INTERACTION BETWEEN FUSARIUM WILT AND ROOT-KNOT NEMATODES IN TOMATO AND THE POTENTIAL OF INTEGRATED STRATEGIES IN MANAGEMENT OF THE DISEASE COMPLEX

Mwangi Margaret Wanjiru

B. Ed (Science) M.Sc. (Microbiology)

A80/94827/2014

A Thesis Submitted in Fulfillment of the Requirements for the Award of the Degree of Doctor of Philosophy in Crop Protection

Department of Plant Science and Crop Protection, Faculty of Agriculture, University of Nairobi

2018

DECLARATION

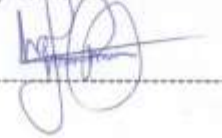
This thesis is my original work and has not been submitted for award of a degree in any other university

Margaret Wanjiru Mwangi — DATE

13/12/2018

This thesis has been submitted with our approval as university supervisors

Dr. William M. Muiru
Department of Plant Science and Crop Protection
University of Nairobi — DATE

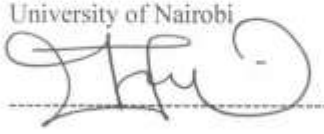


13/12/2018

Prof. Rama D. Narla
Department of Plant Science and Crop Protection
University of Nairobi — DATE

R.D Narla — 13.12.2018

Prof. John W. Kimenju
Department of Plant Science and Crop Protection
University of Nairobi — DATE



13/12/2018

Dr. George M. Kariuki (Deceased)
Department of Agricultural Science and Technology
Kenyatta University

DECLARATION OF ORIGINALITY

Name of Student	MARGARET WANJIRU MWANGI
Registration Number	A80/94827/2014
College	Agriculture and Veterinary Sciences
Faculty/ school/ Institute	Agriculture
Department	Plant Science and Crop Protection
Course Name	Crop Protection
Title of Work:	Interaction between fusarium wilt and root-knot nematodes in tomato and the potential of integrated strategies in management of the disease complex

Declaration

1. I understand what plagiarism is and I am aware of the University's policy.
2. I declare that this thesis is my original work and has not been submitted for examination, award of a degree or publication. Where other peoples work, or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.
3. I have not sought or used the services of any professional agencies to produce this work.
4. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his or her own work.
5. I understand that any false claim in respect of this work shall result in disciplinary action, in accordance with University Plagiarism Policy.

Signature …….

Date …….....

DEDICATION

This thesis is dedicated to all the people who supported this work and in memory of the late Dr. George M. Kariuki

ACKNOWLEDGEMENTS

My Doctorial studies were a great success because of people who assisted and supported the work wholeheartedly. My supervisors, Dr. W. M. Muiru, Prof. R. D. Narla, Prof. J. W. Kimenju and the late Dr. G. M. Kariuki worked tiressly, patiently and with total commitment. I am grateful to them and appreciate their guidance, support and all the sacrifices they made. Your valuable guidance, patience and encouragement have made this work come to a successful end.

My special thanks to University of Nairobi (UoN) Technical staff in the Department of Plant Science and Crop Protection and Mr. Kimotho of LARMAT (UoN) for his technical support in the analysis of the soil samples. I am grateful to Mr. Wachira, Mr. Rono, Ruben Mangi, all of Chiromo Campus (UoN), and Morris Muthini of Kenyatta University for their advice and assistance on the molecular work.

I deeply appreciate the contribution and sacrifices made by my husband Isaac, and our children, Loise, John and Joyce. Your prayers, encouragement, and support counted a lot in making this work a success. I am also especially grateful, to A.I.C (African Inland Church) Friday fellowship members, for their prayers that encouraged and carried me through when the going was very tough.

Special thanks to Nairobi Technical Training Institute Central Administration; the Head of Department, Health and Applied Sciences (HAS), Mr. Earnest Gathiru and Technical staff in the Department, for allowing me to do the bench work in the HAS laboratories.

Finally I appreciate Ministry of Agriculture extension officers in Mwea West Sub County who were always available to give guidance when in the field.

TABLE OF CONTENTS

DECLARATION ..... **Error! Bookmark not defined.**
DECLARATION OF ORIGINALITY iii
DEDICATION iv
ACKNOWLEDGEMENTS v
TABLE OF CONTENTS vi
LIST OF TABLES xi
LIST OF FIGURES xiii
LIST OF PLATES xiv
LIST OF APPENDICES xv
ABBREVIATIONS xvi
ABSTRACT xvii
CHAPTER ONE 1
INTRODUCTION 1
1.1 Introduction 1
1.2. Problem statement 3
1.3 Justification 4
1.4 Objectives 5
1.4.1 Overall objective 5
1.4.2 Specific objectives 5
1.5 Hypotheses 6
CHAPTER TWO 7
LITERATURE REVIEW 7
2.1 Tomato (*Lycopersicum esculentum* Mill) Production 7
2.2 *Fusarium oxysporum* f. sp. *lycopersici* (Snyder and Hansen) 7
2.2.1 Characterisation of Fusarium oxysporum 8
2.3 Diversity of soil nematodes 9
2.3.1 Root-knot nematodes 10
2.4 Synergistic interactions of Fusarium wilt pathogen and root-knot nematodes 11
2.5 Management of Fusarium wilt and root-knot nematodes 11
2.5.1 Cultural control methods 11
2.5.2 Chemical methods of management 13

2.5.3 Biological control 13
2.5.3.1 *Paecilomyces lilacinus* (Thom) Samson syn. *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson 14
2.5.3.2 *Trichoderma* spp. 15
2.5.4 Integrated approaches in the management of Fusarium wilt-root-knot nematodes disease complex 15
CHAPTER THREE 19
TOMATO PRODUCTION PRACTICES AND OCCURRENCE OF DISEASES IN MWEA WEST SUB COUNTY 19
ABSTRACT 19
3.1 Introduction 20
3.2 Materials and Methods 21
3.2.1 Study area 21
3.2.2 Soil sampling 22
3.2.3 Nematode extraction and identification 24
3.2.4 Data analysis 24
3.3 Results 25
3.3.1 Socio economic characteristics of respondents 25
3.3.2 Crops grown and cropping patterns in the study area 27
3.3.3 Diseases and pests that affect tomato crop and control methods 29
3.3.4 Sources of seedlings and agricultural information 32
3.3.5 Soil nematode trophic groups in the rhizhosphere of tomato plants 33
3.4 Discussion 34
CHAPTER FOUR 38
MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF ISOLATES OF *FUSARIUM* SPP. FROM INFECTED TOMATO PLANTS 38
Abstract 38
4.1 Introduction 39
4.2 Materials and Methods 41
4.2.1 Sample collection and isolation of Fusarium spp. from tomato stems 41
4.2.2 Preparation of inoculum for pathogencity tests 42
4.2.3 Preparation of soils for pathogenicity tests in greenhouse experiments 43
4.2.4 Planting of tomato seeds 43
4.2.5 Pathogenicity tests 43

4.2.6 Molecular characterization of Fusarium isolates 44
4.2.6.1 DNA extraction protocol 44
4.2.6.2 PCR amplification 46
4.2.6.3 Purification of PCR products 46
4.2.6.4 Sequencing of PCR products 47
4.2.6.5 Editing, assembly and alignments of the DNA sequences 48
4.3 Results 49
4.3.1 Isolation of Fusarium oxysporum from tomato stems 49
4.3.2 Cultural characteristics of Fusarium spp. isolates 49
4. 3. 3 Microscopic characteristics 49
4.3. 4 Pathogenicity testing 50
4.3.5 Molecular analysis of isolates 55
4.4 Discussion 57
CHAPTER FIVE 60
EVALUATION OF REACTION OF DIFFERENT TOMATO CULTIVARS TO MELOIDOGYNE JAVANICA AND FUSARIUM WILT 60
Abstract 60
5.1 Introduction 61
5.2 Materials and Methods 64
5.2.1 Identification of Meloidogyne species used in the experiments 64
5.2.2 Planting of tomato seeds 65
5.2.3 Preparation and maintenance of second stage juveniles (J2s) inocula 65
5.2.4 Preparation of inoculum for testing the reaction of different tomato cultivars to Fusarium wilt isolates 66
5.2.5 Preparation of *F. oxysporum* f. sp. *lycopersici* (FOL) inoculum 67
5.2.6 Experimental design and application of the treatments 68
5.2.6.1 Reaction of tomato cultivars to *M. javanica* 68
5.2.6.2 Susceptibility of tomato cultivars to four isolates of Fusarium wilt of tomato 69
5.2.6.3 Interaction of *F. oxysporum* f. sp. *lycopersici* and different inoculum levels of M. javanica in two tomato cultivars Rambo F1 and Prostar F1 69
5.2.7 Data collection 70
5.2.7.1 Extraction of juveniles from the roots 71

5.2.7.2 Data analysis 71
5.3 Results 72
5.3.1 Identification of *Meloidogyne* species 72
5.3.2 Reaction of tomato cultivars to *M. javanica* and the effect of the infestation on growth 72
5.3.3 Reaction of different tomato cultivars to four isolates of Fusarium wilt 74
5.3.4 Effect of *F. oxysporum* f. sp. *lycopersici* infection and different M. javanica inoculum levels on galling index, nematode populations in the roots and disease severity in the tomato cultivars 75
5.3.5 Effect of inoculating *F. oxysporum* f. sp. *lycopersici* and different inoculum levels of M. javanica on plant growth parameters in cultivars Prostar F1 and Rambo F1 77
5.4 Discussion 79
CHAPTER SIX 83
MANAGEMENT OF FUSARIUM WILT AND ROOT-KNOT NEMATODES DISEASE COMPLEX BY USE OF ANTAGONISTIC FUNGI, PLANT RESISTANCE AND ORGANIC AMENDMENTS 83
Abstract 83
6.1 Introduction 84
6.2 Materials and Methods 86
6.2.1 Interaction of biocontrol fungi and F. oxysporum f. sp. lycopersici in dual culture plates 86
6.2. 2 Planting of tomato seeds 87
6.2.3 Preparation of *F. oxysporum* f. sp. *lycopersici* (FOL) inoculum 88
6.2.4 Source and production of *P. lilacinum, T. harzianum* inocula 88
6.2.5 Preparation of the J2s and inoculation of tomato plants 89
6.2.6 Experimental design and treatment application 90
6.2.7 Data collection 91
6.2.7.1 Extraction of juveniles from the roots 91
6.2.7.2 Estimation of F. oxysporum f. sp. lycopersici fungal propagules in the soil and roots of tomato plants 91
6.2.7.3. Data analysis 92
6.3 Results 93

6.3.1 Antagonism of *T. harzianum and P. lilacinum* against *F. oxysporum* f. sp. *lycopersici* in vitro 93
6.3.2 Effect of *T. harzianum, P. lilacinum* and neem treatment in controlling Meloidogyne javanica and *F. oxysporum* f. sp. *lycopersici* in tomato cultivars 95
6.3.3 Effect of biological control agents on *F. oxysporum* f. sp. *lycopersici* disease severity, propagules and M. javanica J2s in sterile and non-sterile soils 98
6.4 Discussion 100
CHAPTER SEVEN 104
GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS 104
7.1 GENERAL DISCUSSION 104
7.2 CONCLUSION 108
7.3 Recommendations 109
REFERENCES 111
APPENDICES 129

LIST OF TABLES

Table 3.1 Demographic characteristics of respondents from Mwea West Sub County 26

Table 3.2. Relationship between type of land ownership and allocation of land to different uses 27

Table 3.3. Percent of farmers growing different crops and cropping patterns in Mwea West Sub County 28

Table 3.4. Relationship between type of land ownership and cropping systems in Mwea West Sub County 28

Table 3.5 Relationship between cropping system and diseases and pests on tomato crop.....30

Table 3.6 Chemicals' applied into the soil to control nematodes in Mwea West Sub County.....30

Table 3.7 Relationship between source of information and pesticides applications by farmers in Mwea West Sub County 33

Table 3.8 Abundance, composition of nematode genera in the rhizosphere of tomato plants 34

Table 4.1 Characteristics of *Fusarium oxysporum* isolated from diseased tomato plants collected from MweaWest Sub County fields.....51

Table 4.2 Rate of growth of isolates on PDA and virulence of the isolates on tomato cultivar "Money Maker" 53

Table 4.3 Isolates identified using TEF-1α sequences and Fusarium ID and MLST databases and % similarity value to the reference isolate.....56

Table 5.1 Reproduction factor, number of galls and juvenile populations per root system induced by treatment with *M. javanica* under greenhouse conditions on six tomato cultivars 73

Table 5.2 Effect of infestation with *M. javanica* on the dry weight of shoot and root in different tomato cultivars 74

Table 5.3 Reaction of six tomato cultivars to four Fusarium wilt isolates under greenhouse conditions 75

Table 5.4 Number of galls and juvenile populations in the roots in two tomato cultivars in different treatments of *M. javanica* in combination with FOL under greenhouse conditions 76

Table 5.5 Effect of FOL and different inoculum levels of *M. javanica* on disease severity in tomato cultivars Prostar F1 and Rambo F1 under greenhouse conditions 77

Table 5.6 Effect of inoculation with FOL and different inoculum levels of *M. javanica* on plant growth parameters in cultivars Prostar F1 and Rambo F1 78

Table 6.1 Fungal mycelial growth in dual culture plates compared with controls and results of paired T sample tests. 93

Table 6.2 Effect of different treatments on FOL propagules, *M. javanica* juveniles and growth in cultivar Prostar F1 97

Table 6.3 Effect of different treatments on FOL propagules, *M. javanica* juveniles and growth in cultivar Rambo F1 97

Table 6.4 Effect of fungal antagonists and organic amendments on FOL propagules and *M. javanica* J2s in sterile and non sterile soils 99

Table 6.5 Effect of TH, PL and neem on growth of tomato plants inoculated with *M. javanica* and FOL pathogen in sterile and non sterile soils 99

LIST OF FIGURES

Fig 3.1 Map of Mwea West Sub County indicating routes followed during field survey.....23

Fig. 3.2 Average Farm sizes in Mwea West Sub County 26

Fig 3.3 Tomato varieties grown by farmers in Mwea West Sub County 28

Fig. 3.4 Prevalence of wilt, other diseases and pests on tomato crop.....30

Fig. 3.5 Farmers indication on proportion of crops affected by wilting.....31

Fig. 3.6 Farmers indication of ways of controlling wilting 31

Fig. 3.7 Method employed for controlling nematodes.....32

Fig. 4.1 Phylogenetic tree generated using by MEGA 7 software. Maximum Parsimony Analysis 57

Fig .5.2 Effect of different nematode inoculum levels on disease severity in cultivar Prostar F1 under greenhouse conditions.....76

Fig. 6.1 *In vitro* percentage inhibition of FOL by TH and PL.The I bars signify 5% error. 94

Fig. 6.2 Effect of different treatments on FOL and root-knot disease complex in greenhouse environment.....96

Fig. 6.3 Effect of TH, PL and a combined application (PL-TH) in controlling FOL in Fusarium wilt and root-knot disease complex in greenhouse environment.. 98

LIST OF PLATES

Plate 4.1a isolate F16 (FOL) Upperside isolate F16 (FOL) Reverse side 54

Plate 4.1b isolate F41(FOL) Upperside isolate F41(FOL) Reverse side 55

Plate 5.1 Perineal pattern of *M. javanica* x40 magnification.....72

Plate 6.1a Antagonism between *F. oxysporum* f. sp. *lycopersici* (FOL) and *T. harzianum* (TH).....94

Plate 6.1b Antagonism between *F. oxysporum* f. sp. *lycopersici* (FOL) and *P. lilacinum* (PL) 95

Plate 6.1c Dual culture of *P. lilacinum* (PL) and *T. harzianum* (TH).....95

LIST OF APPENDICES

APPENDIX 1 QUESTIONNAIRE.....129

Appendix 2 Composition of Different Media.....133

Appendix 3 Physico- chemical properties of soil used in greenhouse experiments.....133

Appendix 4 Disease severity scale done based on length of discoloured vascular tissue 133

