CHAPTER 3

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

3.1 ABSTRACT

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

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

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

3.2 INTRODUCTION

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

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

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

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

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

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

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

3.3 MATERIALS AND METHODS

3.3.1 Determination of SWE concentration for seed priming

Site description

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

Experimental design and treatments

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

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

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

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

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

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

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

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

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

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

Site description

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

Experimental design and treatments

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

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

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

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

Site description

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

Experimental design and treatments

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

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

Data collection

Disease assessment

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

Plant biomass

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

Yield

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

3.3.4 Sources of extracts, inoculants and Si

Seaweed extract

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

Fungal inoculants

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

Silicon

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

3.3.5 *Verticillium* inoculum production

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

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

3.3.6 Data analysis

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

3.4 RESULTS

3.4.1 Determination of SWE concentration for seed priming

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

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

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

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

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

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

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

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

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

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

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

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

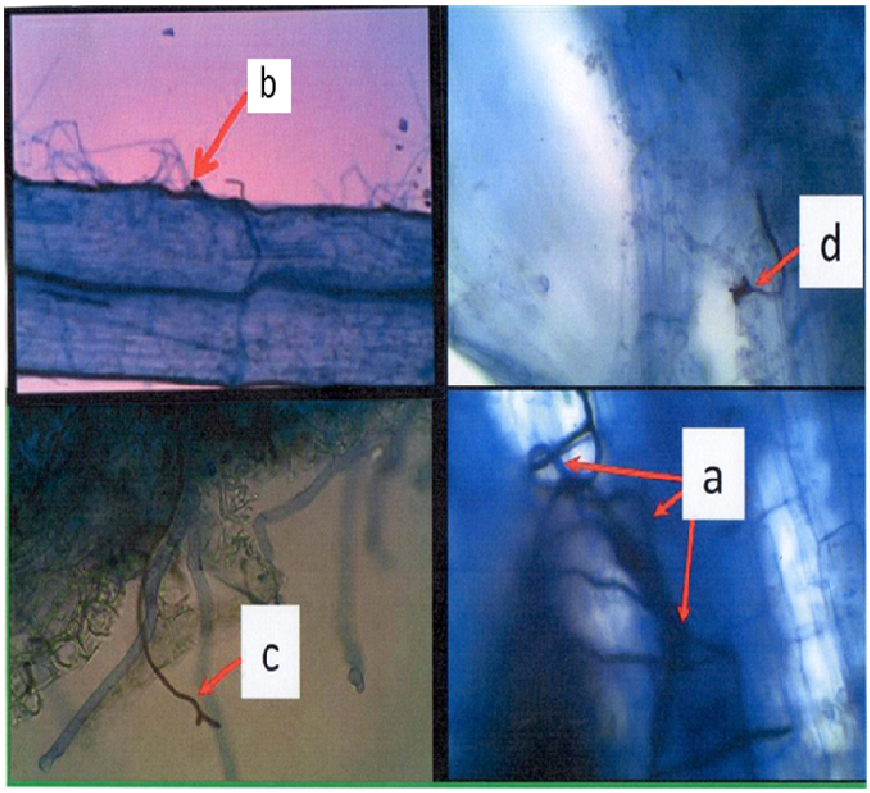

Figure 3.1 Illustration of mycorrhiza structure (a) intracellular mycelia, (b) spores, (c) extracellular mycelia and (d) appresoria confirming the presence of mycorrhizal colonisation in tomato roots inoculated with the mixture of *Trichoderma harzianum* and arbuscular mycorrhizal fungi

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

3.5 DISCUSSION

3.5.1 Determination of SWE concentration for seed priming

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

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

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

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

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

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

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

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

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

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

3.5.4 Conclusions

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