

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

By

Bombiti Nzanza

Submitted in partial fulfilment of Doctor of Philosophy degree

Agronomy

Department of Plant Production and Soil Science

in the Faculty of Natural and Agricultural Sciences University of Pretoria

Pretoria

Supervisor : Dr Diana Marais

Co-supervisor : Prof Puffy Soundy

December 2011

DECLARATION

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

B Nzanza

December 2011

ACKNOWLEDGMENTS

I am ever grateful to God, the source of my strength, for all the privileges, blessings and graces.

I am heavily thankful to my supervisor, Dr Diana Marais for her support, guidance and patience throughout this study. The contribution of my co-Supervisor, Prof Puffy Soundy has been invaluable, for which I am extremely grateful.

I would like to thank my parents for their endless love, support and encouragement. I owe sincere and earnest thankfulness to my uncle, Jean Bakomito and his wife Christiane for their motivation and encouragement. My brothers, sisters, cousins and nephews deserve my wholehearted thanks as well.

My deepest gratitude also goes to my boss, Tommie van Zyl, for giving me the opportunity to complete this thesis. I am obliged to many of my colleagues at ZZ2 who supported me. Special thanks to Piet Prinsloo and Burtie van Zyl.

I am grateful to Dr Karen Surridge-Talbot for her immense contribution in the microbial community structure study. I thank Jacques Marneveck and his staff for their technical assistance. I am indebted to Poly Kayembe and Mireille Asanzi for helping with the experimental trials.

Especially, I would like to give my special thanks to my wife Mireille and my two sons, Nathan and Tommie, whose patient love and endless support enabled me to complete this study.

TABLE OF CONTENTS

DECLARATION

ACKNOWLEDGEMENTS

LIST OF TABLES ix

LIST OF FIGURES xiv

LIST OF ACRONYMS AND ABBREVIATIONS xvi

ABSTRACT xviii

CHAPTER 1: GENERAL INTRODUCTION 1

1.1 Rationale 1

1.2 Objectives 5

1.3 Research approach and thesis outline 6

CHAPTER 2: LITERATURE REVIEW 8

2.1 Arbuscular mycorrhizal fungi 8

2.1.1 Taxonomy and benefits 8

2.1.2 Mycorrhizal root colonisation as affected by AMF inoculation 9

2.1.3 Effect of nursery inoculation with AMF on plant growth 11

2.1.4 Effect of nursery inoculation with AMF on fruit yield and quality 13

2.1.5 Effect of nursery inoculation with AMF on disease control 15

2.2 *Trichoderma* 16

2.2.1	Taxonomy and benefits	16
2.2.2	<i>Trichoderma</i> spp. and plant growth promotion	17
2.2.3	<i>Trichoderma</i> spp. and yield and fruit quality	18
2.2.4	<i>Trichoderma</i> spp. and biological control of plant diseases	19
2.3	Arbuscular mycorrhizal fungi and <i>Trichoderma</i>	21
2.3.1	Interactions and root colonisation	21
2.3.2	Plant growth promotion	24
2.3.3	Disease control	26
CHAPTER 3: GROWTH, YIELD AND <i>VERTICILLIUM</i> WILT INCIDENCE OF TOMATO (<i>SOLANUM LYCOPERSICUM</i>) AS INFLUENCED BY DIFFERENT PRE-SOWING TREATMENTS		28
3.1	Abstract	28
3.2	Introduction	29
3.3	Materials and methods	32
3.3.1	Determination of SWE concentration for seed priming	32
3.3.2	Effect of pre-sowing treatments on tomato seedling growth and development	34
3.3.3	Effect of pre-sowing treatments on growth, yield and <i>Verticillium</i> incidence of tomato	35
3.3.4	Sources of extracts, inoculants and Si	37

3.3.5	<i>Verticillium</i> inoculum production	37
3.3.6	Data analysis	38
3.4	Results	38
3.4.1	Determination of SWE concentration for seed priming	38
3.4.2	Effect of pre-sowing treatments on tomato seedling growth and development	39
3.4.3	Effect of pre-sowing treatments on growth, yield and <i>Verticillium</i> incidence of tomato	42
3.5	Discussion	44
3.5.1	Determination of SWE concentration for seed priming	44
3.5.2	Effect of pre-sowing treatments on tomato seedling growth and development	45
3.5.3	Effect of pre-sowing treatments on growth, yield and <i>Verticillium</i> incidence of tomato	46
3.5.4	Conclusions	48
CHAPTER 4: TOMATO (<i>SOLANUM LYCOPERSICUM</i> L.) SEEDLING GROWTH AND DEVELOPMENT AS INFLUENCED BY <i>TRICHODERMA HARZIANUM</i> AND ARBUSCULAR MYCORRHIZAL FUNGI		49
4.1	Abstract	49
4.2	Introduction	50