Appendix 5 Ingredients and their approximate amount in neem.....134

Appendix 6 Production of *P. lilacinum* on maize flour- sand media.....135

ABBREVIATIONS

DNA	Deoxyribonucleic Acid
FAO	Food Agriculture Organization
FOL	*Fusarium oxysporum* f. sp. *lycopersici*
ICIPE	International Centre for Insect Physiology and Ecology
J2s	Second stage larvae (J2s)
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PL	*Paecilomyces lilacinus* syn. *Purpureocillium lilacinum*
PL-TH	*Paecilomyces lilacinus* and *Trichoderma harzianum*
RKN	Root-knot nematodes
SNA	Spezieller Nahrstoffarmer Agar
TH	*Trichoderma harzianum*
TEF-1α	Translation elongation factor 1-alpha gene
UNEP	United Nations Environmental Program

ABSTRACT

Root-knot nematodes (RKN), *Meloidogyne* spp. and *Fusarium oxysporum* f. sp. *lycopersici* (FOL) that cause vascular wilt, are among the most important diseases affecting tomato production in Kenya. The aim of this study was to investigate interactions between RKN and FOL in tomato cultivars, and explore methods of their control. A survey was conducted in Mwea West Sub County on tomato production practices and diseases that affect the tomato crop. Soil was sampled from 119 farms and was analysed for prevalence of different types of nematodes genera. Experiments were carried out in the greenhouse to test six different tomatoes cultivars reaction to *Meloidogyne javanica* and four different isolates of Fusarium wilt, and also test effect of interaction between *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and *M. javanica* at different inoculum densities of 500, 1000, 2000 and 3000 second stage juveniles (J2s) in two tomato cultivars Rambo F1 and Prostar F1. Efficacy of the biological control agents *Trichoderma harzianum* (TH) and *Purpureocillium lilacinum* (PL) and organic amendment neem in the management of FOL and the root-knot nematode *M. javanica* was assessed under greenhouse conditions. Field survey data showed that RKN had a prevalence rate of 3.8%, while the actual study in field established that among all nematode genera, *Meloidogyne* spp. was the most abundant at 15.86%. *Fusarium verticillioides* was isolated from infected tomato stems collected from tomato farms together with *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum.* In the evaluation of different tomato cultivars reaction to *Meloidogyne javanica,* the results showed that, Rambo F1, Roma VFN, Prostar F1 and Cal J were better hosts to *M. javanica* with high galling indices and greater reductions in growth, while Kilele F1 and Oxly were less susceptible. The tomato cultivars Prostar F1, Roma VFN had 100% disease incidence scores when inoculated with isolate F41 of FOL with cultivar Rambo F1, being the least susceptible, at an incidence rate of 16.7%. When the cultivar Prostar F1 was inoculated with the isolate of FOL and different inoculum levels of *M.*

javanica, there was progressive reduction in growth and increase in FOL disease severity with increasing nematode inoculum levels. In cultivar Rambo F1, the FOL disease severity was insignificant even at high nematode inoculum levels and there was less reduction in growth in this cultivar compared to Prostar F1. *In vitro* dual culture plate tests showed significant mycelia inhibition of FOL by TH and PL. The treatments, TH, PL, and PL-TH significantly controlled FOL disease in the cultivar Prostar F1. The resistance of the cultivar Rambo F1 sufficed to prevent infection by FOL. The treatments TH, PL, PL-TH significantly controlled the *M. javanica* J2s in Prostar F1 and Rambo F1 tomato cultivars. Neem enhanced the efficiency of the fungal biological control agents such that the treatments application with neem and fungal antagonists' performed better than those without neem in the control of FOL wilt disease and *M. javanica* J2s. The fungal antagonists and neem were equally effective in controlling Fusarium wilt in sterile and non sterile conditions while being less effective in the control of *M. javanica* in the cultivar Prostar F1. The survey established the need to create awareness on RKN and FOL to prepare farmers to be receptive to new methods of control of FOL and RKN. There is need of surveillance by all agriculture stakeholders to check on the progress of *F. verticillioides* as a new pathogen that can infect tomato and other crops. The possible effects of *M. javanica* and FOL interaction in an FOL resistant cultivar Rambo F1, implies that the use of a resistant cultivar integrated with neem and a fungal antagonist is a viable option for the control of FOL and RKN disease complex. A combined application of biological control agents should only be used after pretesting to ensure that they do not inhibit one another and are compatible, especially if their modes of action against the pathogen are similar. The fact that biological control agents and neem were equally effective in controlling Fusarium wilt in non sterile soil as in sterile, although not as effective in the control of RKN in non sterile soil conditions, suggest there is potential to use them in non sterile natural soil conditions, as an alternative to use of chemicals pesticides.

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Tomato (*Lycopersicum esculentum* Mill) belongs to the family Solanaceae. In temperate climates it is a perennial crop while in tropical it is an annual crop. It has a weak woody stem and must be supported during growth. It can grow up to a height of 3 metres. Tomato contains vitamin B and C and minerals such as potassium (Beecher, 1998). It also contains lycopene, an anti-oxidant that protects the consumer against carcinogenic substances (Yang *et al*., 2006).

Root-knot nematodes (RKN) are parasitic to most plants (Regaieg *et al.,* 2010). Damage to global agricultural crops due to RKN infestation is estimated to be around US$ 80 billion annually (Li, 2007). Root-knot nematodes infection in tomato plants causes stunting, yellowing, wilting and galls or swelling of the roots (Yazdi *et al.,* 2012). Heavy infestation leads to yield loss and poor crop quality (Kenya Ministry of Agriculture and Livestock development, 2003).

Fusarium oxysporum f. sp. *lycopersici* (FOL) causes vascular wilt in tomatoes. It is a destructive pathogen to tomatoes grown in the field and also in greenhouses. Yield loss in the range of 30% to 40% has been reported in tomato growing areas (Kirankumar *et al.,* 2008). The disease caused by this fungus is characterized by wilted plants, yellow leaves and epinasty of petioles. In older plants there is stunted growth, yellowing of lower leaves and browning of the vascular system (Agrios, 2005).

Disease complexes are formed by an association of two organisms that infect a crop at the same time causing significant loss in yields. The pathogens belonging to *Fusarium* spp. and RKN (*Meloidogyne* spp.) form a complex disease in most crops (Bhagawati *et al*., 2000; Bhagawati *et al*., 2007). The importance of this disease complex has been observed in cotton

whereby RKN increased Fusarium wilt disease severity in different cultivars. The effect was least in cultivars with a high resistance to Fusarium wilt (Katsantonis *et al*., 2003). Root-knot nematodes have been reported to advance Fusarium wilt disease to even breaking resistance in tomato resistant cultivars (Suhardi *et al.*, 1980; Hadavi and Sahebani, 2008).

The disease complex formed due to interaction of RKN and Fusarium wilt pathogen demands a well planned disease management programme for sustainable tomato production. Therefore management options should aim at strategies that will tackle both the Fusarium wilt pathogen and RKN. Main pesticides have been used in the management of RKN and the FOL pathogen individually or when jointly combined with other methods. Most of the chemicals that have previously been used in the control of plant diseases and pests have adverse effect on the environment and leave residues in crop plants that are harmful to human beings. With time these chemicals are being phased out due to the hazardous effects they have on the environment (Pest Control Products Board, 2017). Such is the case for methyl bromide used widely in fumigation in the management of soil pathogens, in particular in the management of RKN (Giannakou and Anastasiadis, 2005) but has been banned all over the world since 2005 (UNEP, 2001).

Breeding for resistance has been widely used to develop cultivars that are resistant to Fusarium wilt pathogen and RKN. However, there is a high resistance and this has led to the development of more virulent strains of the pathogen overcoming resistance in crops. There is also breakdown of resistance in wilt resistant cultivars and enhanced by nematode infestation (Suhardi *et al.*, 1980; Hadavi and Sahebani, 2008).

Other control measures have been suggested in order to replace the highly toxic and potentially polluting chemicals (Mudawi and Idris**,** 2015). These include organic amendments, botanicals and biological control agents (Oostendrop and Sikora, 1989).

Organic amendments such as manures release nutrients into the soil and also improve the soil physical properties. Most organic amendments have a negative effect on soil borne pathogens. Botanicals are products derived from plants that are harmful to disease causing microorganisms. Biological control agents are microorganisms such as bacteria, fungi and viruses that are known to have a negative effect on soil pathogens by mycoparasitism, production of metabolites or even enhancing the plant ability to resist disease either by systemic or induced resistance. To improve on efficiency of biological control in the management of fungi and RKN, it is recommended that two or more agents are combined. Meyers and Robert (2002) documented the effect of mixed biological agents against plant pathogens. These combinations have resulted in enhanced control as the agents have different modes of action against the plant pathogens (Walid *et al*., 2010). Biological control agents are eco-friendly, and are effective in the control of pathogens that infect plants.

1.2. Problem statement

Diseases and pests lower yields in the tomato crop (Wanjohi *et al*., 2018). Root knot nematodes *Meloidogyne* spp. cause 30 to 40% yield losses in tropical countries (Charchar *et al*., 2003). In Kenya, there is lack of recognition of RKN as important in causing losses in yields of the tomato crop (Kariuki *et al*., 2010). Fusarium wilt is a fungal pathogen that causes loss in yield of the tomato crop ranging from 30% to 40% in tomato growing areas (Kirankumar *et al.,* 2008). Infection of crops with RKN, *Meloidogyne* spp. and Fusarium wilt pathogen can result in formation of a disease complex that causes considerable loss in crop yield (Bhagawati *et al.*, 2000, 2007). Fusarium wilt severity and incidence in tomato crop has been reported to be more severe due to simultaneous infection by RKN and *F. oxysporum* f. sp. *lycopersici* (Onkendia *et al*., 2014). Chindo *et al*. (2010) reported that *M. incognita* increased the severity of Fusarium wilt in tomato, while Jain and Jitendra (2010) observed an

interaction between *M. incognita* and FOL that resulted in early expression of wilt symptoms. In tomato production in Kenya, RKN and Fusarium wilt pathogen has been reported in tomato growing areas (Kariuki *et al*., 2010; Mugo, 2012).

The background of this study is Mwea, one of the major regions in Kenya known to produce large volumes of tomatoes (Wanjohi *et al*., 2018). Water has been harnessed from the rivers Ragati, Rwamuthumbi, Thiba, Rupangazi and Nyamidi into irrigation canals and is used in intensive cultivation of tomatoes and other crops such as French beans and rice (Mugo, 2012). The area has an average rainfall of 1100-1250 mm per year and a temperature range of 12^{o}c to 26 ^{o}c.

In Kenya, there is low level of awareness in the use of resistant varieties, nematicides, cultural and biological methods to control RKN (Kimenju *et al.*, 2008). Some carbamate acaricides used in the control of nematodes such as carbofuran have restricted use in Kenya due to their toxicity and harmful effect on the environment (Pest Control Products Board, 2017). Lack of control of RKN and Fusarium wilt means they continue to build up in the soil increasing the adverse effects of the disease complex.

1.3 Justification

Tomato is an important cash crop for both smallholder farmers and medium-scale commercial farmers (Varela *et al.*, 2003). Therefore, timely interventions must be done in order to control RKN and Fusarium wilt pathogen that can lead to huge losses in the yields (Bhagawati *et al.*, 2000, 2007). In addition to this, the possibility of formation of a disease complex necessitates this study in order to understand the disease and explore methods of managing it.

Restrictions on the use of synthetic chemicals due to high levels of pesticide residues necessitated the need to explore other safer alternative strategies. The best approach for the

management of this disease complex is by developing integrated strategies that have different mechanisms of control of RKN and Fusarium wilt pathogen. Such strategies should have different combinations of biological control agents, botanicals, plant resistance, organic amendments, fungicides and nematicides. In such an integrated approach, the components will have different modes of action such as antagonism; creating a favourable environment for competition by beneficial microorganisms; improving the health of the plant; systemic and induced resistance. A single control method is not considered as effective as a combination containing more than one strategy. This integrated approach shifts from reliance on chemicals in disease control to other methods that do not have a negative effect on the environment (Singh, 2002).

This study was therefore undertaken with the following objectives:

1.4 Objectives

1.4.1 Overall objective

The broad objective of the present study is to study the interaction and effective management strategies of Fusarium wilt pathogen and root-knot nematodes disease complex. The specific objectives of the study are:

1.4.2 Specific objectives

1. To identify disease constraints encountered by the farmers during tomato cultivation in Mwea West Sub County, Kirinyaga County.

2. To determine prevalence of *Meloidogyne* spp. in the rhizosphere of tomato plants.

3. To identify Fusarium wilt pathogens that cause vascular wilt in tomato.

4. To determine the reaction of selected popular tomato cultivars to Fusarium wilt pathogen and root-knot nematodes

5. To evaluate the effect of combining host resistance, biological control agents and organic amendments in the management of Fusarium wilt and root- knot disease complex.

1.5 Hypotheses

1. There are no disease constraints encountered by the farmers during tomato cultivation in Mwea West Sub County.

2. *Meloidogyne* spp. is not the most prevalent genera in the rhizosphere of tomato plants.

3. Different tomato cultivars do not react differently to Fusarium wilt pathogen and RKN producing different levels of disease.

4. An integrated package incorporating resistance, biological control agents and neem is not more effective in the control of fusarium wilt and root-knot disease complex compared to application of only one single method.

CHAPTER TWO

LITERATURE REVIEW

2.1 Tomato (*Lycopersicum esculentum* Mill) Production

Tomato is an important horticultural crop in many countries. In Kenya and other East African countries, its production is only surpassed by the potato crop (FAO, 2005; Maerere *et al*., 2006). Soups, sauces, juices and tomato pastes are some of the products processed from tomatoes (FAO, 2000).

Tomato fruits are sold fresh in markets while others are taken to processing industries. Money Maker, Zawadi, Marglobe, Fortune Maker and Kentom F are examples of varieties grown for fresh market in Kenya while processing varieties include Roma VF, Rutgers 10x, Cal-J and M 82 (Kenya Ministry of Agriculture and Livestock development, 2003). In Kenya, major constraints in tomato production include high prices of inputs, poor crop management practices, and inefficient transportation due to poor road infrastructure. Other constraints are related to marketing of the crop, diseases and pests (Kenya Ministry of Agriculture, 2006). Bacteria, fungi and viruses are the major pathogens that cause diseases in the tomato crop. Wilts in tomato crop are mainly by the bacterium, *Ralstonia solanacearum* and fungi belonging to *Fusarium* spp. and *Verticillium* spp. Blights in tomato are mainly caused *Alternaria solani* and *Phytophthora* spp. Root-knot nematodes, aphids, African bollworm and the tomato leaf miner are examples of pests that affect tomatoes (Wanjohi *et al*., 2018). One of the most devastating pests of tomato that has been reported recently is *Tuta absoluta* that can cause up to 100% loss in yields (Ndalo *et al*., 2015).

2.2 *Fusarium oxysporum* f. sp. *lycopersici* (Snyder and Hansen)

Fusarium oxysporum f. sp. *lycopersici* (FOL) is a soil borne pathogen that causes vascular wilt in tomato. The pathogen belongs to *Fusarium oxysporum* species group. *Fusarium*

oxysporum is an abundant, fungal species complex with numerous morphologically indistinguishable plant pathogenic and non pathogenic strains (Jacobs *et al*., 2013). Host specificity by pathogenic *F. oxysporum* determine their group of formae speciales (Lievens *et al.*, 2009). More than 120 formae speciales have been described (Di Pietro *et al*., 2003; van der Does *et al*., 2008; Lievens *et al.*, 2009).

Three races of FOL pathogen have been identified (Cai *et al*., 2003; Tanyolaç and Akkale, 2010; Inami *et al.*, 2012). These are Race 1, 2 and 3. Race one is widely distributed and has been reported in most geographical areas of the world. Race two is growing in significance as it has also been reported in major tomato growing areas in the world (Malafaia *et al*., 2013). Only a few areas in the world have reported on race 3 of the FOL pathogen (Reis *et al*., 2005).

2.2.1 Characterisation of *Fusarium oxysporum*

Morphological identification relies on culturing of the fungus followed by identification based on extensive taxonomical knowledge (Saikia and Kadoo, 2010). Identification of the fungus based on morphological criteria is difficult and sometimes can be erroneous, which may complicate timely disease management decisions (Saikia and Kadoo, 2010). Molecular biology has provided new methods in the identification of fungal pathogens (Paminondas and Paplomatas, 2004). Some of the most used methods in molecular analysis are RAPD analysis and sequence based characterisation. The RAPD analysis is a reliable tool for differentiating species of a genus and complementary to methods based on morphology and pathogenic characteristics (El-Fadly *et al*., 2008).

