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.

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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.