AMF + Biochar added; M_0B_0 (Sample 4) = no AMF + no Biochar added

The average number of dominant bands found between the pro- and eukaryotes screened for diversity shows higher diversity within the fungi, although this was not significant. Similarities between samples within the profile are indicated by branch lengths (Figure 7.6). The dendrogram forms two distinct clades/groupings. Clade I contains only fungal samples, whereas clade II only contains bacterial samples, with the exception of combined AMF and biochar (Sample 3, M_1B_1). Focusing on clade I, AMF-treated sample (sample 1, M_0B_1) and untreated-AMF sample with biochar added (sample 4, M_1B_0) grouped together, whereas on clade II, AMF-treated samples (sample 3, M_1B_1 and sample 4, M_1B_0) grouped together. Interestingly, combined AMF and biochar sample (M_1B_1) did not share common fungal species with any other treatments.

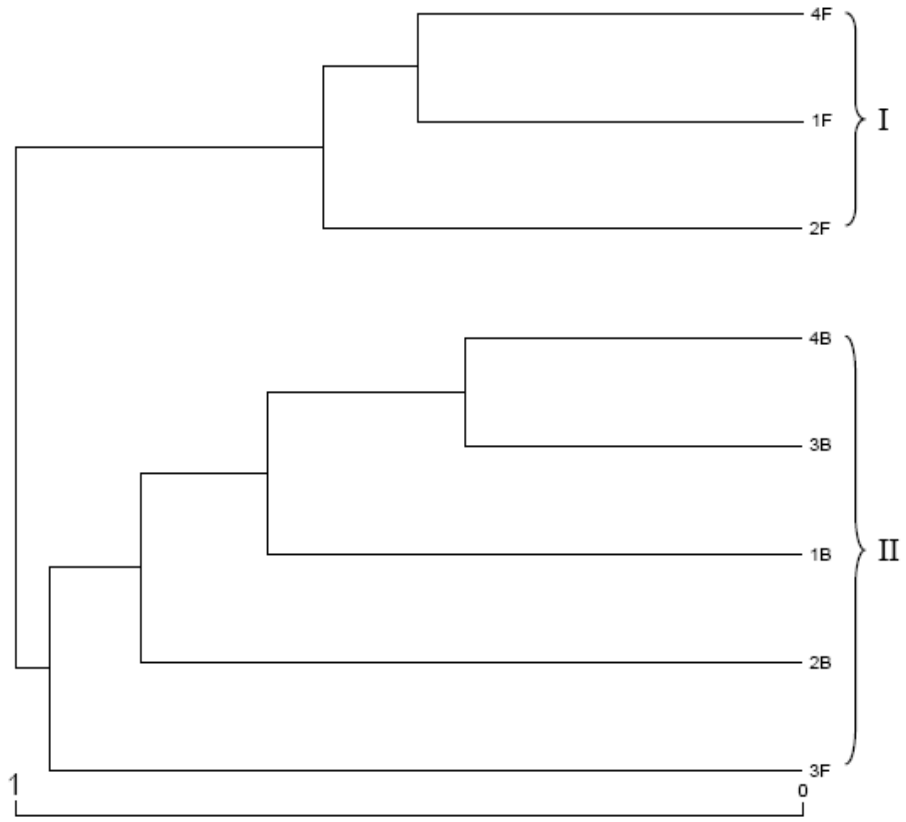

Figure 7.6 Cluster analysis of the banding pattern in Fig. 4, using a jaccard matching, group average setting to separate bacterial (B) and fungal (F) populations on the basis of community differences

Sample 1 = no AMF + Biochar added (M_0B_1); Sample 2 = no AMF + no Biochar added (M_0B_0);
Sample 3 = AMF + Biochar added (M_1B_1); Sample 4 = no AMF + no Biochar added (M_0B_0)

Tentative identification of the bands cut from the DGGE gel as indicated by arrows above (Figure 7.3) and confirmed in the phylogenetic tree (Figure 7.7) are presented in Table 7.6. There was 94% DGGE confidence AMF-treated (M_1B_0) soil sample contained *Penicillium pinophilum*, which was not found in any other samples.