The RAPD-PCR technique has been used extensively in the molecular characterisation of *F. oxysporum* isolates (Bogale, 2006). This method has also been used to evaluate genetic diversity of *F. oxysporum* f. sp. *psidii* and *F. solani* isolates, that cause Fusarium wilt on

guava (Gupta *et al*., 2010) and analysis of *F. oxysporum* isolates causing wilt in cucumber (Vakalounakis and Fragkiadakis, 2008). The technique requires only small amounts of genomic DNA (Baleiras *et al.,* 1995) and no sequence information about the target DNA is required (Semagn *et al.,* 2006). Although the RAPD technique has been successfully used in many studies for detection and identification of *F. oxysporum* isolates as well as to evaluate their genetic diversity, it is not easy to reproduce the methodology in new circumstances (Saikia and Kadoo, 2010).

Sequence based characterisation relies on obtaining sequences of specific gene regions and comparing them with reference sequences in public data bases (Saikia and Kadoo, 2010). Genetic diversity and phylogenetic relationships among formae speciales of *F. oxysporum* has been studied based on the translation elongation factor (TEF)-1α gene, mitochondrial small subunit (mtSSU) ribosomal RNA gene, intergenic transcribed spacer (ITS) region, rDNA intergenic spacer (IGS) region (Lievens *et al.* 2008). From such studies, formae speciales; *vasinfectum, radicis lycopersici, phaseoli, melonis lycopersici, lini, dianthi, cucumerinum, asparagi,* were shown to be polyphyletic (Lievens *et al*., 2009)

2.3 Diversity of soil nematodes

Nematodes have a significant role in ecosystems through nutrient cycling which influences primary productivity. They play a big role in decomposition of organic matter in the soil, and therefore play a key role in nutrient cycling in food webs affecting plant growth and yield (Philip *et al*., 1995). Plant parasitic nematodes directly affect plant growth vigor and yield (Yeates *et al*., 2009). Microbial feeding nematodes determine breakdown of organic matter and cycling of nutrients in agro ecosystems (Vauramo and Setälä, 2010).

Nematodes belong to different trophic groups based on feeding habits (Yeates *et al.,* 1993). There are those that feed on fungi, bacteria and plants, while others are predators (Yildiz,

2012). Those that feed on fungi are referred to as fungivores while those that feed on bacteria are bacteriovores. Abundance of nematode trophic groups has been reported to vary with different land management systems. Addition of biochar significantly increased the number of fungivores, but decreased the populations nematodes parasitic to plants (Zhang *et al.*, 2013). When cover crops belonging to families Poaceae and Fabaceae were used in banana ecosystems, the population of bacteriovores increased more than all other nematode groups (Djigal *et al*., 2012). Plant-parasitic, fungivore and bacterivore nematodes were also abundant in grazed and ungrazed sites (Yildiz, 2012).

2.3.1 Root-knot nematodes

Nematodes that cause root knots also referred to as galls belong to the genus *Meloidogyne.* The root-knot nematodes (*Meloidogyne* spp.) are the most widespread and economically important nematodes that infect tomatoes. *Meloidogyne arenaria* (Neal) Chitwood, *M. javanica* (Treub) Chitwood and *M. incognita* (Kofoid and White) are the most common species that infect tomatoes (Rumbos *et al.,* 2011). These species cause galls on roots, stunted growth, wilting, and low yield (Yazdi *et al.*, 2012). Charchar *et al.* (2003) reported a 30 to 40% yield losses due to *Meloidogyne* spp. in tropical countries, while Serfoji *et al.* (2010) observed that RKN lowers production in the tomato crop. At temperatures of 27^{o}C, the populations of RKN (*Meloidogyne* spp.) raise to maximum within 25 days in susceptible crops by the time the crop mature. Significant reduction in growth has been reported in tomato plants infested with RKN *Meloidogyne* spp. (Esfahani and Pour, 2006). Root galling interferes with absorption of mineral salts and water and this reduces growth in plants (Ahmed *et al.,* 2009).

2.4 Synergistic interactions of Fusarium wilt pathogen and root-knot nematodes

Disease complexes involving nematodes and fungal pathogens may cause significant crop losses than each on its own (Hussey and McGuire, 1987). In different cotton cultivars, RKN increased Fusarium wilt disease severity symptoms. The effect was least in cultivars with a high resistance to the disease (Katsantonis *et al*., 2003). Maheshwari *et al.* (1995) reported increased chlorosis and in some cases vascular discolouration above the collar in five wilt resistant cultivars in chickpea, due to concomitant infection with Fusarium wilt and RKN.

Synergistic effects of concomitant inoculations of the pathogen FOL and the RKN have been documented. In concomitant inoculation of RKN and FOL, severity of the wilt was significantly increased and plant growth and yield reductions were also considerably higher, compared to the sum of individual effects of the pathogens (Khan and Akram, 2000). In split-root assay experiments in tomato plants, research conducted showed that, *M. javanica* infection in one part of a root resulted in suppression of tomato defences in all parts of the root and therefore intensified infection by the Fusarium wilt pathogen. There was possibility of systemic induced susceptibility to the fungus by local nematode infection (Hadavi and Sahebani, 2008).

The mechanism by which RKN enhance vascular wilt has been a subject for debate and research. Exudates released by the plant roots are believed to attract both soil pathogens and the RKN to the rhizosphere of the plant (Badri and Vivanco, 2009). Injuries caused by the RKN as they penetrate the plant roots could then provide entry points for the fungal pathogens (Bhagawati *et al*., 2000).

2.5 Management of Fusarium wilt and root-knot nematodes

2.5.1 Cultural control methods

Various methods have been suggested for the management of RKN and Fusarium wilt pathogen. In the control of RKN, long crop rotational programmes including non host plants

and leaving land fallow during the dry season have been recommended. Other cultural methods in the control of RKN and Fusarium wilt pathogen include the use of resistant varieties and organic amendments. Crop rotation is limited as farmers have small field sizes and cannot afford to practice long-term crop rotation (Sally *et al.,* 2006). Very many crops are hosts of RKN (Regaieg *et al.,* 2010) making crop rotation difficult. Leaving land fallow might also not be feasible, unless weeds are removed because many of them are hosts of RKN (Davis and Webster, 2005). Crop rotation and leaving land fallow is not applicable for the management of FOL as the pathogen forms chlamydospores, which remain for a long period in the soil.

Disease control by host resistant varieties is among the best methods in the management of plant pathogens (Sheu and Wang, 2006), including FOL (Fesenko *et al*., 2013). Tomato cultivars resistant to race 1, 2 and 3 of FOL and to RKN have been developed. Tomato cultivars that are resistant to *M. arenaria*, *M. javanica* and *M. incognita* have the Mi-gene (Roberts and Thomason, (1989) as cited by Verdejo-lucas *et al*. (2009), and this has been the source of resistance for many years. Resistance to RKN by the Mi-gene in tomato cultivars has often failed at high temperature above 28^0C, as the Mi gene is unstable at high temperatures (Kolashian, *et al.,* 1996). Virulent races of *Meloidogyne* spp. will be selected if planting of resistant cultivars is done continuously. Thus the duration of the resistance may be limited (Noling, 2010) as new races of pathogens overcoming host resistance develop (Tzortzakakis *et al*., 2005). There have been reports of emergence of virulent strains of *Meloidogyne* spp. able to break the Mi-gene tomato resistance (Verdejo-Lucas *et al*., 2012).

Organic amendments such as poultry manures, oil seed cakes of neem and groundnut that have been known to increase agricultural productivity have also been found to be suppressive to RKN (Alam *et al.,* 1980). It has been reported that neem cake has anti- nematicidal, anti-

bacterial, anti-fungal properties (Lokanadhan *et al*., 2012). Kimaru *et al.* (2004) observed that neem cake powder had fungistatic effect on Fusarium wilt of tomato.

2.5.2 Chemical methods of management

Application of chemical nematicides such as furadan, mocap or nemacur and methyl bromide and use of organic amendments have been investigated in the control of RKN (Kenya Ministry of Agriculture and Livestock development, 2003). Most of the nematicides have already been banned due to the harmful effects on the environment (UNEP, 2001; Chitwood, 2002). Application of agrochemicals such as thiophanate, thiabendazole and benomyl, has been used to control Fusarium wilt. However, sustainable use of fungicides in Fusarium wilt management is difficult due to development of resistant isolates and damaging effects of the fungicides on the natural environment (Pritesh and Subramanian, 2011).

2.5.3 Biological control

Development of resistance to chemicals by fungi and nematodes and the harmful effects of chemicals on the environment have led to the search for new methods of control (Hirooka and Ishii, 2013). One of the most researched alternatives to chemical control is biological control. In the narrowest sense, biological control involves suppressing pest and pathogens with other organisms. Most research on biological control has been done on fungi and bacteria (O'brien, 2017). Bacteria that have been investigated for the control of Fusarium wilt and RKN include those belonging to the group of Rhizobacteria. Rhizobacteria, a soil bacterium found in the root rhizosphere that colonizes the root system (Badri and Vivanco, 2009). Certain strains of Rhizobacteria are able to suppress a variety of nematodes and diseases and stimulate plant growth. Amin *et al.* (2014) reported increased growth, and production of peroxidases, polyphenol oxidases and phenols in tomato plants infested with *M.*

incognita but treated with Rhizobacteria. *Bacillus thuringiensis*, also a rhizobacteria remarkably suppressed nematode infestations by inhibiting the hatching of juveniles, reducing the number of gall masses and preventing formation of root knots (Jafri *et al*., 2015). *Pasteuria penetrans* a bacterium has also been reported in the control RKN. Three isolates of *P. penetrans* as a blend and as individuals were found to be effective in suppressing the root-knot nematode, *M. javanica* in the roots and in the soil in tomato (Zareen *et al*., 2002).

Fungi like *Fusarium oxysporum, T. harzianum, Paecilomyces lilacinus* and *Pochonia chlamydosporia* (Nagesh *et al*., 2006; Pau *et al*., 2012, Luambano *et al*., 2011; Anil and Garampalli, 2013; Kamali *et al*, 2015; Nawer, 2015) have been used in the control of RKN. Endophytic, non pathogenic *F. oxysporum* showed its effectiveness *M. incognita* on tomato plant. The strain, *F. oxysporum* 162 reduced *M. incognita* infestations significantly, in both Fusarium wilt resistant and susceptible tomato cultivars by induced systemic resistance (Dababat *et al*., 2008). Endophytic fungi *Gliocladium* spp. reduced *Melodoigyne* spp. juveniles and the intensity of its damage in infected plants (Amin, 2014). A non pathogenic *F. oxysporum* isolate significantly lowered the disease severity in soil infested with Fusarium wilt pathogen (Nawar, 2015). *Pochonia chlamydosporia* parasitizes the females and eggs of RKN and cyst nematodes (Atkins *et al.*, 2003). Luambano *et al*. (2011) reported that some isolates of *P. chlamydosporia* were as efficacious as PL-plus a commercial product of *P. lilacinus* in controlling RKN in greenhouse environment.

2.5.3.1 *Paecilomyces lilacinus* (Thom) Samson syn. *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson

Paecilomyces lilacinus also known as *Purpureocillium lilacinum,* a saprophytic fungus found in the soil and that has good potential for the control of nematodes by parasitizing their eggs

(Jatala, 1986). *Paecilomyces lilacinus* has been reported to effectively control RKN in different crops (Ahmad and Khan, 2004; Kiewnick and Sikora, 2006). Control of nematodes is by mycoparasitism, whereby chitinases are produced that destroy the chitinous layer resulting in inhibition of embryonic development (Chan *et al.*, 2010) .

2.5.3.2 *Trichoderma* spp.

Trichoderma spp. (*T. harzianum* and *T. viride*) have widely been used in the control of plant pathogens including nematodes. Windham *et al*. (1989) reported the reduction of egg production by RKN following soil treatment with a *Trichoderma* spp. conidial suspension. *Trichoderma* spp. may also promote plant growth and have the ability to colonize root surfaces and the cortex (Sharon *et al.*, 2001). *Trichoderma harzianum* induces systemic resistance against invasion by RKN (Naserinasab *et al*., 2011) and plant diseases (Harman *et al*., 2008).

Trichoderma spp. through production of fungal metabolites inhibited the hatching of RKN nematode eggs and through production of hydrolytic enzymes in mycoparasitism, *T. viride* and *T. harzianum* destroyed the gravid females (Jegathambigai *et al*., 2011). *Trichoderma harzianum* was antagonistic to *Phytophthora infestans* (Fatima *et al*., 2015), reduced mycelia growth of *Fusarium graminearum* (Foroutan, 2013), and inhibited *Macrophomina phaseolina* and *F. oxysporum* f. sp. *lycopersici*, (Javaid *et al*., 2014) in dual culture. It also inhibited the growth of the *F. oxysporum* f. sp. *lycopersici in vitro* and was effective in suppressing it in greenhouse environment (Selvakumar *et al*., 2014).

2.5.4 Integrated approaches in the management of Fusarium wilt-root-knot nematodes disease complex

There is need for environmentally and ecologically friendly methods in the control of Fusarium wilt-RKN disease complex in tomato, for sustainable production (Elanchezhiyan *et*

al., 2018). An integrated approach using management options that have different modes of action that tackle the Fusarium wilt pathogen and RKN would be highly applicable. A single control method is not considered as effective as a combination containing more than one strategy. Biological control agents have been found to be inconsistent and lacking in persistence (Agbenin, 2011). To overcome the problem of persistence and inconsistencies, application of two or more biological control agents has been recommended (Meyers and Robert, 2002). Application of different compatible disease suppressive microorganisms to mimic the natural environment may improve on biological control. The combination of biological control agents is an alternative to improve on biological management of RKN and Fusarium wilt pathogen.

Meyers and Robert (2002) reported the effect of combining biological agents against plant pathogens. These combinations have resulted in enhanced control as the agents have different modes of action against the plant pathogens (Guetsky *et al.,* 2001). However, it has also been reported that application of biological control agents whose antagonisms include antibiosis, competition and induced systematic resistance might have these mechanisms directed towards the other antagonist, with either zero control, or control similar to one of the biological control agents (Boer *et al*., 1998).

Trichoderma harzianum combined with *Bacillus subtilis* was very effective in the management of vascular wilt compared to single applications of the biological control agents (Pandey and Gupta, 2012). Some authors have suggested that in dealing with Fusarium wilt and root-knot nematode complex, biological control agents should be selected with reference to specific organisms. A study by Khan and Akram (2000) had an integrated approach using biological control agents namely, *P. lilacinus, Gliocladium virens, Pseuodomonas fluorescens* and *Bacillus polymyxa* performed better than any of the components applied alone. Integrated approaches have also involved biological control organisms when combined

with organic amendments. A combination of neem cake powder, *P. lilacinus* and *T. harzianum* was very effective in controlling Fusarium wilt and *M. incognita,* such that the plants were free from nematodes and Fusarium pathogen and did not wilt till harvest. Those that received *T. harzianum* only started wilting six weeks after transplanting (Nagesh *et al*., 2006). Srivastava and Tewari (2011) reported that an integrated approach containing the fungus *T. harzianum, P. lilacinus* with karanj oilseed cake was the most effective in increasing yield and reducing the incidence of Fusarium wilt and RKN. Sheep manure as an organic amendment enhanced *Trichoderma harzianum* suppression of Fusarium pathogen in tomato (Barakat and AL-Masri, 2009).

Apart from combinations of biological control agents combined with amendments, other approaches involving mutualistic management options have been investigated. Observations were made where solarisation integrated with karanj oil-seed cake, *T. harzianum* and VAM *Glomus etunicatum* on soils heavily infested with Fusarium wilt and RKN increased germination, growth parameters and reduced *M. incognita* in the soil compared to a treatment with the same components but was unsolarized (Goswami *et al.*, 2013).

Mugo (2012) reported that a combination of three components namely, neem kernel cake powder (NKCP), organic matter and fungicides proved to be more effective in the control of Fusarium wilt in tomatoes as compared to any of the component applied alone, or even when two of the components were applied together. An experiment where seed treatment with *T. harzianum* was applied together with soil application of neem cake powder and foliar spray of carbendazim, had the lowest disease severity and highest fresh and shoot dry weights compared to other treatments applied singly or applied together in different combinations (Singh *et al*., 2015).

