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.