Table 7.6 Tentative identification of denaturing gradient gel electrophoresis (DGGE) bands sequenced according to BLAST results from the NCBI GenBank database

Seq. no.	Species	Accession no.	Similarity (%)	Associated literatures	Samples
1	Ascomycete sp.	DQ683976	96	Conley *et al.* (2006)	M_0B_1
2	*Mortierella elongata*	GU446646	98	Bukovska (2009)	M_1B_0
3	*Penicillium pinophilum*	HQ589152	94	Iskandar *et al.*(2009)	M_1B_0
4	Uncultured Chlorophyta	HQ219393	81	Monchy *et al.*(2007)	M_1B_0
5	*Leptosphaeria* sp.	AM921719	90	Marquez *et al.* (2008)	M_1B_0
6	*Alternaria* sp.	EF432296	98	Mwangi *et al.*(2009)	M_0B_0
7				No match	M_0B_0
8				No match	M_0B_0
9				No match	M_0B_1
10	*Sporormiella septenaria*	GQ203790	90	Kruys & Wedin (2009)	M_0B_1
11				No match	M_0B_1

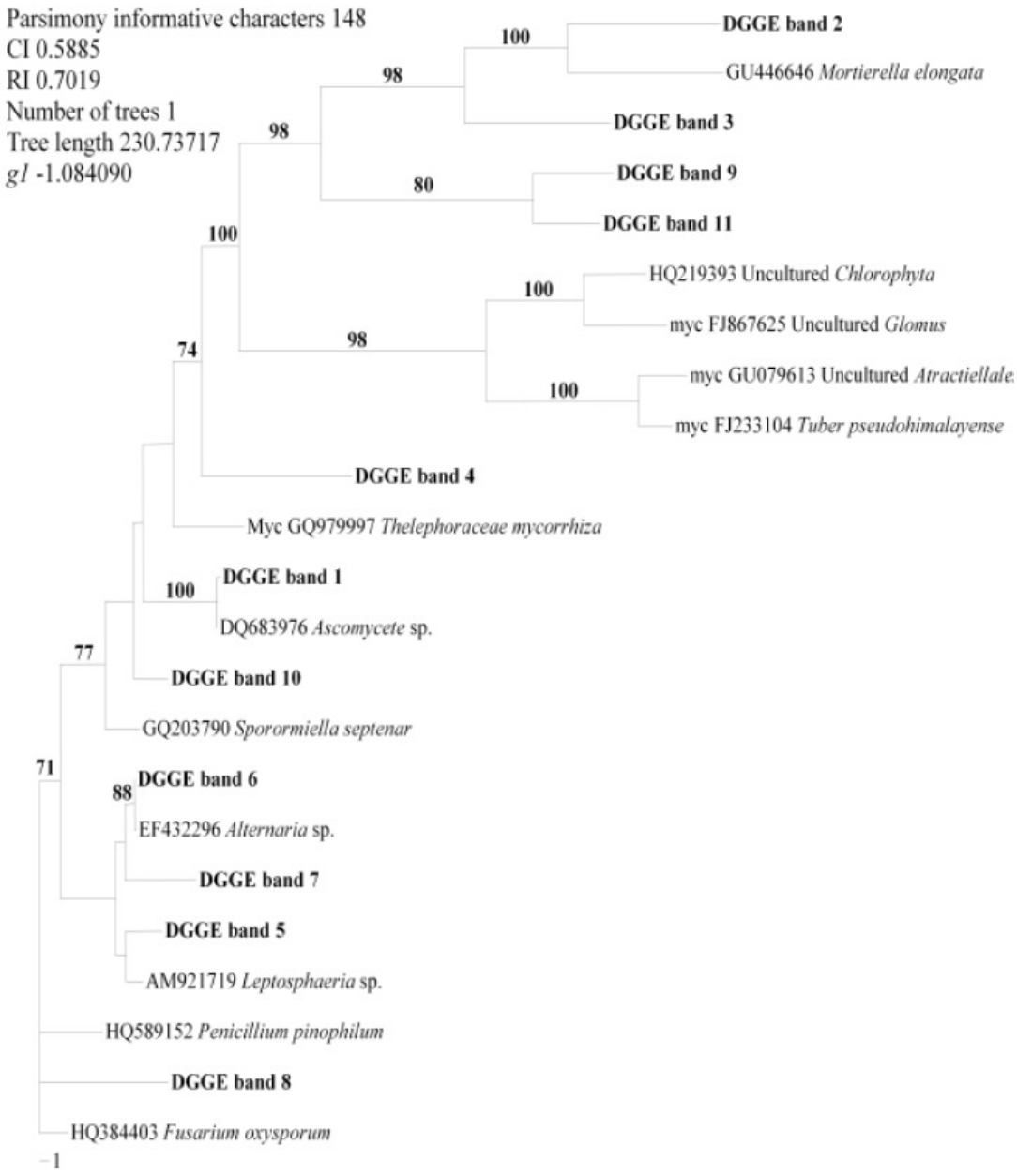

Figure 7.7 Phylogram of the denaturing gradient gel electrophoresis (DGGE) bands sequenced for tentative identification of fungi found in Mittal soil samples

7.5 DISCUSSION

7.5.1 Effect of AMF-inoculated plants and biochar-amended soil on tomato production

The addition of biochar to the planting hole of AMF-inoculated tomato seedlings did not increase the percentage of root colonisation, growth, yield or yield components of tomato plants. However, this combination influenced shoot P content. Effects of biochar addition to soil on root colonisation by AMF have been contradictory. Ishii and Kadoya (1994) observed increased percentage of root colonised by AMF on citrus. Wallstedt *et al*. (2002) argued that biochar could reduce root mycorrhizal colonisation by decreasing nutrient availability or creating unfavourable nutrient ratios in soils. In this study, biochar had no effect on mycorrhizal colonisation rate probably due to four reasons: (i) low seedling mycorrhizal colonisation (< 11%) before transplanting (ii) soil disturbance during production, (iii) use of synthetic fertilisers, especially P and (iv) application of pesticides, more especially copper-based products, which were used for the control of bacterial diseases. All these factors have been correlated with low mycorrhizal root colonisation in field production (Martin, 2007).