A combination of vermicompost, *Glomus aggregatum* and *Bacillus coagulans* in glasshouse experiment in pot soils that were highly infested with *Meloidogyne* spp. juveniles produced

maximum growth and highest reduction in RKN population, compared to other treatments with single applications or combinations of two components applied together (Serfoji *et al*., 2010). However there is very limited research on management of Fusarium wilt and RKN that integrate tomato resistance with other strategies.

CHAPTER THREE

TOMATO PRODUCTION PRACTICES AND OCCURRENCE OF DISEASES IN MWEA WEST SUB COUNTY

ABSTRACT

Tomato is an important crop in Mwea West Sub County, Kirinyaga County, Kenya. Mwea is an area in Kenya where there is intensive tomato cultivation. A survey was carried out in the area to investigate tomato production practices, diseases and pests that hinder tomato crop. The study endeavoured to establish farmers' knowledge on Fusarium wilt disease and root-knot nematodes and the methods used to control them. Data was collected from two hundred and eighteen randomly selected small holder producers who were equally distributed in the study area. One hundred and nineteen soil samples were also collected from the study area for analysis of nematode groups in order to establish the prevalence of *Meloidogyne* spp. root-knot nematodes. Eighty five point three (85.3%) percent of the farmers were males while fourteen point seven (14.7%) percent were females. The farmers (71.6%) indicated that tomato was the most important crop grown for income generation in the area. Popular tomato cultivars grown in this area include Safari, Kilele F1, Prosta F1, Rambo F1 and Rio- Grande. Diseases affecting tomato crop were; late blight (*Phytophthora infestans*) and early blight (*Alternaria solani*), fungal wilts (*Fusarium* sp. *Verticillium* sp. *Rhizoctonia* sp.) and bacterial wilt (*Ralstonia* s*olanacearum*). Plant parasitic nematodes and pests (thrips, aphids, and spider mites) were also reported in the study area. There was a significant ($P<0.05$) association between the variables like land ownership and cropping system as the highest proportion of those who did intercropping were those who leased land for tomato cultivation. There was no evidence that farmers applied any fungicides into the soil in order to control Fusarium wilt. Only about 3.8% of the farmers recognised nematodes as a threat to tomato production while field study data showed that *Meloidogyne* spp. was the most prevalent genera with the highest

relative abundance of 15.86%. *Meloidogyne* spp. is the most damaging of the plant parasitic nematodes as they form giant cells in the roots resulting in root galls that affect the ability of plants to absorb nutrients and are therefore considered a potential threat to tomato production in the area. There is need of creating awareness on RKN and Fusarium wilt that can cause yield losses in tomato production.

3.1 Introduction

Tomato is an important horticultural crop in many countries. In Kenya and other East African countries, its production is only surpassed by the potato crop (FAO, 2005; Maerere *et al*., 2006). The crop is an important part of the diet for most households in Kenya (Waiganjo *et al.,* 2006). Soups, sauces, juices and tomato pastes are some of the products processed from tomatoes (FAO, 2000). In Kenya, tomato is an important crop for small holder farmers.

Tomatoes exist as determinate and indeterminate types. Varieties like Eden, Onyx, Monyalla and Cal J are examples of determinate varieties that produce fruit for a relatively short period (Musyoki *et al.,* 2005). Indeterminate tomato varieties include Marglobe, Nemonneta and Anna F1 that produce fruit continuously for a relatively long time (Odame, 2009). Hybrid varieties of tomatoes that produce high yields have been developed (Anastacia *et al.*, 2011). Other cultivars developed much earlier, such as Rio grande, Money Maker, Roma VF and Marglobe are still widely grown because of important quality traits such as fruit colour. These are open-pollinated varieties that lack Plant Variety Protection (Fufa, 2009).

Diseases and pests are a major constraint in tomato production. Diseases that are common include bacterial wilt by *Ralstonia solanacearum,* fungal wilts caused by *Fusarium oxysporum* f. sp. *lycopersici* and *Verticillium* sp. tomato blights caused by *Alternaria solani* and *Phytophthora infestans* (Varela *et al*., 2003). The pests that affect tomatoes include whiteflies (*Bemisia tabaci*); thrips (*Frankliniella* spp.); African bollworm (*Helicoverpa*

armigera); aphids (*Myzus* spp.); spider mites (*Tetranychus evansi*) and RKN (*Meloidogyne* spp.). Plant parasitic nematodes have been reported to cause up to 20.6% yield loss in tomatoes (Sasser and Freckman, 1987). Root-knot nematodes *Meloidogyne* spp. are among the plant parasitic nematodes and are major pests of tomatoes (Sikora and Fernandez, 2005; Serfoji *et al*., 2010). Charchar *et al*. (2003) reported that root-knot nematodes cause 30% to 40% yield losses in tropical regions. Apart from plant parasitic nematodes, other nematode groups in the soil are bacteriovores, fungivores and omnivore/predators (Yeates *et al*., 2009).

A survey was carried out in the area to investigate tomato management practices, diseases and pests that hinder tomato production with special emphasis on awareness and management of nematode and wilt diseases. A field study was also carried out to in order to determine the prevalence of *Meloidogne* spp. that has potential to cause damage to the tomato crop.

3.2 Materials and Methods

3.2.1 Study area

A survey was carried in the month of October 2014 in Mwea West Sub County, Kirinyaga County, Kenya. This was done among farmers living in Baricho located at longitude $37^0$14 '27"E and latitude $0^0$33'7"S, Kagio ($37^0$15' 12"E, $0^0$37'27"S), Kangai ($37^0$15'12"E, $0^0$37'18 "S) and Kandongu (latitude, $37^0$17'38" E, latitude $0^0$39'45"S) (Fig 3.1.) The survey involved interviewing the farmers by use of questionnaires.

The survey included 218 randomly selected tomato growers. Out of those 218 farmers only 119 had a crop of tomato at the time of the survey. The other 99 farmers must have had planted a crop of tomatoes within a period of two years. Frequency tables were used to estimate the sample size (Krejcie and Morgan, 1970). Before launching the survey, the questionnaire was pretested and improved accordingly.

3.2.2 Soil sampling

A field survey was also carried out in119 farms in the month of October 2014 in the same area. Soil samples were collected in farms along Kagio-Kandongu road, Kagio-Kangai road and around Baricho as shown in Fig. 3.1. The sampling was done on tomato farms located 1-2 km apart. The estimate of the number of soil samples was calculated using the formula by Krejcie and Morgan (1970) (equation 1). A total of 595 soil samples were taken from 119 farms. This was in excess of calculated sample size of 384, this was to take into account all farmers who had previously been interviewed and had a crop of tomato. Soil samples were obtained from the rhizosphere of tomato plants. Five soil samples were collected from each of the farms in a zigzag pattern, to take into consideration the irregular distribution of nematodes.

Soil samples were taken from the top 20 cm layer using a trowel from the rhizosphere of a tomato plant. Five subsamples were collected at equal distance along the circumference of the rhizosphere and were mixed up to make a composite sample and put in a polythene bag. The five soil samples, about 400 g from five different tomato plants in the same farm, were kept in a cool box before transportation to the laboratory. In the laboratory, samples from each site were mixed thoroughly and 200 g from each site, used for nematode extraction and analysis.

(Equation 1)

$$\text{Sample size (S)} \qquad S = \frac{Z^2 \times P(1-P).}{D^2}$$

Where Z= reliability coefficient, fixed at 1.96 for 95% confidence level.

D=0.05, is degree of accuracy expressed as a proportion

P= 0.5, the population proportion is estimated at 0.5, this would give maximum sample size

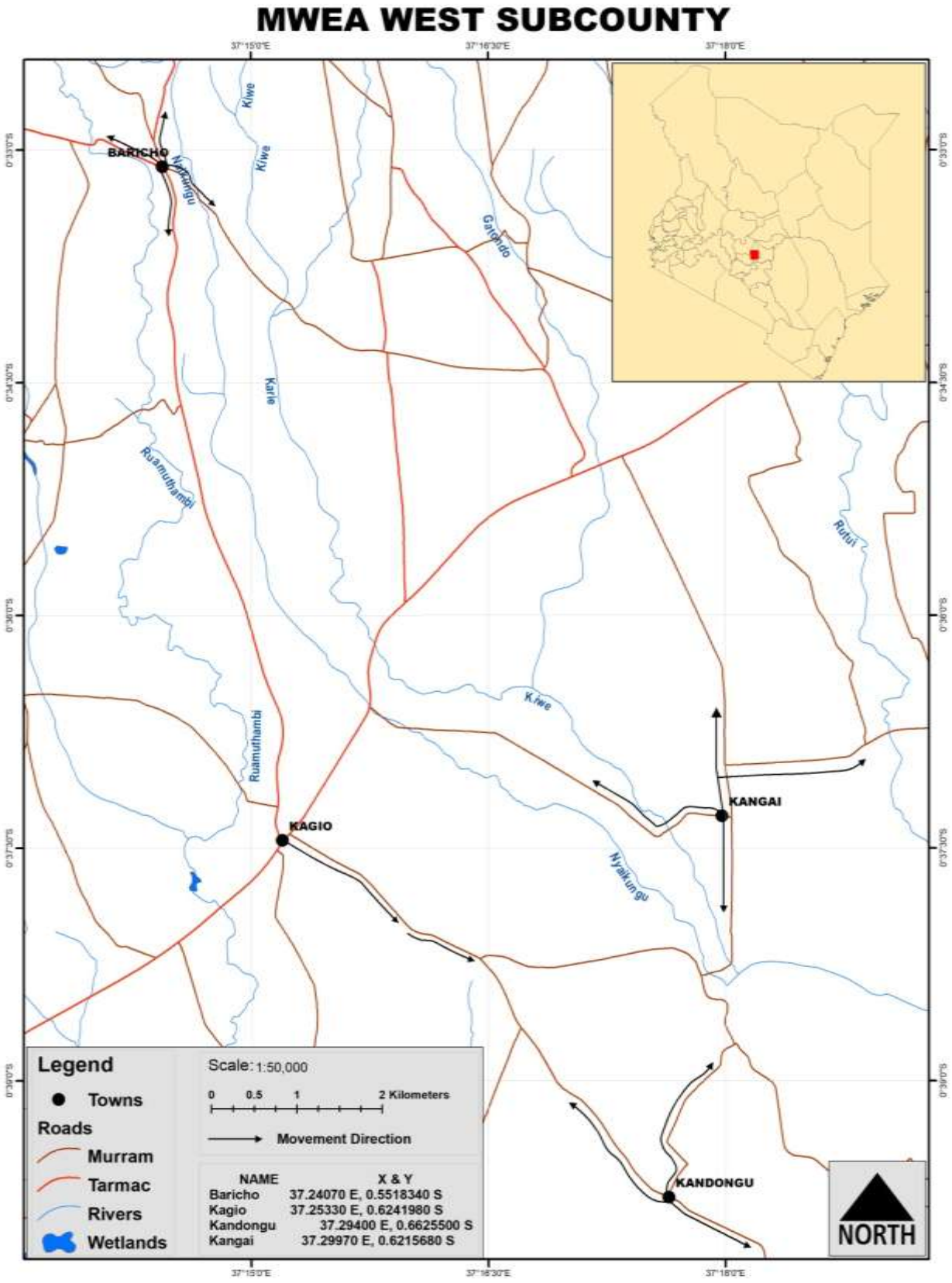


Fig 3.1 Map of Mwea West Sub County indicating routes followed during field survey

3.2.3 Nematode extraction and identification

Nematodes were extracted from the soil using sieving and centrifugation technique (Jenkins, 1964). A soil sub-sample of 200 g was drawn from the composite sample and was put in a 20 litre bucket where 4-5 litres of water were added. The suspension was stirred and passed through a 2 mm sieve into a second bucket. The suspension was passed through a series of sieves namely; 250 µ, 150 µ and a 38 µ. Nematodes were collected in 38 µ sieve, and were backwashed to form a 30 ml suspension which was further concentrated by centrifugation at 1750 revolutions per minute (rpm) for seven minutes. The pellet obtained was suspended by topping up to the 30 ml mark using sucrose solution (450 g/l) and centrifuged again at 1750 rpm for three minutes. The supernatant was concentrated using the 38 µ sieve to three millilitres.

The nematodes were killed by heating the suspension in a water bath at 60^{o}C and preserved in Triethanolamine formalin. Aliquots of one millilitre were obtained from the suspension and put into a counting dish where they were counted systematically using the gridlines. Nematodes observed from a particular grid were picked with a sharpened tooth pick and were placed on a glass slide and observed at x40 and x100 (oil emulsion) under a compound microscope. They were identified and grouped to order, family and the genus level with the help of a Plant Nematology Field Guide (Mekete *et al*., 2012). Total nematode numbers in each sample representing different genera were counted. Nematodes were classified according to trophic groups (bacteriovores, fungivores, omnivores/predators and plant parasitic) based on known feeding habits as described by Yeates *et al*. (1993).

3.2.4 Data analysis

The data was put into categories, coded and entered into Statistical Programmes for Social Sciences [SPSS (IBM SPSS Statistics 20)]. A spreadsheet was developed and all information

gathered using the questionnaires entered. Statistical Programmes for Social Sciences (SPSS) data analysis options including frequency tables, bar charts were used in descriptive analysis of data. Cross tabulation and Chi-square tests statistic was used to test association between variables at significance level of ($P<0.05$).

3.3 Results

3.3.1 Socio economic characteristics of respondents

The study showed male dominance in tomato production in the study area. Eighty five point three (85.3%) percent of the farmers were males while 14.7% percent were females (Table 3.1). Fifty six (56%) percent of farmers interviewed were between the ages of 20 to 40 years, while 28.4% were in the age group of 41 to 50 years, and the rest (15.6%), were above 51 years. The farmers in the study area were literate with 47.7% of the respondents having primary education, 45.9% secondary education and 6.5% had tertiary education. Acquisition of land for tomato cultivation was not a major concern in the area of study. Most farmers planted tomatoes on their own land (43.1%), (45%) used family land, while 11.9% leased land (Table 3.1).

There was an association between the type of landowner and the major use of land, whether for income generation or providing food for the family (Chi-Sq =11.65 DF=2 P-Value = 0.03). Seventy four point two percent (74.2%) of the landowners indicated that the main use of land was income generation and this proportion was significantly ($P<0.05$) different from those who leased land, whose sole purpose (100%) was income generation (Table 3.2). Farm sizes ranged from 0.25acres – 14 acres with majority of farmers having a farm size in the range of 0.25 to 4 acres as shown in Fig. 3.2.

