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).