In this study, biochar had no positive effect on yield or yield components with or without AMF inoculation. Similarly, Graber *et al*. (2010) did not find any effect of biochar on the number of flowers or fruit yield of tomato grown in a soil-less medium. However, Steiner *et al*. (2007) observed increased yield in rice and sorghum with an application of 11 t ha^{-1} biochar over two years in an oxisol in Brazil. Similar results were observed for maize following three repeated applications of 7 t ha^{-1} of biochar over two growing seasons in Kenyan soils cropped to maize for up to 100 years (Kimetu *et al*., 2008). Even with 20 t ha^{-1} biochar applied, Major *et al*. (2010)

only found a significant yield response in maize in subsequent cropping years. Despite the clear evidence that increased yield is usually observed in subsequent years, some authors found positive results in the first year. For instance, in cherry tomato, Hossain *et al*. (2010) reported a 20% yield increase with combined biochar and fertiliser. In their studies, Hossain *et al*. (2010) used a low pH chomosol with 10 t ha^{-1} of biochar applied. The absence of a clear yield increase in our study could partly be attributed to the soil used (acid), application rate (5 t ha^{-1}), one growing season and application frequency.

Generally, K and Na are affected by salinity, nematodes and AMF (Graham & Sylvesten 1989; Mashela & Nthangeni, 2002). In this study, AMF inoculation did not affect shoot K content, probably due to low mycorrhizal root colonisation. The lower shoot K content in biochar-amended transplants was likely due to enhanced N and P by biochar resulting in an imbalance ratio of N/K and P/K in the rhizosphere, which then reduced K uptake. Shoot P content was the only mineral nutrient whose uptake was decreased by both AMF inoculation and biochar application probably due to the use of P fertilisers and non-stressed growing conditions during this study.

7.5.2 Effect of AMF and biochar amendment on fungal and bacterial populations

Results of this study showed that AMF-treated plants with or without biochar addition, shared specific bacterial species with each other, but which they did not share with other treatments, suggesting that AMF might influence bacterial community development in the rhizosphere. Generally, plant growth-promoting rhizobacteria (PGPR), which are important contributors to

overall plant growth and nutrition, are often associated with mycorrhizal hyphae (Garbaye, 1994; Vestergard *et al*., 2008; Rooney *et al*., 2009). In addition, some bacterial communities specifically attach to dead hyphae, whereas others use exudates from living hyphae as a growth substrate (Rooney *et al*., 2009). In this study, the AMF-treated sample had the highest dominant bacterial band. Albertsen *et al*. (2006) observed increased bacterial and saprophytic fungal biomass in the presence of AMF *G. intraradices*, whereas Andrade *et al*. (1997) found higher numbers of bacteria in AMF-untreated plant roots. According to Garbaye (1991), AMF might alter root exudation in the rhizosphere and therefore, indirectly affect bacterial growth.

Cluster analysis showed that fungal diversity of AMF-untreated (M_0B_1) and biochar-amended (M_1B_0) treatments were closer, when compared to other samples. Surprisingly, combination of AMF and biochar (M_1B_1) did not share common fungal species with M_0B_1 or M_1B_0, suggesting that biochar might modify the mycorrhizosphere community. Biochar contains organic pyrolytic byproducts, including phenolic and polyphenolic compounds, which might inhibit soil organisms including AMF (Warnock *et al*., 2010).

Tentative identification of DGGE band suggested that *Alternaria* sp. was found in the untreated control only. Physical field scouting supported this finding, as untreated plots had the highest disease incidence of early blight (*Alternaria solani*), *Fusarium* and *Verticillium* wilts when compared to other treatments. In this study, *Penicillium pinophilum* was found in AMF-treated sample (M_1B_0) only. Rando *et al*. (1997) classified *P. pinophilum* as a minor pathogen due to growth retardation observed in tomato. Fan *et al*. (2008) observed AMF symbiosis in strawberry roots when inoculated with *P. pinophilum*. However, Hempel (2009) questioned the finding that

P. pinophilum was capable of forming AMF symbiosis and called for further investigations with other plants. Synergistic effects between AMF and *Penicillium* spp. have been reported on wheat and maize (Babana & Antoun, 2006; Chandanie *et al*., 2006; Zaidi & Khan, 2007).