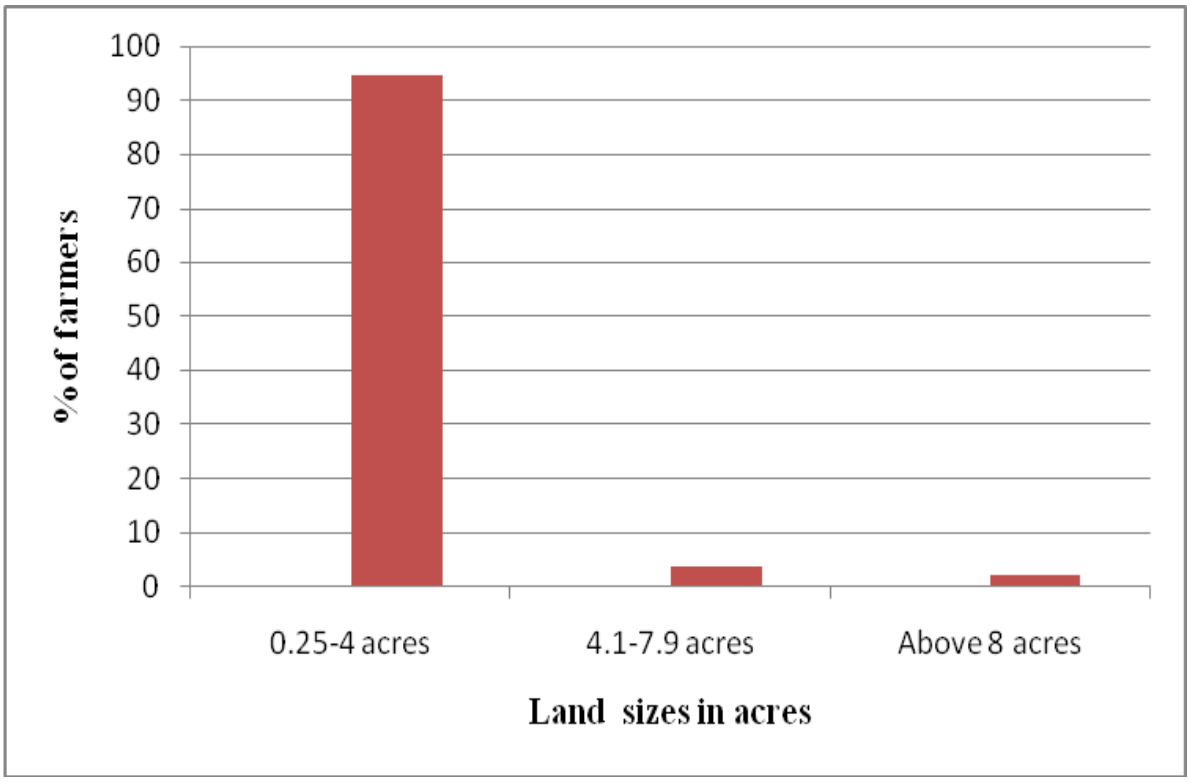


Fig. 3.2 Average Farm sizes in Mwea West Sub County

Table 3.1 Demographic characteristics of respondents from Mwea West Sub County

Characteristic	Number of farmers interviewed	% of farmers
Gender		
Male	186	85.3
Female	32	14.7
Age in years		
20-30	56	25.7
31-40	67	30.7
41-50	61	28
51 and above	32	15.6
Educational level		
Primary	104	47.7
Secondary	100	45.9
Tertiary	14	6.5
Type of land ownership		
Own	94	43.1
Family owned	98	45.0
Leased	26	11.9

Table 3.2. Relationship between type of land ownership and allocation of land to different uses

Type of land ownership	Respondents allocating land to food security	Respondents allocating land to income generation	Total number of farmers interviewed
Own	28 (19.8%)	66 (74.2%)	94
Family owned	18 (18.4%)	80 (81.6%)	98
Leased	0 (0.0%)	26 (100%)	26
Total	46	172	218

Chi-Sq =11.65 DF=2 (P<0.05) Number in parenthesis is the calculated percentage of respondents

3.3.2 Crops grown and cropping patterns in the study area

Tomato was the most important crop grown by the farmers interviewed (71.6%) followed by maize (13.8%) and French beans at 11.9% (Table 3.3). Tomatoes and French beans were mainly grown for income generation while maize was for household consumption.

The survey showed that (79.8%) of the farmers practiced mono-cropping while 20.2% intercropped the tomato crop with maize and beans (Table 3.3). There was an association between the type of cropping pattern and the type of landowner (Chi-Sq = 7.842 DF=2 (P< 0.05) (Table 3.4)]. Thirty eight point five percent (38.5%) of those who rented land practised intercropping and this was significantly (P<0.05) different from 13.8% who practised intercropping and who were the land owners. The farmers indicated that they practised mono-cropping to avoid disease transmission and competition from other crops, while those who intercropped with other plants did so in order to minimize the risks of crop failure and maximize on returns. The farmers also indicated that they practiced crop rotation and they rotated the tomato crop with maize, beans, French beans and sweet potatoes. The farmers grew a wide variety of tomato cultivars, and varieties, Safari and Kilele F1 were the most popular with frequencies of 30.35% and 26.6% respectively (Fig. 3.3).

Table 3.3. Percent of farmers growing different crops and cropping patterns in Mwea West Sub County

Crops grown	Number of farmers	% of farmers
Tomatoes	156	71.6
French beans	26	11.9
Maize	30	13.8
Beans	2	0.9
Bananas	2	0.9
Rice	2	0.9
Cropping pattern		
Mono-cropping	174	79.8
Intercropping	44	20.2

Table 3.4. Relationship between type of land ownership and cropping systems in Mwea West Sub County

Type of land ownership	Monocropping	Intercropping	Total number of farmers interviewed
Own	81(86.2%)	13 (13.8%)	94
Family owned	77(78.6%)	21(21.4%)	98
Leased	16(61.5%)	10(38.5%)	26
Total	174(79.8%)	44(20.2%)	218

Chi-Sq =7.842 DF=2 ($P<0.05$) Number in parenthesis is the calculated percentage of the respondents

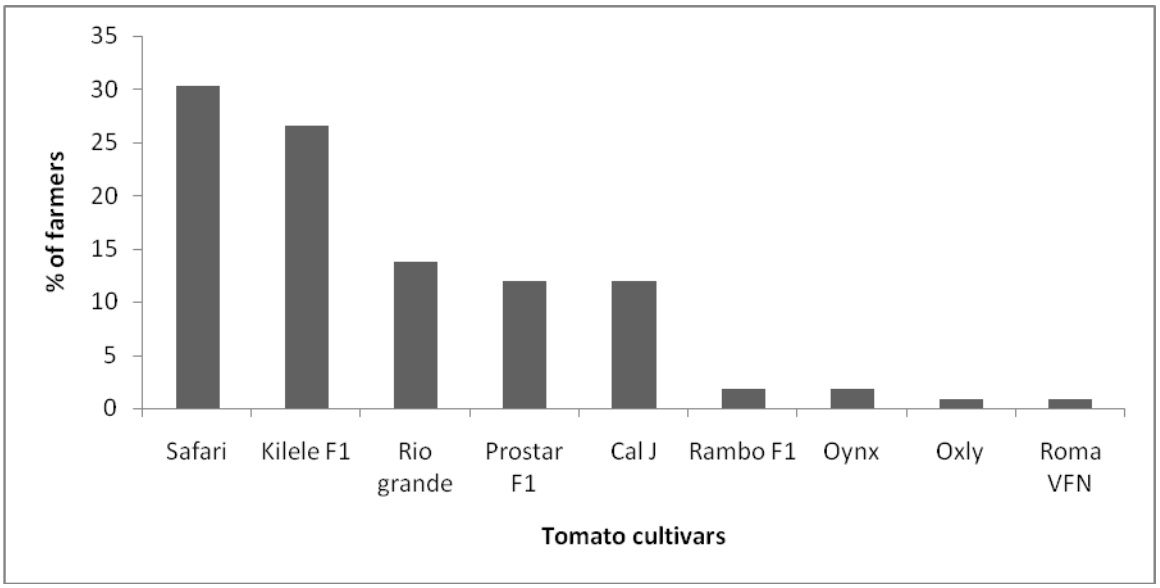


Fig 3.3 Tomato varieties grown by farmers in Mwea West Sub County

3.3.3 Diseases and pests that affect tomato crop and control methods

The most important diseases recorded were early and late blight (53.9%) followed by tomato wilt diseases (32%), nutritional disorders (6.6%), root-knot nematodes (3.8%) and pests (3.8%) as shown in Fig 3.4. There was no relationship between cropping system and the diseases and pests that were prevalent in the tomato farms [Chi- sq =3.3 DF=4 (P> 0.05) (Table 3.5)].

Farmers indicated that blight disease could easily be controlled by regular spraying using fungicides like Metalaxyl (Ridomil) and Mancozeb (Dithane M45). Ninety one (91%) percent of the farmers had observed wilting of tomatoes in their farms. Observation of tomato plants in the field and preliminary investigations of diseased plant materials in the laboratory indicated that wilting was caused by the bacterium *Ralstonia solanaceaerum* or by fungi like *Rhizoctonia solani*, *Verticillium* sp. or *Fusarium oxysporum* f. sp. *lycopersici*. Wilting would cause yield losses of 1-10 % according to 57.8 % of the farmers (Fig 3.5).

Seventy four (74%) percent of the farmers were aware that wilting was caused by a pathogen found in the soil. Others (21%) indicated that it was spread in irrigation water, while still others (5%) did not know the causes of the wilting. The farmers indicated that they used various methods to control wilting (Fig 3.6). The findings indicate that 40.6% uprooted the diseased plants, while 21.8% sprayed chemicals such as Metalaxyl (Ridomil). Other methods of controlling wilting included crop rotation, use of resistant varieties and watering (Fig 3.6). A small percentage of the farmers (15.8%) did not use any method to control the wilt. Various methods were used by farmers to control nematodes namely; applying chemicals (58.2%), watering (19.2%), use of resistant varieties (5.8%), adding manure (3.8%), crop rotation (1.9%) and adding ash (1.9%) (Fig.3.7). The survey observed that 9.6% of respondents did not know of any methods that could be used to control nematodes. Findings

of this study indicate that 48.6% of the farmers applied pesticides into the soil while 51.4% did not (Table 3.6). The most popular pesticide applied in the soil was Ethoprop (Table 3.6).

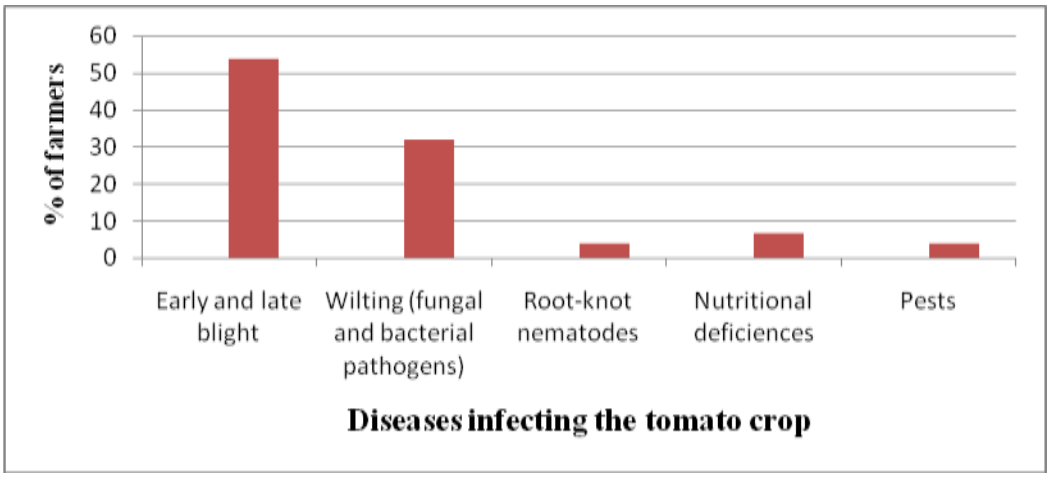


Fig. 3.4 Prevalence of wilt, other diseases and pests on tomato crop

Table 3.5 Relationship between cropping system and diseases and pests on tomato crop

Type of cropping system	Blight	Wilt	Nematodes	Nutritional	Pests	Total number of farmers interviewed
Monocropping	89 (52.4)	59(34.7)	6(3.5)	10 (5.9)	6 (3.45)	170
Intercropping	26(60.5)	9(20.9)	2(4.7)	4 (9.3)	2(4.65)	43
Total	115(53.9)	68(32)	8(3.8)	14 (6.5)	8(3.8)	213

Chi- sq =3.3 DF=4 0.509 (P.> 0.05). Number in parenthesis represents the calculated percentage

Table 3.6 Chemicals' applied into the soil to control nematodes in Mwea West Sub County

Chemical name	Brand name	Percent of farmers
Ethoprop	Mocap®	14.7
Pyrethroid	Alpha®	11.9
Imidacloprid	Gaucho-xt®	8.2
Dinocap	Karathane®	4.6
Carbosulphan	Marshall 250EC®	4.5
Dimethoate	Dimeton® 40 EC	3.2
Mancozeb	Agrithane® WP	1.4