4.3	Materials and methods	51
4.3.1	Site description	51
4.3.2	Experimental design and treatments	51
4.3.3	Data collection	53
4.3.4	Data analysis	54
4.4	Results	54
4.4.1	Root colonisation by fungi	54
4.4.2	Growth variables	56
4.4.3	Biomass production	59
4.4.4	Shoot chemical analysis	61
4.5	Discussion	64
	CHAPTER 5: YIELD AND NUTRIENT CONTENT OF GREENHOUSE PRODUCED TOMATO (<i>SOLANUM LYCOPERSICUM</i> L.) AS INFLUENCED BY <i>TRICHODERMA HARZIANUM</i> AND <i>GLOMUS MOSSEAE</i> INOCULATION	68
5.1	Abstract	68
5.2	Introduction	69
5.3	Materials and methods	71
5.3.1	Site description	71
5.3.2	Experimental design and treatments	71

5.3.3	Data collection	72
5.3.4	Data analysis	73
5.4	Results	73
5.4.1	Yield and fruit size distribution	73
5.4.2	Tomato fruit mineral content	75
5.4.3	Phytochemical analysis	77
5.5	Discussion	78
CHAPTER 6: RESPONSE OF TOMATO (<i>SOLANUM LYCOPERSICUM</i> L.) TO NURSERY INOCULATION WITH <i>TRICHODERMA HARZIANUM</i> AND ARBUSCULAR MYCORRHIZAL FUNGI UNDER FIELD CONDITIONS		81
6.1	Abstract	81
6.2	Introduction	82
6.3	Materials and methods	84
6.3.1	Site description	84
6.3.2	Experimental design and treatments	85
6.3.3	Data collection	87
6.3.4	Data analysis	87
6.4	Results	88
6.4.1	Mycorrhizal and <i>Trichoderma</i> root colonisation	88

6.4.2	Shoot and root dry mass	88
6.4.3	Yield and yield components	89
6.4.4	Fruit size	90
6.4.5	Vitamin C and TSS	91
6.5	Discussion	92
CHAPTER 7: EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGAL INOCULATION AND BIOCHAR AMENDMENT ON GROWTH AND YIELD OF TOMATO (<i>SOLANUM LYCOPERSICUM</i> L.)		96
7.1	Abstract	96
7.2	Introduction	97
7.3	Materials and methods	99
7.3.1	Effect of AMF-inoculated plants and biochar-amended soil on tomato production	99
7.3.2	Effect of AMF and biochar amendment on fungal and bacterial populations	102
7.4	Results	103
7.4.1	Effect of AMF-inoculated plants and biochar-amended soil on tomato production	103
7.4.2	Effect of AMF and biochar amendment on fungal and bacterial populations	107

7.5 Discussion	115
7.5.1 Effect of AMF-inoculated plants and biochar-amended soil on tomato production	115
7.5.2 Effect of AMF and biochar amendment on fungal and bacterial populations	116
7.5.3 Conclusions	118
CHAPTER 8: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS	119
REFERENCES	123
APPENDICES	169

LIST OF TABLES

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	40
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	42
Table 3.3	Effect of nursery treated seedlings with seaweed extracts, silicon and fungal inoculants on the <i>Verticillium</i> wilt incidence on tomato	43
Table 3.4	Effect of nursery treated seedlings with SWE, silicon and fungal inoculants on marketable yield and dry matter production of tomato	44
Table 4.1	Partitioning of the treatment sum of squares (SS) derived from ANOVA for the root colonisation of 6-week old tomato seedlings as influenced by <i>Trichoderma harzianum</i> and arbuscular mycorrhizal fungi	55
Table 4.2	Percentage root colonisation of 6-week old tomato seedlings as influenced by <i>Trichoderma harzianum</i> and arbuscular mycorrhizal fungi	56
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 <i>Trichoderma harzianum</i> and arbuscular mycorrhizal fungi	58
Table 4.4	Plant growth variables of 6-week old tomato seedlings as influenced by <i>Trichoderma harzianum</i> and arbuscular mycorrhizal fungi	60
Table 4.5	Results of ANOVA (P values) executed for the shoot mineral nutrient content for the 2008 growing season	61
Table 4.6	Macronutrient shoot contents of 6-week old tomato seedlings as influenced by <i>Trichoderma harzianum</i> and arbuscular mycorrhizal fungi	62