7.5.3 Conclusions

In conclusion, the addition of biochar in the planting hole during transplanting of AMF-inoculated seedlings had no effect on root colonisation, yield or yield components, or most of the shoot nutrients measured. However, the treatment reduced shoot P content. Findings in this study also suggest that biochar amendment might modify the rhizosphere, resulting in the altered development of microorganisms. Consequently, biochar should first be researched in detail before attempting any combination with AMF.

CHAPTER 8

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The aim of this study was to investigate the potential use of *T. harzianum* and AMF mixture as pre-sowing treatment in improving tomato seedlings quality, yield and fruit quality of tomato. To attain this goal, growth chamber, greenhouse, nethouse and field experiments were conducted. Major findings are presented below, followed by recommendations for future studies.

Trichoderma harzianum and AMF mixture was compared with SWE as well as Si in improving seedling growth and development, fruit yield and *Verticillium* wilt incidence on tomato plants. Because SWE contains growth hormones, susceptible to inhibit seedling germination and development, a preliminary study comparing two types of SWE derived from *Ecklonia maxima* and *Ascophyllum nodosum* at different concentrations, found 10% dilution with *E. maxima* as ideal SWE pre-sowing treatment. Combining *T. harzianum* and AMF mixture with *E. maxima* extract and or Si had no effect on seedling growth and development of tomato. However, *Ecklonia maxima* inhibition of AMF root colonisation of tomato seedlings suggested that fungal mixture should not be combined with SWE as pre-sowing treatment. Investigations on the influence on *T. harzianum* and AMF mixture, *E. maxima* and Si, each applied alone on the incidence of *Verticillium* wilt, confirmed the potential of the fungal mixture in reducing the deleterious effect of the disease during the early season. However, *T. harzianum* and AMF mixture could not improve tomato yield when compared with control plants. Evidences are that *T. harzianum* and AMF interact on each other and the nature of interactions, which are synergistic, antagonistic or neutral, depends on strains, inoculation time and crops. In this study,

combination of *T. harzianum* with four AMF species as single inoculation might have reduce the efficacy of each fungus in improving seedling quality and increasing tomato yield. Further study looked at *T. harzianum* and AMF (*G. mosseae*), each alone or in combination.

Trichoderma harzianum and AMF (*G. mosseae*) were inoculated alone or in combination before sowing or two weeks after sowing, and their effects on seedling growth and development were evaluated. Interestingly, interactions between *T. harzianum* and AMF (*G. mosseae*) on root colonisation were neutral. In this study, high *T. harzianum* root colonisation and low AMF root colonisation observed are simply indications of each fungus colonisation capacity rather antagonism between the two fungi. Findings of this study suggested that *T. harzianum* and AMF (*G. mosseae*) could simultaneously be applied to improve seedling growth and development, except when both fungi are applied two weeks after sowing. Another major finding in this study is the capacity of each fungus to induce seedling growth and development, confirming the potential use of each as biofertiliser and pre-sowing treatment on tomato.

Investigations were carried out to find out whether those benefits could be translated into increased yield and fruit quality under greenhouse conditions. There was no evidence of increasing yield or yield components of tomato plant following *T. harzianum* and AMF inoculation. However, increased percentage of extra-large fruit by *G. mosseae* confirmed previous studies that AMF could increase crop fruit size. The lowering of fruit K content in late AMF inoculation supported suggestions that early AMF inoculation was preferable than late inoculation. Although Vitamin C and fruit lycopene varied among treatments, there was no clear evidence of influence of *T. harzianum* and/or AMF on fruit phytochemical contents. Findings of

this study suggested that *T. harzianum* and AMF have negligible effect on yield of tomato under greenhouse conditions.

In South Africa, the large majority of fresh produce tomato originates from open field production. Experiments were conducted to investigate the influence of inoculation with *T. harzianum* and AMF on field-grown tomato. Growth promotion following microbial inoculation observed in previous studies with seedlings or greenhouse production was confirmed under field conditions. However, as previously observed, increased dry matter production was not translated into increased yield or yield components of tomato. Interestingly, when observing yield of first four weeks harvest, pre-inoculated seedlings increased early yield of tomato, which suggest that *T. harzianum* and AMF have the potential to influence yield of tomato. In this study, the role of AMF in increasing the percentage of extra-large fruit was confirmed. Inoculation with AMF also increased Vitamin C, while AMF alone or in combination with *T. harzianum* increased fruit TSS. However, the non-response of the fruit qualities during subsequent years confirmed suggestions that variations in fruit quality are not restricted to the impact of fungal inoculation. Although, AMF performed better than *T. harzianum* or combined *T. harzianum* and AMF, there was no indication of any antagonistic effect between the two fungi. The major setback in this study was the inability of obtaining high root mycorrhizal colonisation, despite inoculation in the nursery.