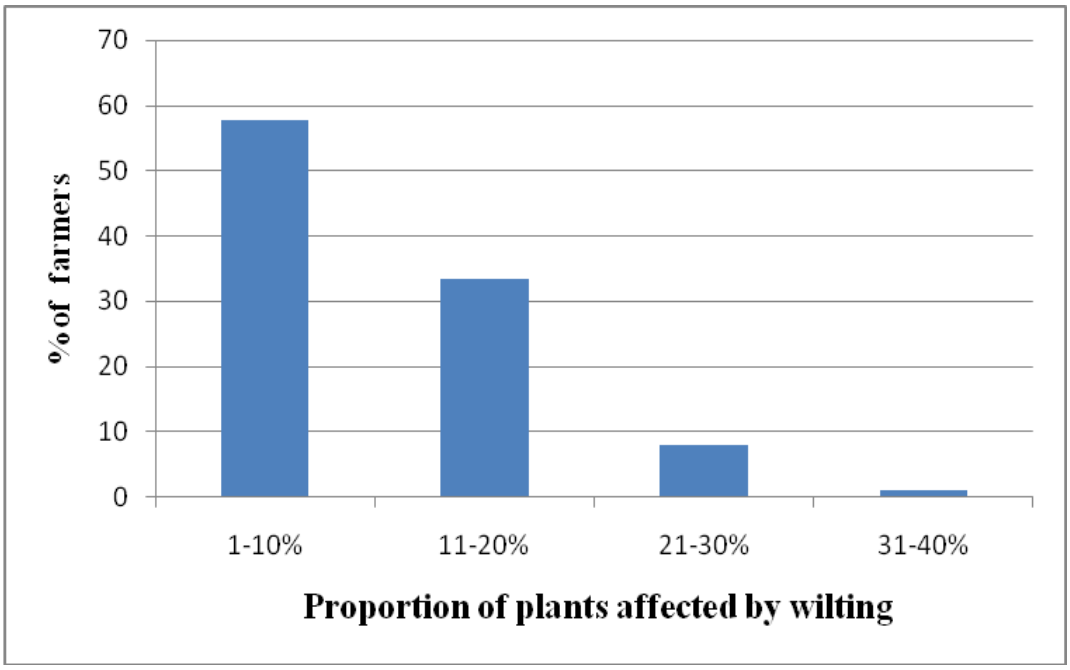


Fig. 3.5 Farmers indication on proportion of crops affected by wilting

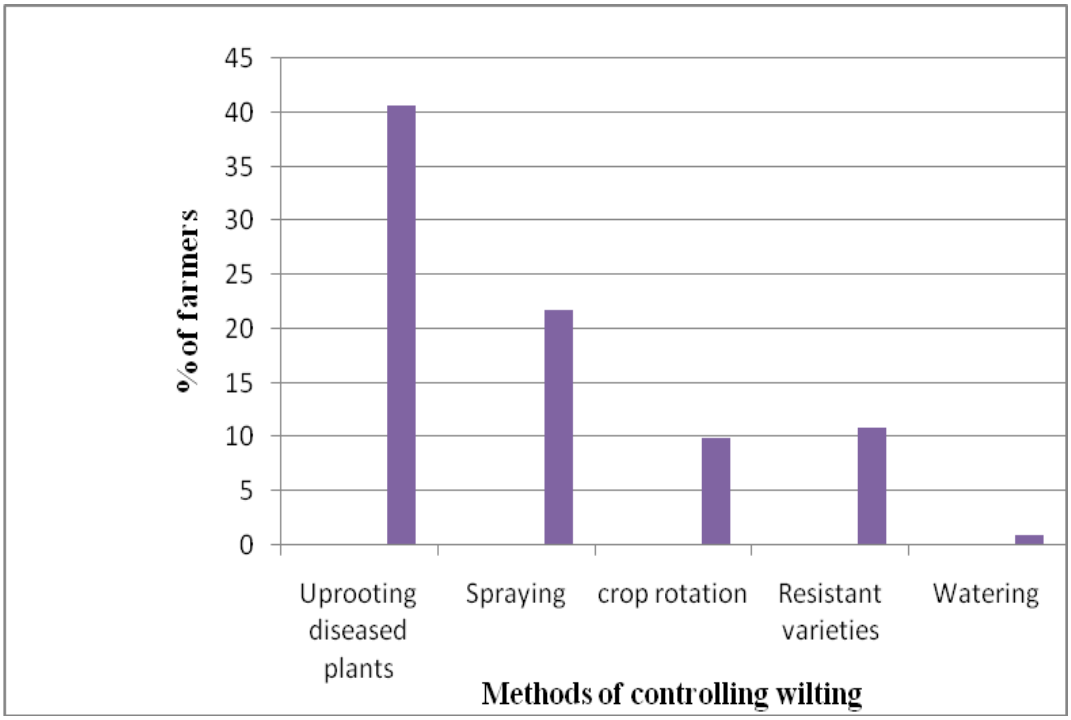


Fig. 3.6 Farmers indication of ways of controlling wilting

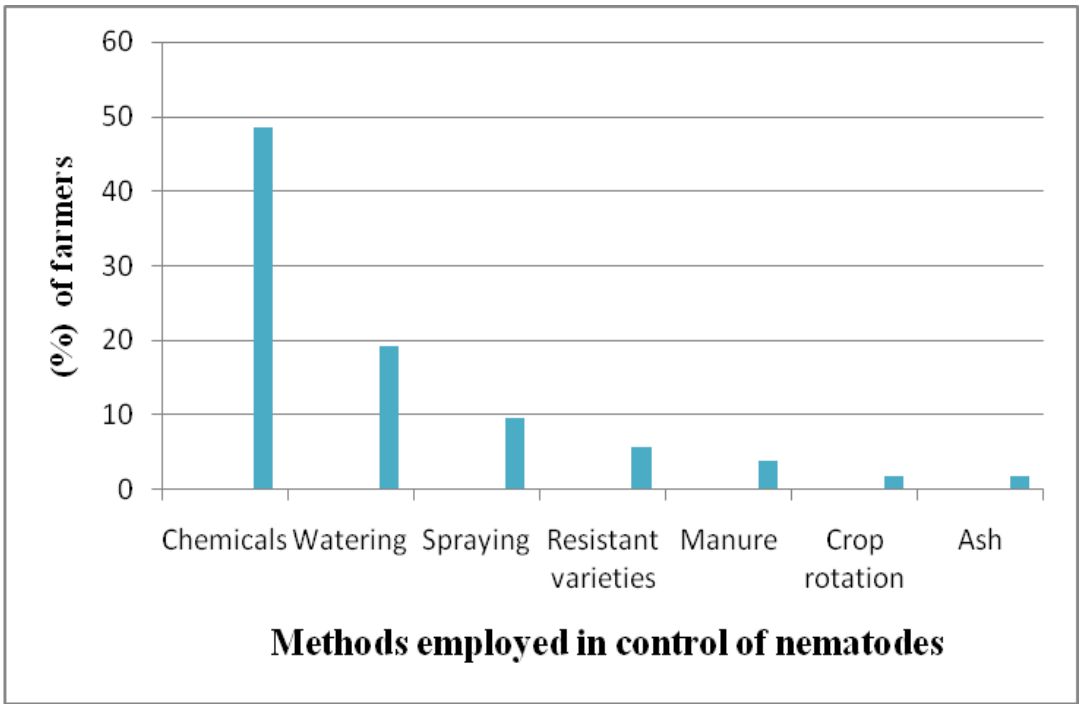


Fig. 3.7 Method employed for controlling nematodes

3.3.4 Sources of seedlings and agricultural information

Eighty one point six (81.6%) percent of the farmers grew their seedlings for planting. Farmers indicated that this is due to the convenience and ready availability, and also to avoid disease transmission from other nurseries.

Farmers (40.4%) had access to agricultural information in the past one year, mainly from government staff through agricultural extension officers and field days. Other important channels of getting information included other farmers (38.4%), agro vet-shops (12.1%) and mass media at 9.1% (Table 3.7).

There was a significant ($P<0.05$) relation, (Chi-sq =9.628 DF= 3 ($P<0.5$) between the source of agricultural information and whether or not to apply pesticides (Table 3.7). Most farmers (77.8%) whose source of agricultural information was mass media applied pesticides and this was significantly different ($P<0.05$) from 48.7% who also applied pesticides and whose source of agricultural information was government staff. Similarly, 51.3% of respondents whose source of agricultural information was government staff did not apply pesticides and

this was significantly (P<0.05) different from those whose source of information was the mass media who did not apply pesticides {(22.2%) (Table 3.7)}

Table 3.7 Relationship between source of information and pesticides applications by farmers in Mwea West Sub County

Source of agricultural information	Percent of farmers	Yes	No	Total number of farmers interviewed
Government staff	40.4	38(48.7)	40(51.3)	78
Other farmers	38.4	36(50)	36(50)	72
Mass Media	9.1	14(77.8)	4(22.2)	18
Agrovet shops	12.1	18(75)	6(25)	24
Total	100	106	86	192

Chi- sq =9.628 DF=3 (P < 0.05). Number in parenthesis represents the calculated percent of respondents

3.3.5 Soil nematode trophic groups in the rhizhosphere of tomato plants

Plant parasitic and bacteriovores were the most abundant trophic groups at 51.46% and 33.42%, respectively. A total of 25 nematode genera were identified. The most abundant and frequent genera of plant parasitic nematodes were *Meloidogyne*, *Tylenchus*, and *Pratylenchus.* The *Meloidogyne* spp. was the most abundant (relative abundance of 15.9%) while *Tylenchu*s spp. was the most frequently isolated (76.3%). The most abundant bacteriovores genera were *Cephalobus* and *Eucephalobus* with a relative abundance of 12.1%, 9.26% and a frequency of 67.2% and 53.8% respectively (Table 3.8). The most abundant predators-omnivores were in the genus *Discolaimus* and *Dorylaimus* at a relative abundance of 2.6% and 2.2%, respectively. The only fungivores genera were *Aphelenchus* and *Aphelenchoides* with a relative abundance of 5.5% and 0.1% and a frequency of 42.9% and 1.7% respectively.

Table 3.8 Abundance, composition of nematode genera in the rhizosphere of tomato plants

Trophic group	Genus	Relative Abundance (%)	Frequency of isolation (%)
Plant parasitic	*Meloidogyne*	15.86	67.2
	Tylenchus	11.54	76.3
	Pratylenchus	11.42	54.6
	Helicotylenchus	4.83	26.9
	Rotylenchus	2.7	17.6
	Filenchus	2.29	26.1
	Tylenchorhynchus	1.66	15.1
	Scutellonema	0.67	13.4
	Criconema	0.42	4.2
	Hoplolaimus	0.05	1.7
	Ditylenchus	0.02	0.84
Bacteriovore	*Cephalobus*	12.07	67.2
	Eucephalobus	9.26	53.8
	Wilsonema	5.44	11.6
	Rhabditis	5.24	48.7
	Leptolaimus	0.76	9.2
	Alaimus	0.36	8.4
	Cervidellus	0.29	8.4
Predator/Omnivore	*Leptonchus*	3.15	26.9
	Discolaimus	2.56	27.7
	Dorylaimus	2.18	24.4
	Labronema	1.03	13.0
	Prodorylaimus	0.62	10.9
Fungivore	*Aphelenchus*	5.49	42.9
	Aphelenchoides	0.11	1.7

3.4 Discussion

This study in Mwea West Sub County observed that men were the ones mainly involved in tomato production. This may be due to the fact that tomato production is capital intensive and men generally have greater access to capital than women. Anang *et al.* (2013) reported a similar outcome of males' majority in tomato production in Wenchi Municipal District of Ghana.

Majority of the farmers were young (20 – 40 yrs) and in their energetic and productive years. This is an indication that there may be a high potential for increasing tomato production in the area. Asare-bediako *et al*. (2007) reported a similar age distribution of tomato farmers at

Bontanga irrigation project in Ghana. The results indicate that the farmers in the area were literate and could understand disease and pest management practices that affect tomatoes. Literacy is an important characteristic that influence production (Awan *et al*., 2012). Farmers in the area may easily adopt improved farming practices as they can fully comprehend the implications of such practices.

Data collected on tomato varieties showed preference by the farmers for high yielding tomato cultivars such as Cal J, Kilele F1, Prostar F1 that are tolerant and resistant to diseases. Indeterminate varieties and hybrids such as Kilele F1, Prostar F1 were preferred by farmers due to a longer period of harvesting and high yields.

Farmers rotated the tomato crop with non-solanaceous crops like maize and beans. The effectiveness of such crop rotation programmes in reducing tomato diseases might have been reduced by the fact that farm sizes here were very small (0.25-4 acres), and could not afford the long rotational cycles (3 years) that is recommended to avoid pest problems and diseases common in tomatoes and other similar crops (Sally *et al.*, 2006).

The largest proportion of farmers who practised intercropping had leased land. Farmers who leased and practised intercropping possibly did so to minimize the risks of one crop failing. It would be expected that there is a relation between prevalence of pests and diseases and cropping pattern in tomato farms but this was not the case in this study. Possibly, the farmers had done intercropping for a short period of time.

A few farmers lacked knowledge on application of chemicals and their target pests. They indicated that fungicides were applied into the soil to control nematodes. A similar observation that farmers do not have a thorough understanding of disease control was also made in Kamuli, Uganda, on a survey carried out among small scale tomato farmers (Tusiime, 2014). Tinyami *et al.* (2014) gave a report on a survey carried out in Buea Municipality in Cameroon indicating that farmers did not have a thorough understanding of

wilting disease symptoms and nematodes on the tomato crop. In this study, only about 3.8% of the farmers recognised nematodes as a threat to tomato production. Oruko and Ndun'gu (2001) did a study in Mwea in Kirinyaga district (Kirinyaga County) and reported that only 2.5% of farmers recognized nematodes as an important disease constraint in tomato production. In this study there was no evidence that farmers applied any fungicides into the soil in order to control Fusarium wilt. A study on prevalence of Fusarium wilt in Kirinyaga district (Kirinyaga County) by Mugo (2012) also indicated that the farmers had a poor understanding of Fusarium wilt. Similarly, Tinyami *et al.* (2014) gave a report on a survey carried out in Buea Municipality in Cameroon indicating that farmers did not have a thorough understanding of nematodes and diseases that caused wilting symptoms on the tomato crop. One of the methods advocated by farmers in the control of RKN was addition of manure. Organic amendments like manure have been used in the control of RKN (Lokanadhan *et al.*, 2012). The proportion of farmers who applied pesticides into soil was higher for those who got agricultural information from mass media than those who got it from government staff. The probable reason for this could be due to the fact that mass media will aggressively advertise their products, while government staff will not necessarily emphasize on chemical control but will advocate for an integrated approach incorporating other methods like crop rotation.

Plant parasitic nematodes were the most predominant trophic group comprising mainly of *Meloidogyne* spp., *Tylenchus* spp., and *Pratylenchus* spp. *Meloidogyne* spp. is the most damaging of the plant parasitic nematodes as they form giant cells and root galls affecting the ability of plants to provide nutrients to the above ground parts (Ahmed *et al.,* 2009). This may result in low yields in the crop. Charchar *et al*. (2003) reported that root-knot nematodes cause 30 to 40% yield losses in tropical regions. The plant parasitic genus of *Tylenchus* spp. though also abundant and frequent in most farms does not present a great threat to tomato

production because it is considered a weak parasitic group (Akyazi *et al.,* 2013). The high abundance of plant parasitic nematodes may be because of continuously growing crops in the same fields, which encourages their multiplication. An abundance of plant parasitic groups was observed in fields where tomato crop was grown continuously (Zheng *et al*. 2011). Akyazi *et al*. (2013) also reported a high abundance of plant parasitic groups in agricultural soils and hazelnut orchards respectively.

The bacteriovores were next in abundance to plant parasitic nematodes. This indicates decomposition of organic matter bacteria. Ferris and Matute (2003) reported that bacteriovores increase due to rapid build up of bacteria and a low C/N in the soil. Another researcher reported that bacteriovores were as abundant as plant parasitic nematodes in tomato fields where the crop was continuously grown (Zheng *et al*. 2011). Bacteriovores were also reported to be abundant as plant parasitic in agricultural soils (Wasilewska, 1979) and hazelnut orchards (Akyazi *et al*., 2013). The population of omnivores/predators and fungivores were low, with the fungivores being the least abundant. The population of fungi in the soil was low depicting a situation where there was less recalcitrant organic material that would favour decomposition by fungi (Briar, 2007).

There was low level of awareness on presence of root-knot nematodes and Fusarium wilt disease and methods of their control. The study established that RKN is prevalent in the area and therefore the need to create awareness on RKN, FOL and their management strategies.

CHAPTER FOUR

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF ISOLATES OF *FUSARIUM* SPP. FROM INFECTED TOMATO PLANTS

Abstract

Fusarium oxysporum f. sp. *lycopersici* (FOL) is a major fungal pathogen of the tomato crop. It is important to characterize isolates of *Fusarium* spp. that cause wilt of tomato in order to develop appropriate management strategies in the control of the wilt. The objective of this study was to isolate *Fusarium* spp. from disease tomato stems. The isolates were then identified using morphological and molecular procedures. Deoxyribonucleic Acid (DNA) of eleven of the isolates was extracted by growing them in potato dextrose broth (PDB). Isolates were characterized macroscopically, based on colour of mycelia, pigments produced in the media and rate of growth, while microscopic identification was based on the features of macroconidia, microconidia and chlamydospores. Molecular characterization of the isolates was done by PCR amplification and sequencing of the Translation Elongation Factor1-alpha (TEF-1α) gene. One hundred and one isolates were identified as being of *Fusarium oxysporum* by their macroscopic and microscopic characteristics. Eleven of the isolates were characterized by sequencing of TEF-1α gene and identified as those of *F. oxysporum* f. sp. *lycopersici* (F41, F66), *F. oxysporum* (F68, F99, F100, F101) and *F. verticillioides* (F1, F11, F84, F86, F98) using MLST databases. Isolates identified as *F. oxysporum* by use of morphology were characterised as those of *F. verticillioides* by molecular means. *Fusarium verticillioides* is a new pathogen that infects the tomato crop. Continuous growing of the maize crop in fields used for tomato crop production may have led to build up *F. verticillioides* inoculum. There is need for surveillance by all agricultural stakeholders in order to check on the progress of this pathogen in tomato and other crops.

4.1 Introduction

Fusarium oxysporum f. sp. *lycopersici* (Snyder and Hansen) is a soil borne pathogen that causes vascular wilt in tomato and is common in most tomato-growing areas of the world (Staniazsek *et al*., 2007; Mishra *et al.,* 2010; Jordão do Amaral *et al*., 2013). The pathogen infects tomatoes in greenhouses and in the field with estimated yield losses of 30% to 40% (Kirankumar *et al*., 2008). The disease is considered as one of the main soil-borne systemic diseases (Schwarz and Grosch, 2003).

Fusarium oxysporum f. sp. *lycopersici* (FOL) belongs to *Fusarium oxysporum* species complex. This species complex has numerous morphologically indistinguishable, saprophytic non-pathogenic, and plant pathogenic strains that infect a wide range of crops. The pathogenic strains are grouped into formae speciales, based on the host plant that they infect (Di Pietro *et al*., 2003).

Fusarium oxysporum f. sp. *lycopersici* persists in the soil and infects the tomato crop causing vascular wilt. The pathogen enters the plant through the roots and then spreads to the whole plant through the vascular system. The mycelium advances through the root cortex intercellulary and enters the xylem vessels through pits (Agrios, 2005). The fungal microconidia are carried upwards in the transpiration stream and upon reaching the end wall they germinate to form a tiny germ tube which squeezes into the next vessel (Smith, 2007). While inside the host plant the fungus produces toxins required for pathogenesis. Fusaric acid, a powerful toxin to plants, may be responsible for some of the symptoms seen in wilt diseases (Smith, 2007). Fusaric acid chelate iron leading to iron deficiency in plants and cell membrane impermeability (Parmar *et al.,* 2010). The fungus also produces extra cellular enzymes which degrade the cell wall. Tomatinase enzyme is produced by the pathogen and it detoxifies α-tomatine, a saponin produced by tomato plants (Ito *et al*., 2005; Pareja-Jaime *et al*., 2008) and this could be the way the pathogen avoids the plant glycoalkaloid barrier

(Lairini and Ruiz-Rubio, 1997). Polygalacturonase enzymes which depolymerize pectin a major component in plant cell wall and middle lamella (Di Pietro *et al.*, 1998) are also produced. Tyloses develop by the bulging of adjacent parenchyma cells to form cross walls that block the pathogen (Smith, 2007). The vessels are eventually blocked by mycelium, spores, gels, gums and tyloses. As a result, the water transport within the plant breaks down leading to development of Fusarium wilt disease (Agrios, 2005).