Table 4.7	Micronutrient shoot contents of 6-week old tomato seedlings as influenced by <i>Trichoderma harzianum</i> and arbuscular mycorrhizal fungi	63
Table 5.1	Results of ANOVA (p values) executed for the yield and yield components of tomato plants	74
Table 5.2	Number of fruit, yield and marketable yield of tomato as influenced by <i>Trichoderma harzianum</i> and arbuscular mycorrhizal fungi	74
Table 5.3	Fruit size of tomato as influenced by <i>Trichoderma harzianum</i> and arbuscular mycorrhizal fungi	75
Table 5.4	Results of ANOVA (p values) executed for the chemical and phytochemical contents of tomato fruit	76
Table 5.5	Chemical fruit contents of tomato as influenced by <i>Trichoderma harzianum</i> and arbuscular mycorrhizal fungi	76
Table 5.6	Phytochemical fruit content of tomato as influenced by <i>Trichoderma harzianum</i> and arbuscular mycorrhizal fungi	77
Table 6.1	Dry matter content and root colonisation of field-grown tomato as influenced by <i>Trichoderma harzianum</i> and arbuscular mycorrhizal fungi	88
Table 6.2	Yield and yield components of field-grown tomato as influenced by <i>Trichoderma harzianum</i> and arbuscular mycorrhizal fungi	89
Table 6.3	Fruit size class of field-grown tomato as influenced by <i>Trichoderma harzianum</i> and arbuscular mycorrhizal fungi	90
Table 6.4	Vitamin C content and TSS of field-grown tomato fruit as influenced by <i>Trichoderma harzianum</i> and arbuscular mycorrhizal fungi	91
Table 7.1	Chemical and physical characteristics of biochar produced from <i>Eucalyptus globulus</i>	101
Table 7.2	Growth variables of tomato as influenced by arbuscular mycorrhizal fungi and	104

biochar

Table 7.3	Percentage of mycorrhiza root colonisation, mean yield and yield components of tomato as influenced by arbuscular mycorrhizal fungi and biochar	105
Table 7.4	Shoot nutrient content of tomato as influenced by arbuscular mycorrhizal fungi	106
Table 7.5	Phosphorus shoot content of tomato as influenced by interactive effect of arbuscular mycorrhizal fungi and biochar	107
Table 7.6	Tentative identification of denaturing gradient gel electrophoresis (DGGE) bands sequenced according to BLAST results from the NCBI GenBank database	113

LIST OF FIGURES

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 <i>Trichoderma harzianum</i> and arbuscular mycorrhizal fungi	41
Figure 4.1	Tomato seedlings growing into PVC pipe supported by a cylinder base	53
Figure 6.1	Illustration of tomato plants transplanted into double rows on raised beds in an open field	86
Figure 7.1	Tris-acetate –EDTA (TAE) agarose gel (1.5%) showing high-quality, clean genomic DNA extracted from soil samples	107
Figure 7.2	Tris-acetate –EDTA (TAE) agarose gel (1.5%) showing 5µl of PCR product from each of the 16S bacterial gene amplifications	108
Figure 7.3	Denaturing gradient gel electrophoresis (DGGE) gel showing species diversity of bacteria (B) and fungi (F) from soil samples run at 40-60% denaturants. PCR product is separated according to base-pair sequence differences to determine community richness and diversity of microorganisms based on these fingerprints	109
Figure 7.4	Graphic representation of the denaturing gradient gel electrophoresis (DGGE) gel in Figure 7.3 depicting the band pattern, indicating species diversity within bacterial (B) and fungal (F) populations, produced by each of the samples	110
Figure 7.5	Number of dominant bacterial or fungal species per sample visible from denaturing gradient gel electrophoresis (DGGE) bands	110
Figure 7.6	Cluster analysis of the banding pattern in Figure 7.4, using a jaccard matching, group average setting to separate bacterial (B) and fungal (F) populations on the basis of community differences	112

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

114

LIST OF ACRONYMS AND ABBREVIATIONS

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



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

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

BY

BOMBITI NZANZA

SUPERVISOR: DR D MARAIS

CO-SUPERVISOR: PROF P SOUNDY

DEPARTMENT: PLANT PRODUCTION AND SOIL SCIENCE

DEGREE: PhD

ABSTRACT

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

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