Although no actual evidence exist to support the premise that high mycorrhizal root colonisation could increase crop yield, strategies to improve mycorrhizal root colonisation should not be overlooked. Indications are that biochar could serve as refuge for AMF against soil predators and increase mycorrhizal root colonisation of crop. In this study, when AMF-inoculated seedlings

were transplanted with biochar, no effects on root colonisation yield or yield components of tomato were observed. However, assessment of the microbial communities in the rhizosphere showed that AMF-inoculated plants shared specific bacterial species with each other suggesting that AMF might influence bacterial community such as PGPR, which are associated with improved plant growth , nutrient uptake and disease control in the rhizosphere. Results of this study also showed that when AMF was applied simultaneously with biochar, the fungal community differed with the rest of the treatments, suggesting that biochar might modify the mycorrhizosphere.

Finally, for commercial fresh produce tomato farmers, nursery inoculation with *T. harzianum* and AMF during sowing could be considered as an effective integrated nutrient and disease management strategy. However, the persistence of low AMF root colonisation in this and numerous previous findings present opportunities for further studies into strategies to improve the situation through using crop specific AMF species, and by investigating the effect of AMF species alone or in combination. Investigations should not be restricted *in vitro* or to seedlings but expanded to field conditions as well. The fact that biochar had an effect on the mycorrhizosphere also opened new avenues on understanding the interactions between AMF and biochar. Arbuscular mycorrhizal fungi are well-documented for stress alleviation, such as salinity and drought, so are *Trichoderma spp*., particularly in disease control. Future studies should investigate the combined effect of *Trichoderma* and AMF on stress (salinity, drought and disease) alleviation and the resultant effect on growth promotion, yield and fruit quality. Similar studies should also be conducted under nutrient stressed conditions, especially where P is limited.

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APPENDICES

Appendix A Table 3.1 ANOVA data for the effect of seaweed extracts types and ratios on the germination, fresh mass and radicle length of tomato seedlings in a growth chamber

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Mean Germination Time	0.725965	72.67368	3.210360	4.417500	Treatment	5.41	0.0002
Germination index	0.969193	11.30386	1.790407	15.83889	Treatment	3.0171	<.0001
Speed of germination	0.981541	14.50422	0.074536	0.513889	Treatment	0.1256	<.0001
Fresh mass	0.742130	26.44139	0.025927	0.09805	Treatment	0.0437	<.0001
Radicle length	0.924474	18.23436	1.412150	7.744444	Treatment	2.3797	<.0001

*Coefficient of variation; **Root MSE = Root Mean Squared Error; ***LSD = Least significant difference

Appendix A Table 3.2 ANOVA data for the effect of nursery application of seaweed extracts, silicon and *Trichoderma harzianum* and arbuscular mycorrhizal fungi mixture on growth and yield aspects of tomato seedlings

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Plant height	0.093723	6.500249	0.722611	11.11667	Treatment	0.8432	0.7593
Root length	0.035124	13.40788	1.557102	11.61333	Treatment	1.8169	0.9817
Dry shoot mass	0.059805	11.17777	0.097179	0.869396	Treatment	0.1134	0.9180
Dry root mass	0.089089	11.58737	0.022144	0.191104	Treatment	0.0258	0.7844
Total biomass	0.046634	9.376077	0.099484	1.061042	Treatment	0.1161	0.9583

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 3.3 ANOVA data for the effect of nursery application of seaweed extracts , silicon and *Trichoderma harzianum* and arbuscular mycorrhizal fungi mixture on *Verticillium* wilt incidence, dry matter content and yield of tomato

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Disease index (10 weeks)	0.129008	69.73124	12.94386	18.56250	Treatment	10.65	0.1048
Disease symptom (10 weeks)	0.233449	37.78520	32.27486	85.41667	Treatment	26.555	0.0080
Disease index (20 weeks)	0.066507	26.78781	15.02908	56.10417	Treatment	12.365	0.3822
Dry shoot mass	0.198544	11.97295	9.003341	75.19738	Treatment	15.584	0.7726
Dry root mass	0.008126	34.26967	5.266435	15.36763	Treatment	9.1156	1.0000
Plant biomass	0.137444	12.39081	11.22174	90.56500	Treatment	19.424	0.9100
Yield	0.651384	12.13971	0.540950	4.456042	Treatment	0.6312	<.0001

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 4.1 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on mycorrhizal and *Trichoderma* root colonisations of 6-week old tomato seedlings