The first symptom of Fusarium wilt is usually the yellowing and drooping of a single leaf, or leaflet usually found on the lowest part of the stem. This usually happens on only one side of the plant. Later at the time of fruitation, the plant wilts and dries up (Tanyolaç and Akkale, 2010). There is also browning of the vascular system which can extend up to the apex (Michielse and Rep, 2009).

Fusarium oxysporum can be differentiated from other *Fusarium* sp. by microscopic and macroscopic characteristics. Macroconidia are slightly curved (sickle cell shape), with 3-5 septations (Nelson *et al.*, 1983, Leslie and Summerell, 2006). All isolates produce microconidia which are oval or kidney shaped, mainly single celled with a few having two cells. Most isolates will also produce chlamydspores in aged cultures. They have white to pale violet mycelia producing dark purple pigment on PDA (Nelson *et al.*, 1983; Leslie and Summerell, 2006).

Identification by molecular methods and knowledge of the genetic diversity of pathogenic *Fusarium* species is important in the management of the disease. Deoxyribonucleic Acid (DNA) sequences can be used to identify individuals, using existing databases as a reference. In recent years, numerous DNA based methods have been used to study variability in pathogenic Fusarium population. Translation elongation factor 1-alpha gene (TEF-1α) DNA sequence is preferred as a genetic marker in the identification of *Fusarium* spp. The internal transcribed spacer (ITS) region ribosomal DNA selected as the official ‘barcode’ locus for

Fungi (O'Donnell *et al.*, 2015), does not resolve at species level for most of *Fusarium* spp., while there are other genetic markers like the TEF-1α, that are able to discern up to species level for most of the *Fusarium* spp. (Bruns, 2001; Gazis *et al.*, 2011).

Deoxyribonucleic Acid (DNA) sequences from TEF-1α gene are most informative for species-level identifications for most Fusarium because they resolve at species-level (www.cbs.knaw.nl). The sequences are well represented in the databases (Geiser *et al*., 2004). A dense sampling of this gene across the breadth of the Fusarium genus is represented in Fusarium-ID databases **(**http://isolate.fusariumdb.org**)** and Fusarium Multilocus Species Typing (MLST) databases (CBS Knaw Fungal Biodiversity Centre http://www.cbs.knaw.nl/Fusarium). These databases house broadly sampled, well-characterized phylogenetically informative sequences (O'Donnell *et al*., 2015). The isolates can be obtained from Fusarium Research Centre (http://plantpath.psu.edu/ facilities/fusarium), the Agricultural Research Service Culture Collection (NRRL, http://nrrl.ncaur.usda.gov/cgi-bin/usda) or the (CBS Knaw Fungal Biodiversity Centre) (O'Donnell *et al*., 2015).

The study was carried out with the aim of characterizing *Fusarium oxysporum* isolates using morphological and molecular means.

4.2 Materials and Methods

4.2.1 Sample collection and isolation of *Fusarium* spp. from tomato stems

Fungal isolation was done from diseased tomato stems collected from farms at Mwea West Sub-County in Kenya. There were a total of 225 stem pieces obtained from 119 farms. These were obtained by purposeful sampling of diseased tomato plants showing symptoms of wilting and vascular discolouration during the field study. The stem pieces from each diseased plant were thoroughly washed in tap water and then aseptically cut into one

centimetre long pieces. The stem pieces were surface sterilized in 2% sodium hypochlorite solution for 30 seconds. They were then rinsed with three changes of sterile distilled water and dried using sterile serviette. Five sterilized stem segments were aseptically placed on petri-dishes with Potato Dextrose Agar (PDA). This was done in duplicate (Waudo *et al.*, 1995). The inoculated plates were amended with antibiotics [ampicillin (50 mg), chlortetracycline (50 mg), penicillin (50 mg), streptomycin (50 mg) and 28 mg of pentachloronitrobenzene (PCNB)]. They were incubated at room temperature (24^{o}C ± 2) for 10 days. Fungal colonies with white to pink mycelia that are characteristic of *Fusarium* spp. were sub cultured on PDA at room temperature. After one week, the isolates were cultured on SNA (Spezieller Nahrstoffarmer Agar), a media with low nutrients to allow sporulation of the fungus (Burgess *et al.*, 1994). After 14 days, microscopic examination of the cultures was done. A portion of mycelia was picked using a needle placed on a slide and was stained using lactophenol in cotton blue. This was then observed under a compound microscope at, X10, X40 objective and X100 under oil emersion. Macroconidia, microconidia, phialides and chlamydospores were important characteristics in the identification of the isolates (Nelson *et al.*, 1983). Cultural growth characteristics including colour of mycelia, pigmentation on media and radial growth were determined by growth of the isolates on PDA medium. Single spore isolates were made and were preserved as agar slants on PDA at 4^{o}C for molecular identification. The isolates were tested for their pathogenicity on a tomato cultivar, "Money Maker".

4.2.2 Preparation of inoculum for pathogencity tests

One week old *Fusarium oxysporum* f. sp. *lycopersici* (FOL) grown on PDA plates were flooded with 10 ml of sterile distilled water and the conidia dislodged with a sterilized glass rod bent to the shape of a hockey stick. The conidial suspension was filtered through a double

muslin cloth to remove media and mycelia. The count of propagules in the filtrate was estimated by serial dilution of the filtrate and plating. The filtrate was adjusted to a concentration of $1x10^7$ spores / ml.

4.2.3 Preparation of soils for pathogenicity tests in greenhouse experiments

Soil mixed with sand was used as the substrate for growing tomato plants in the greenhouse. The soil was collected from Kabete field station and mixed with sand in the ratio 3:1. The mixture was sterilised at 121^{o}C for 15 minutes and the procedure repeated after a period of three days. The soils were left to stand at least two weeks and then placed in seedling trays measuring. The contents of the soil are shown in appendix 3.

4.2.4 Planting of tomato seeds

Seeds of tomato cultivar "Money Maker" were surface disinfected in 0.5% sodium hypochlorite solution for 3 minutes and afterwards rinsed with three changes of sterile distilled water before sowing. The seeds were sown in seedling trays in the sterilised soil sand medium. The seedlings were maintained in a glasshouse and were watered regularly. Each tray could accommodate 150 seedlings, the space between one seedling and another being 2 cm. The seedlings took one week to germinate from the soil and were transplanted after 21 days.

4.2.5 Pathogenicity tests

Twenty one days old seedlings of tomato cultivar Money Maker were inoculated by the standard root dip method (Reis *et al.*, 2005). Seedlings were uprooted, shaken to remove adhering soil particles and washed carefully under running water. Two centimetre of the root system from the apex was cut off with sterile scissors and then dipped in a spore suspension

of approximately 10^7 conidia/ml suspension for each of the test isolates for 10-15 minutes. In the control treatment, plants were dipped in sterile distilled water. The inoculated seedlings were planted in polythene sleeves (5x7x150 cm) containing soil to sand in the ratio 3.1. Each polythene sleeve had one seedling. The test for each isolate was replicated four times and was laid in a randomised complete block design in the greenhouse. Plants were supplied with Di-ammonium phosphate (DAP) fertilizer at planting time and were top dressed with Calcium ammonium nitrate (CAN) fertilizer four weeks after transplanting. Both fertilizers were applied at the rate of one gram per sleeve. Plants were watered every day, for the first few weeks and every day during the last few weeks of growth. Disease severity was assessed 45 days after transplanting. Disease severity was assessed using a severity scale of 1-4, a modification of a wilting index by Akram *et al*. (2014), where 1 represented no symptom, 2= wilting and yellowing covered less than 25%, 3= wilting and yellowing less than 50%, 4= wilting and yellowing more than or equal to 50%.

4.2.6 Molecular characterization of Fusarium isolates

4.2.6.1 DNA extraction protocol

Eleven isolates that were the most pathogenic were selected for the molecular analysis. Each test isolate that had been preserved in agar slants was subcultured in PDA and the fungus left to grow at room temperature ($24^oC \pm 2$) for seven days. Five mycelia discs derived from colony margins of seven day old cultures were inoculated separately in 100 ml potato dextrose broth (PDB) in 250 ml Erlenmeyer flasks for each of the isolates. The cultures were maintained in the dark and were agitated for seven days on a rotatory shaker at 50 rpm at room temperature (24 ± 2^oC). Mycelia from 100 ml PDB cultures were collected by filtration over a funnel using suction and washed with distilled water. The DNA extraction was done for each isolate according to the procedure adapted from Sambrook *et al*. (1989). Fifty

milligrams of mycelium were transferred to a 1.5 ml eppendorf tube. The mycelium for each sample was suspended in 600 µl Cetyltrimethylammonium bromide (CTAB) lysis buffer containing: 0.1M NaCl, 0.5M Tris-HCl (pH 8.0), 50mM EDTA and 2% CTAB, 0.2% mercaptoethanol and 0.1 mg/ml proteinase K. Two glass beads for crushing of cell walls were also added. The tubes were then placed into a mixer mill 301 (Retsch) and homogenized for two and a half minutes at maximum speed.

The tubes were incubated in a water bath for one hour at 65ºC. Afterwards, 600 µl of phenol was added and the suspension mixed by vortexing gently for 10 seconds. Then, the tubes were centrifuged for 20 minutes at 14,000 rpm. After centrifugation, the top aqueous layer was transferred to new tubes. To remove any RNA contamination, 5 µl (1mg/ml) RNase was added to the DNA solution and incubated for five hours at room temperature. This procedure was followed by the addition of 600 µl of phenol: chloroform to each sample. The samples were vortexed briefly, and centrifuged for 10 minutes in a micro centrifuge. The aqueous layer was transferred to a new tube and extracted again with chloroform, by mixing with 600 µl chloroform and centrifuging for 10 minutes at 14,000 rpm. The supernatant was transferred to new eppendorf tubes, and 60 µl of 3M sodium acetate was added to the recovered aqueous solution and mixed by vortexing. Deoxyribonucleic acid was then precipitated from the solution with 750 µl of ice cold ethanol by incubating the samples in a freezer at -20ºC overnight.

Samples were then centrifuged at 4°C for 10 minutes at 14,000 rpm. The pellets were washed twice with cold 70% ethanol, air-dried, and then re-suspended in 80 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0). The quality and concentration of the DNA was determined by running 5 µl of the DNA on 1% agarose gel stained with ethidium bromide in 1X TAE buffer, at 80V for 45 minutes. The visualization was done on UV light and photographed

using the UVR transilluminator apparatus. The DNA obtained was quantified and its purity verified by a spectrophotometer.

4.2.6.2 PCR amplification

The isolated DNA products were amplified by Polymerase chain reaction (PCR). The PCR amplification of the translation elongation factor 1-alpha (TEF-1α) gene was performed using the following primers: ef1 (5'-ATG GGT AAGGA (A/G) GAC AAG AC-3') and ef2 (5'-GGA (G/A) GT ACC AGTG/C) AT CAT GTT-3') (O'Donnell *et al.*, 1998). The PCR mixture with a total volume of 25 µl consisted of 2x Eppendorf Master Mix (Taq DNA polymerase 0.125 µl (1.25 U), 30 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$; 0.1% Igepal-CA630; 0.2 mM dNTP), 0.6 µM of each primer, and 2 µl of fungal DNA (Mohammed *et al*., 2016).

A Gene Amp 9700 thermocycler (Applied Biosystems, Waltham, USA) was used for PCR amplifications with the following amplification cycles: initial denaturation at 94°C for 85 seconds followed by 35 cycles of denaturation at 95°C for 35 seconds, annealing at 59°C for 55 seconds and extension at 72°C for 90 seconds, followed by final extension for 10 minutes at 72°C. PCR products were detected by electrophoresis in a 1.5% agarose gel in TBE buffer (Mohammed *et al*., 2016).

4.2.6.3 Purification of PCR products

Ten microliters of PCR products obtained for each sample were transferred to 1.5 ml Eppendorf tubes. To the product, one microlitres of 3M sodium acetate and 30 µl 99.5% Ethanol were added and gently mixed. The mixture was left standing at room temperature for 15 min, before centrifugation at 15000 rpm for 20 min. The supernatant was decanted and to the pellet, which contained the desired product, 150 µl of 70% ethanol was added. The

mixture was centrifuged for 20 min at 15000 rpm. The supernatant was decanted carefully while the DNA pellet was vacuum dried for 10 min using the EYELA evaporator. Ten microlitres of Hi-Di formamide was added and the mixture vigorously vortexed to re-suspend the DNA fragments. The products were thereafter kept at -20 ^{o}C overnight.

4.2.6.4 Sequencing of PCR products

The purified PCR products were sent to Inqaba Biotechnical Industries (Pretoria, South Africa) for sequencing. The protocol for sequencing of the PCR products was a modified version of the protocol described in Applied Biosystem's ABI PRISMTM Dye Terminator Cycle Sequencing Ready Reaction Kit manual. In a 0.5 ml eppendorf tube, add 2 µl primers, either forward or reverse primer per reaction was added and Big Dye Terminator v3.1 mix (2 µl per reaction) was added per reaction. One micro litre of PCR product was added and topped up with sterile distilled water to a volume of 10 µl.

The tubes were span briefly to ensure that no solutions were left on the sides of the tubes. The tubes were placed into a PCR machine and the cycle sequencing program was run at: 26 cycles for ten seconds denaturation at 96ºC, five seconds annealing at 50ºC and four minutes extension at 60ºC. Three microlitres each of the PCR products was analyzed on 2% agarose gel to confirm success or failure of sequencing reaction, while the rest were subjected to purification prior to being run in the genetic analyzer machine.

Microplates were prepared and 30 µl of a mixture of ethanol (100%) and Sodium acetate (30 µ EtOH and one microliter NaAc) were put for each of the samples. Then the 10 µ of the cycle sequencing product was added followed by a centrifugation for 10 seconds. This was left on ice for one hour and centrifugation repeated again for one hour and 20 minutes at 2300 rpm. The ethanol and Sodium acetate was removed by emptying the plate over the sink. Then, 70% (250 µ) of ethanol was added followed by centrifugation at 15 minutes at 2300

rpm. The microplates were dried in an oven at 60ºC for 15 minutes. Ten microlitres HiDi formamide was added to each sample and was centrifuged for five minutes at 2300 rpm. The purified sequencing reaction product was transferred immediately on ice to ensure that they remained linear until analyzed. The reaction mixtures, 10 µl in quantity, were transferred to lanes of the MicroAmp optical wells. At the end of the sequencing run, the results in the form of electrophoregrams and deduced textual sequences were copied out and transferred to a PC where the Genetyx Mac program or BIOEDIT Sequence Analysis program for Windows were used to analyze the sequences.

4.2.6.5 Editing, assembly and alignments of the DNA sequences

The trace files generated by the DNA analyser sequencer from Applied Biosystems were imported into CLC main workbench version 6.8.1 for quality check, editing and assembling. Following this procedure, all the trace files were trimmed off to remove low quality bases at the extreme ends of the chromatograms using the default settings of the software.

For each sample, trimmed sequences for the reverse and forward orientations were assembled into contigs against *Fusarium oxysporum* f. sp. *lycopersici* TEF1-alpha gene as the reference for scaffolding using the default assembling algorithm. Corrections of bases on the resultant contigs were manually done to ensure the correct base calling for the subsequent consensus sequences. These ambiguities and disagreements were resolved by referring to the chromatogram files of the respective reads in that consensus sequence and independent of the reference sequence. Clean scaffolds were saved as consensus files in FASTA (FAST- ALL) formats. The sequences were individually blasted for similarity in the Fusarium database (http://isolate.fusariumdb.org) and Fusarium Multilocus Sequence Typing (MLST) database [(http://www.cbs.knaw.nl/Fusarium) (Geiser *et al*., 2004]).

4.3 Results

4.3.1 Isolation of *Fusarium oxysporum* from tomato stems

A total of 101 isolates of *F. oxysporum* were recovered from fusarium wilted plants stem pieces collected from 119 farms. It is only isolates that were morphologically similar to *F. oxysporum* that were characterised. The microscopic features that were important in identification were septation and shapes of micro and macroconidia and structure of the chlamydospores (Nelson *et al.*, 1983). Macroscopic characteristics such as growth rates, pigmentation on media were also important in the identification process (Leslie and Summerell, 2006).