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
% *Trichoderma* (2008)	0.978167	11.59883	7.302967	62.96296	T	4.903	<.0001
					M	4.903	0.8707
					T×M	8.9262	0.2530
% mycorrhiza (2008)	0.584845	70.44750	3.848521	5.462963	T	2.5838	0.5192
					M	2.5838	<.0001
					T×M	4.4406	0.8877
% *Trichoderma* (2009)	0.884715	26.09832	16.23896	62.22222	T	10.902	<.0001
					M	10.902	0.3719
					T×M	14.114	0.8515
% mycorrhiza (2009)	0.385690	79.93650	6.883421	8.611111	T	4.6213	0.9953
					M	4.6213	<.0001
					T×M	3.9176	0.8073

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 4.2 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on plant growth variables of 6-week old tomato seedlings during 2008 growing season

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Shoot length	0.965695	3.879521	0.933905	24.07269	T	0.627	<.0001
					M	0.627	<.0001
					T×M	1.1196	<.0001
Root length	0.835116	6.926261	1.992455	28.76667	T	1.3377	<.0001
					M	1.3377	<.0001
					T×M	2.3918	<.0001
Stem diameter	0.895701	2.778196	0.146550	5.275000	T	0.0984	<.0001
					M	0.0984	<.0001
					T×M	0.1775	<.0001
Dry shoot mass	0.498647	25.03747	2.138246	8.540185	T	1.4355	0.0002
					M	1.4355	0.0364
					T×M	2.5404	0.0045
Dry root mass	0.436730	24.64911	0.592948	2.405556	T	0.3981	0.0003
					M	0.3981	0.3004
					T×M	0.6462	0.0224

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 4.3 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on plant growth variables of 6-week old tomato seedlings during 2009 growing season

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Shoot length	0.478276	11.56668	2.969061	25.66907	T	1.9933	0.0009
					M	1.9933	0.0670
					T×M	3.5086	0.0028
Root length	0.333797	16.23070	4.529839	27.90907	T	3.0412	0.0460
					M	3.0412	0.1920
					T×M	5.3833	0.0235
Stem diameter	0.317632	9.606278	0.470601	4.898889	T	0.3159	0.0098
					M	0.3159	0.0351
					T×M	0.5737	0.4943
Dry shoot mass	0.385323	13.34916	1.278182	9.575000	T	0.8581	<.0001
					M	0.8581	0.5292
					T×M	1.4567	0.4131
Dry root mass	0.207262	13.14408	0.350411	2.665926	T	0.2353	0.0149
					M	0.2353	0.8601
					T×M	0.4082	0.6988

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 4.4 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on macronutrient shoot content of 6-week old tomato seedlings during 2008 growing season

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Nitrogen (N)	0.720602	4.722166	0.213914	4.530000	T	0.2119	0.0143
					M	0.2119	0.0003
					T×M	0.3845	0.1124
Phosphorus (P)	0.468206	13.16453	0.081035	0.615556	T	0.0803	0.6978
					M	0.0803	0.0123
					T×M	0.1458	0.4604
Potassium (K)	0.401253	8.121039	0.228352	2.811852	T	0.2262	0.0648
					M	0.2262	0.6723
					T×M	0.3928	0.3396
Calcium (ca)	0.305944	11.86191	0.507470	4.278148	T	0.5026	0.3635
					M	0.5026	0.1442
					T×M	0.9119	0.8283
Magnesium (mg)	0.280578	12.09942	0.130270	1.076667	T	0.129	0.2593
					M	0.129	0.3427
					T×M	0.2343	0.7653
Sulphur (s)	0.629200	10.74485	0.177409	1.651111	T	0.1757	0.0615
					M	0.1757	0.0055
					T×M	0.3183	0.0807

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 4.5 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on micronutrient shoot content of 6-week old tomato seedlings during 2008 growing season

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Copper (Cu)	0.237408	31.58935	3.615109	11.44407	T	3.5803	0.7363
					M	3.5803	0.2109
					T×M	6.3401	0.8090
Zinc (Zn)	0.654105	11.14818	3.377485	30.29630	T	3.345	0.0686
					M	3.345	0.0163
					T×M	5.8544	0.0125
Manganese (Mn)	0.646686	10.91927	2.624669	24.03704	T	2.5994	0.0696
					M	2.5994	0.0152
					T×M	4.5644	0.0168
Molybdenum (Mo)	0.293211	11.98164	1.731081	14.44778	T	1.7144	0.2328
					M	1.7144	0.9932
					T×M	3.1029	0.3991

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 5.1 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on yield and yield components of tomato

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Number of fruit/plant	0.036930	14.05521	19.99093	142.2315	T	9.3494	0.5404
					M	9.3494	0.3464
					T×M	15.334	0.9810
Total yield	0.059925	17.24880	1.321450	7.661111	T	0.618	0.3827
					M	0.618	0.1459
					T×M	1.0337	0.9783
Marketable yield/plant	0.066497	23.00017	1.367446	5.945370	T	0.6395	0.3780
					M	0.6395	0.1033
					T×M	1.1085	0.9788

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 5.2 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on fruit size of tomato

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Extra-large fruit	0.081515	16.05886	7.219349	44.95556	T	3.3764	0.8905
					M	3.3764	0.0382
					T×M	5.7112	0.7711
Large fruit	0.056502	23.31973	7.152033	30.66944	T	3.3449	0.2097
					M	3.3449	0.5877
					T×M	5.8641	0.7927
Medium fruit	0.187827	33.50834	4.692719	14.00463	T	2.1947	0.3587
					M	2.1947	0.0032
					T×M	3.8269	0.0798
Small fruit	0.033262	33.83728	3.495266	10.32963	T	1.6347	0.4999
					M	1.6347	0.8199
					T×M	2.9095	0.8061

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 5.3 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on phytochemical content of tomato fruit

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Antioxidant activity	0.200505	2.015294	0.101804	5.051593	T	0.1008	0.5130
					M	0.1008	0.3624
					T×M	0.1737	0.9090
Lycopene content	0.665776	13.95257	2.110372	15.12533	T	2.0901	0.0229
					M	2.0901	0.0129
					T×M	3.779	0.0203
Vitamin C	0.390852	13.47633	3.294215	24.4444	T	3.2625	0.0837
					M	3.2625	0.4523
					T×M	5.5715	0.4120

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 5.4 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on chemical content of tomato fruit

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Calcium (Ca)	0.555556	15.11858	0.017638	0.116667	T	0.0182	0.0458
					M	0.0182	0.0534
					T×M	0.0315	0.1296
Phosphorus (P)	0.432130	15.87560	0.055976	0.352593	T	0.581	0.5762
					M	0.581	0.0839
					T×M	0.1007	0.1908
Potassium (K)	0.632363	15.01586	0.253268	1.686667	T	0.2508	0.0678
					M	0.2508	0.00340
					T×M	0.3197	0.1097
Magnesium (Mg)	0.391724	13.46578	0.019052	0.141481	T	0.0189	0.0446
					M	0.0189	0.1643
					TXM	0.0344	0.9965

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 6.1 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on dry matter content and root colonisation of field-grown tomato during 2008 growing season

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
% Mycorrhizal root colonisation	0.874483	39.25276	4.494441	11.45000	Treatment	6.0259	<.0001
% *Trichoderma* root colonisation	0.989399	9.657312	4.900024	50.73900	Treatment	6.5697	<.0001
Dry shoot mass	0.634377	10.17165	2.862860	28.14550	Treatment	3.8384	0.0009
Dry root mass	0.258629	8.047310	0.415120	5.158500	Treatment	0.5566	0.1770

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 6.2 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on yield and yield components of field-grown tomato during 2008 growing season

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Number of fruit/plant	0.059656	7.211939	10.55107	146.3000	Treatment	14.146	0.7979
Early yield/plant	0.534623	24.16640	0.539032	2.230500	Treatment	0.7227	0.0056
Total yield/plant	0.294686	8.120264	0.699723	8.617000	Treatment	0.9382	0.1244
Marketable yield/plant	0.312807	12.29304	0.815828	6.636500	Treatment	1.0938	0.1032

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 6.3 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on fruit size of field-grown in the 2008 growing season

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Extra-large fruit	0.194879	23.25455	7.873992	33.86000	Treatment	10.557	0.3116
Large fruit	0.653966	17.47355	5.358265	30.66500	Treatment	7.1841	0.0006
Medium fruit	0.453247	36.41466	7.960245	21.86000	Treatment	10.673	0.0191
Small fruit	0.104865	20.71346	2.818067	13.60500	Treatment	3.7783	0.6093

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 6.4 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on vitamin C and TSS content of field-grown tomato in the 2008 growing season

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Vitamin C	0.622369	8.519432	2.254810	26.46667	Treatment	4.2455	0.0418
TSS	0.256167	7.652184	0.399061	5.215000	Treatment	0.535	0.1811

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 6.5 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on dry matter content and root colonisation of field-grown tomato during 2009 growing season

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
% Mycorrhizal root colonisation	0.933758	27.42382	3.016621	11.00000	Treatment	4.0445	<.0001
% *Trichoderma* root colonisation	0.993127	8.222172	3.535534	43.00000	Treatment	4.7403	<.0001
Dry shoot mass	0.655587	8.538775	2.697271	31.58850	Treatment	3.6164	0.0006
Dry root mass	0.248172	6.047684	0.313724	5.187500	Treatment	0.4206	0.1952

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 6.6 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on yield and yield components of field-grown tomato during 2009 growing season

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Number of fruit/plant	0.103643	8.590151	11.77710	137.1000	Treatment	15.79	0.6142
Early yield/plant	0.505769	13.10260	0.275155	2.100000	Treatment	0.3689	0.0089
Total yield/plant	0.341703	5.261477	0.446226	8.481000	Treatment	0.5983	0.0756
Marketable yield/plant	0.524508	7.015642	0.449703	6.410000	Treatment	0.6029	0.0066

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 6.7 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on fruit size of field-grown tomato in the 2009 growing season

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Extra-large fruit	0.452195	16.26688	5.930093	36.45500	Treatment	7.9507	0.0194
Large fruit	0.454103	15.05578	4.129800	27.43000	Treatment	5.537	0.0189
Medium fruit	0.164893	41.05721	8.463738	20.61450	Treatment	11.348	0.3963
Small fruit	0.257538	16.45699	2.550833	15.50000	Treatment	3.42	0.1788

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 6.8 ANOVA data for the effect of *Trichoderma harzianum* and arbuscular mycorrhizal fungi on vitamin C and TSS content of field-grown tomato in the 2009 growing season

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Vitamin C	0.538281	5.847494	1.444818	24.70833	Treatment	2.7204	0.0887
TSS	0.554595	3.275484	0.182428	5.569500	Treatment	0.2446	0.0040

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 7.1 ANOVA data for the effect of arbuscular mycorrhizal and biochar on plant growth variables of tomato

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Plant length	0.067380	4.391962	6.554472	149.2379	M	5.5817	0.6391
					B	5.5817	0.5227
					M×B	9.1552	0.3832
Root length	0.197528	7.662864	4.611671	60.18208	M	3.9273	0.2982
					B	3.9273	0.0690
					M×B	5.9005	0.7669
Dry shoot mass	0.355480	9.935326	1.116110	11.23375	M	0.9505	0.0113
					B	0.9505	0.1034
					M×B	1.1361	0.5661
Dry root mass	0.259325	12.61466	0.255209	2.023113	M	0.2173	0.6213
					B	0.2173	0.0235
					M×B	0.3441	0.3999
Plant biomass	0.316595	8.331453	1.104489	13.25686	M	0.9406	0.0137
					B	0.9406	0.2606
					M×B	1.7852	0.4398

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 7.2 ANOVA data for the effect of arbuscular mycorrhizal fungi and biochar on yield and yield components of tomato

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Marketable fruit/plant	0.088033	23.07987	16.38132	70.97667	M	13.95	0.2141
					B	13.95	0.7032
					M×B	13.95	0.7176
Early yield/plant	0.033810	28.02373	0.480490	1.714583	M	0.4092	0.5897
					B	0.4092	0.5394
					M×B	0.3011	0.9231
Marketable yield/plant	0.038824	17.39789	1.097807	6.310000	M	0.9349	0.3906
					B	0.9349	0.9181
					M×B	1.0408	0.8717
Total yield/plant	0.048388	16.85298	1.236587	7.337500	M	1.0531	0.3563
					B	1.0531	0.7374
					M×B	1.810	0.9221

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 7.3 ANOVA data for the effect of arbuscular mycorrhizal fungi and biochar on macronutrient shoot content of tomato

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Nitrogen (N)	0.087236	3.867294	0.157528	4.073333	M	0.1341	0.3887
					B	0.1341	0.4922
					M×B	0.1860	0.4312
Phosphorus (P)	0.470272	18.72597	0.066087	0.352917	M	0.0563	0.0742
					B	0.0563	0.0241
					M×B	0.0840	0.0094
Potassium (K)	0.287723	9.048491	0.245591	2.714167	M	0.2091	0.7679
					B	0.2091	0.0275
					M×B	0.2819	0.1418
Calcium (Ca)	0.039899	23.10564	0.456818	1.977083	M	0.389	0.5887
					B	0.389	0.4911
					M×B	0.5773	0.8496
Magnesium (Mg)	0.012466	2.33402	0.069893	0.566667	M	0.0595	0.9540
					B	0.0595	0.6453
					M×B	0.0887	0.8627

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference

Appendix A Table 7.4 ANOVA data for the effect of arbuscular mycorrhizal fungi and biochar on micronutrient shoot content of tomato

Variable	R-square	*CV	**R-MSE	Mean	Source	***LSD	Pr > F
Zinc (Zn)	0.132388	18.99885	6.784173	35.70833	M	5.7773	0.2316
					B	5.7773	0.2544
					M×B	8.2857	0.6998
Cupper (Cu)	0.254335	35.70015	79.78983	223.5000	M	67.948	0.0616
					B	67.948	0.7165
					M×B	95.157	0.1119
Manganese (Mn)	0.240130	23.42686	32.54382	138.9167	M	27.714	0.0922
					B	27.714	0.7015
					M×B	38.592	0.0966
Sodium (Na)	0.033167	13.08201	389.0425	2973.875	M	331.31	0.9269
					B	331.31	0.5860
					M×B	489.36	0.5493

*Coefficient of variation
**Root MSE = Root Mean Squared Error
***LSD = Least significant difference