4.3.2 Cultural characteristics of *Fusarium* spp. isolates

The cultural characteristics of the isolates were observed after culturing on PDA at room temperature and a photo period of 12 hours for one week. Growth on PDA media produced cream to yellowish and white aerial mycelia, cottony in appearance which in some cases became pink and later purple in colour. The isolates showed variation on pigments produced in media, which varied from no pigmentation (creamy white) to dark red pigmentation (Table 4.1, Plate 4.1a, b).

Radial growth on PDA media varied in the different isolates. The isolates could be categorized as 1; fast growing (> 70 mm) radial growth, 2; moderate (50 mm- 70 mm) radial growth, and 3; slow growing with less than 50 mm radial growth within seven days of inoculation on a PDA plate at 24 ± 2^{o}C (Table 4.2).

4. 3. 3 Microscopic characteristics

Microscopic characteristics such as shape and septation of conidia were studied after culturing in SNA medium. Masses of conidiophores growing on sporodochia were produced

in culture. The macroconidia were produced on sporodochia or from monophialides on hyphae, appearing at the tip of the phialide one at a time. The macroconidia in some cases were abundant while in other cases were few or even absent. They were slightly curved (sickle cell shape), and had 3-5 septations. Microconidia accumulated at the tip of the conidiogenus cells, the phialides. They were borne on short monophialides arising from aerial mycelia. All isolates produced microconidia which were oval or kidney shaped mainly single celled with a few having two cells. The resting spores, chlamydospores were produced in cultures that were over two weeks old. Some isolates did not produce chlamydospores. The microscopic characteristics of the isolates are as shown in table 4.1.

4.3. 4 Pathogenicity testing

Variation in severity of symptoms was observed in the aerial parts and within the stem tissues of tomato cultivar Money Maker infected with the different isolates. Early stage symptoms appeared as yellowing of lower leaves and in later stages drooping of leaves was observed. In severe infections, there was browning of vascular bundles and wilting of the plant. Out of the 101 isolates, four were found to be virulent 15 were moderately virulent, 67 were weak isolates in terms of virulence, while 15 were non virulent (Table 4.2). The four virulent isolates produced total discoloration of the vascular bundles on tomato cultivar Money Maker. The 15 moderately virulent showed faint discoloration of the vascular bundles while the 67 weak isolates showed symptoms of the wilt and yellowing of the leaves with no vascular discolouration. The four and 15 moderately virulent and 67 weak isolates were re-isolated from the test plants and were re-inoculated again on tomato “Money Maker” and symptoms similar to those of infected plants observed.

Table 4.1 Characteristics of *Fusarium oxysporum* isolated from diseased tomato plants collected from MweaWest Sub County fields

Isolate	Colour of mycelium	Colour of reverse	Micro-conidia	Macro-conidia	Chlamydospores
F1	White pink	Purple	0	3 septate	Terminal and solitary
F2	Purple	Purple	0	3 septate	Terminal and solitary
F3	White pink	Purple	0	3 septate	Terminal and solitary
F4	Purple	Purple	0	5 septate	Terminal and solitary
F5	Purple	Purple	0	3 septate	Terminal and solitary
F6	Purple	Purple	0	3 septate	Terminal and solitary
F7	Orange	Orange	0	3 septate	Terminal and solitary
F8	Purple	Purple	0	3 septate	Terminal and solitary
F9	Purple	Cream	0	3 septate	Intercalary in chains
F10	White pink	Purple	0	3 septate	None
F11	Cream	Cream	0	None	None
F12	Pink	Dark red	0	None	Terminal and solitary
F13	White pink	Purple	0	3 septate	Terminal and solitary
F14	White pink	Purple	0	4 septate	Intercalary in chains
F15	Purple	Cream	0	3 septate	Terminal and solitary
F16	Pink	Red	0	3 septate	Intercalary in chains
F17	Pink	Red	1	3 septate	Terminal and solitary
F18	White	Cream	0	3 septate	Terminal and solitary
F19	Purple	Purple	0	3 septate	Terminal and solitary
F20	Pink	Dark red	0	3 septate	Terminal and solitary
F21	White	Cream	0	None	Intercalary in chains
F22	Pink	Dark red	1	3 septate	Terminal and solitary
F23	Pink	Red	0	None	None
F24	Pink	Dark red	0	None	Terminal and solitary
F25	Pink	Dark red	0	3 septate	Intercalary in chains
F26	Pink	Dark red	0	3 septate	Terminal and solitary
F27	Purple	Purple	1	3 septate	Terminal and solitary
F28	Purple	Purple	0	3 septate	Terminal and solitary
F29	Pink	Dark red	1	4 septate	None
F30	White pink	Purple	0	3 septate	Terminal and solitary
F31	Orange	Orange	0	3 septate	None
F32	Purple	Purple	0	3 septate	Terminal and solitary
F33	White pink	Purple	1	3 septate	Terminal and solitary
F34	Purple	Cream	0	3 septate	Terminal and solitary
F35	Purple	Cream	0	3 septate	Terminal and solitary
F36	Purple	Cream	0	None	None
F37	White pink	Purple	0	3 septate	Terminal and solitary
F38	White	Cream	0	3 septate	Terminal and solitary
F39	White pink	Purple	1	3 septate	Terminal and solitary
F40	Purple	Cream	0	None	None
F41	White pink	Purple	0	3 septate	None
F42	Orange	Orange	1	4 septate	None
F43	Purple	Purple	0	None	None
F44	Purple	Purple	0	4 septate	None
F45	White pink	Purple	0	3 septate	None
F46	Purple	Cream	1	3 septate	None
F47	Purple	Purple	0	3 septate	Terminal and solitary

Characteristics of FOL isolated from diseased tomato plants collected from MweaWest Sub County fields (Table 4.1 continued)

Isolate	Colour of mycelium	Colour of reverse	Micro-conidia	Macro-conidia	Chlamydospores
F48	Purple	Purple	0	3 septate	Terminal and solitary
F49	Purple	Cream	0	3 septate	Terminal and solitary
F50	White pink	Purple	1	3 septate	Intercalary in chains
F51	White	Cream	0	None	Terminal and solitary
F52	White pink	Purple	0	None	None
F53	Orange	Orange	0	None	Terminal and solitary
F54	White	Pink	1	3 septate	Terminal and solitary
F55	Purple	Purple	0	2 septate	Terminal and solitary
F56	White pink	Purple	0	3 septate	None
F57	White	Cream	0	3 septate	Intercalary in chains
F58	White	Cream	0	3 septate	None
F59	White	Cream	0	3 septate	Terminal and solitary
F60	White pink	Purple	0	3 septate	Terminal and solitary
F61	Orange	Orange	0	4 septate	None
F62	White-cream	Orange	0	4 septate	None
F63	Purple	Purple	0	3 septate	Terminal and solitary
F64	Pink	Dark red	1	None	Terminal and solitary
F65	White pink	Purple	0	3 septate	Intercalary in chains
F66	White	Cream	0	3 septate	Terminal and solitary
F67	White	Cream	0	3 septate	Terminal in pairs
F68	White	Cream	0	3 septate	None
F69	Orange	Orange	0	3 septate	Intercalary in chains
F70	Orange	Orange	1	Many	None
F71	Orange	Orange	0	4 septate	Intercalary in chains
F72	Purple	Purple	0	3 septate	None
F73	Cream	Cream	0	3 septate	Terminal and solitary
F74	White	Cream	0	None	Intercalary in chains
F75	White pink	purple	0	3 septate	None
F76	White	Cream	0	3 septate	Intercalary in chains
F77	Purple	Purple	1	None	Terminal and solitary
F78	Orange	Orange	0	3 septate	None
F79	Orange	Orange	0	4 septate	None
F80	White	Cream	0	3 septate	Intercalary in chains
F81	White	Cream	0	None	Terminal and solitary
F82	Orange	Orange	1	5 septate	Terminal and solitary
F83	White	Cream	0	5 septate	Terminal and solitary
F84	Orange	Orange	0	3 septate	Terminal and solitary
F85	Purple	Purple	1	3 septate	Terminal and solitary
F86	Orange	Orange	0	3 septate	Terminal and solitary
F87	Orange	Orange	0	4 septate	Terminal and solitary
F88	White	Cream	0	None	None
F89	Orange	Orange	0	3 septate	Terminal and solitary
F90	Cream	Cream	0	3 septate	Terminal and solitary
F91	White pink	Purple	0	3 septate	Terminal and solitary
F92	White	Cream	0	3 septate	Terminal and solitary

Characteristics of FOL isolated from diseased tomato plants collected from MweaWest Sub County fields (Table 4.1 continued)

Isolate	Colour of mycelium	Colour of reverse	Micro conidia	Macroconidia	Chlamydospore
F93	White	Cream	0	3 septate	Terminal and solitary
F94	Purple	Cream	0	3 septate	Terminal and solitary
F95	White pink	Purple	0	3 septate	Terminal and solitary
F96	White pink	Purple	0	3 septate	Terminal and solitary
F97	Cream	Cream	0	None	Terminal and solitary
F98	White pink	Cream	0	3 septate	None
F99	White	Cream	0	4 septate	None
F100	Purple	Purple	1	3 septate	Terminal and solitary
F101	White pink	Purple	1	3 septate	None

Septate- number of cross walls in a macroconidia; 0= no septation in microconidia, 1=only one septation in microconidia, none-no macroconidia or chlamydospores, Terminal and solitary= single chlamydospore at the end of hyphae; Intercalary in chains= chlamydospores found between hyphae

Table 4.2 Rate of growth of isolates on PDA and virulence of the isolates on tomato cultivar "Money Maker"

Isolate	Radial growth(mm)	Disease severity	Isolate	Radial growth(mm)	Disease severity
F1	2	3	F25	2	2
F2	2	2	F26	2	2
F3	2	3	F27	2	2
F4	2	2	F28	2	3
F5	1	1	F29	2	2
F6	2	3	F30	2	2
F7	3	2	F31	2	2
F8	2	3	F32	2	2
F9	2	2	F33	2	2
F10	2	2	F34	2	3
F11	2	3	F35	2	2
F12	2	2	F36	2	2
F13	2	3	F37	2	2
F14	1	1	F38	2	2
F15	2	2	F39	2	2
F16	2	2	F40	2	2
F17	2	2	F41	2	4
F18	2	2	F42	2	3
F19	3	2	F43	3	2
F20	3	2	F44	2	2
F21	2	2	F45	2	2
F22	2	2	F46	2	2
F23	2	2	F47	3	2
F24	1	2	F48	2	3

Rate of growth of isolates on PDA and virulence of the isolates on tomato cultivar "Money Maker" (Table 4.2 cont)

Isolate	Radial growth (mm)	Disease severity	Isolate	Radial growth (mm)	Disease severity
F49	2	2	F76	2	2
F50	2	2	F77	1	1
F51	3	2	F78	2	2
F52	2	2	F79	2	2
F53	2	2	F80	3	2
F54	2	1	F81	3	3
F55	2	1	F82	2	1
F56	2	2	F83	2	2
F57	2	2	F84	2	3
F58	2	2	F85	2	3
F59	2	2	F86	3	1
F60	2	2	F87	3	2
F61	2	2	F88	2	1
F62	2	1	F89	2	3
F63	2	2	F90	2	2
F64	2	2	F91	2	2
F65	2	1	F92	2	2
F66	2	4	F93	3	2
F67	2	2	F94	2	1
F68	2	1	F95	2	2
F69	2	2	F96	2	2
F70	2	2	F97	2	1
F71	2	2	F98	2	3
F72	2	1	F99	2	2
F73	2	3	F100	2	4
F74	2	2	F101	2	4
F75	2	2			

1=Fast growing >70 mm; 2=Moderate growth (50-70 mm); 3=Slow growing < 50 mm. High virulence disease severity score=4; moderately pathogenic=3; weakly pathogenic=2 1=non pathogenic

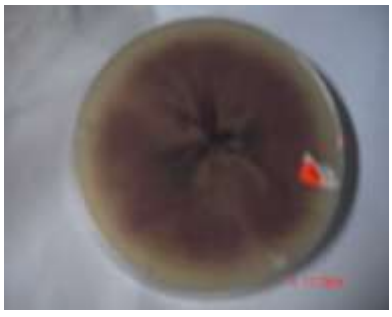


Plate 4.1a isolate F16 (FOL) Upperside isolate F16 (FOL) Reverse side

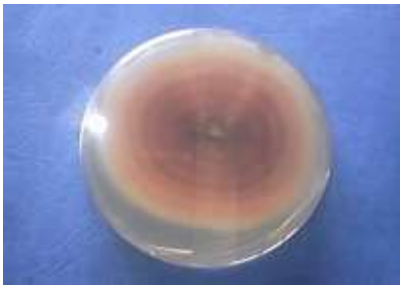


Plate 4.1b isolate F41(FOL) Upperside isolate F41(FOL) Reverse side

4.3.5 Molecular analysis of isolates

The protein contamination test in the DNA extract from the different isolates was verified by taking spectrophotometer absorbance reading at 260 nm and 280 nm and this ratio was calculated to be 1.8 to 2 for all the isolates. The TEF-1α gene primers EfI and Ef2 primers amplified fragments of 570 to 710 bp for all isolates. The identity of isolates was determined by comparing their TEF1 - α sequence with those in Fusarium-ID **(**http://isolate.fusariumdb.org**)** and MLST **(**http://www.cbs.knaw.nl/Fusarium) database (Bogner *et al*., 2016). The BLAST analysis of the TEF1-α gene sequence data, identified the closest match (99–100% similarity) references of the isolates in the Fusarium-ID and MLST databases. The reference description in the Fusarium-ID and MLST databases that was close match for each of the isolates is shown in Table 4.3. Both the Fusarium-ID and MLST databases have included collections from Northern Region Research Laboratory (NRRL) databases in some of the reference descriptions and these are included in the table.

Only two of the isolates were closely matched with isolates of *F. oxysporum* f. sp. *lycopersici*; F41 and F66 using MLST data bases, though the Fusarium-ID databases, simply classified them as *F. oxysporum* (Table 4.3). All other isolates were identified as *F. oxysporum* and *F. verticillioides*, in the MLST databases. A phylogenetic tree constructed to show the evolutionary relationships between the *Fusarium* spp. indicated they could be grouped into four groups (Fig.4.1).

Table 4.3 Isolates identified using TEF-1α sequences in Fusarium ID and MLST databases and % similarity value to the reference isolate

Isolate	Fusarium-ID Reference isolate	Fusarium MLST Reference isolate	% similarity
F1	FD_01856_EF1α [*Fusarium* sp.] NRRL 43608	*Fusarium verticillioides*, NRRL 20960 CBS 119285	100
F11	FD_01856_EF-1 α [Fusarium sp.]	*F. moniliforme*, (NRRL 43608; CBS 130180	100
F41	FD_01199_EF-1 α [*Fusarium oxysporum*] (NRRL 26037)	*F. oxysporum* f.sp. *lycopersici* N8; elongation Factor 1- α (NRRL 34936; CBS12368)	100
F66	FD_01199_EF-1 α [*Fusarium oxysporum*] (NRRL 26037)	*F. oxysporum* f.sp. *lycopersici* N8; elongation Factor 1- α (NRRL 34936; CBS12368)	99
F68	FD_00786_EF-1 α [*Fusarium oxysporum*] (NRRL 38592)	*Fusarium oxysporum* species complex 191 (NRRL 36356)	100
F84	FD_01856_EF1α [*Fusarium* sp.]	*F. moniliforme*, (NRRL 43608; CBS 1301800)	99
F86	FD_01856_EF-1α [*Fusarium* sp.]	*Fusarium verticillioides*, (NRRL 20960; CBS 119285)	99
F98	FD_01856_EF-1α [*Fusarium* sp.]	*F. moniliforme*, (CBS 130180	100
F99	FD_00733_EF-1 α [*Fusarium* sp.] (NRRL 458881)	*F. oxysporum* species complex (NRRL 20433)	99
F100	FD_00789_EF1α [*Fusarium oxysporum*) (NRRL38595)	*Fusarium oxysporum* species complex 191, (NRRL 38593)	99
F101	FD_01215_EF1 α [*Fusarium oxysporum*] (NRRL25356)	*Fusarium oxysporum* (NRRL 36356 ; CBS 21.49)	99

CBS = Centraalbureau voor Schimmelcultures, Utrecth, Netherlands. Northern Region Research Laboratory cultures (NRRL)

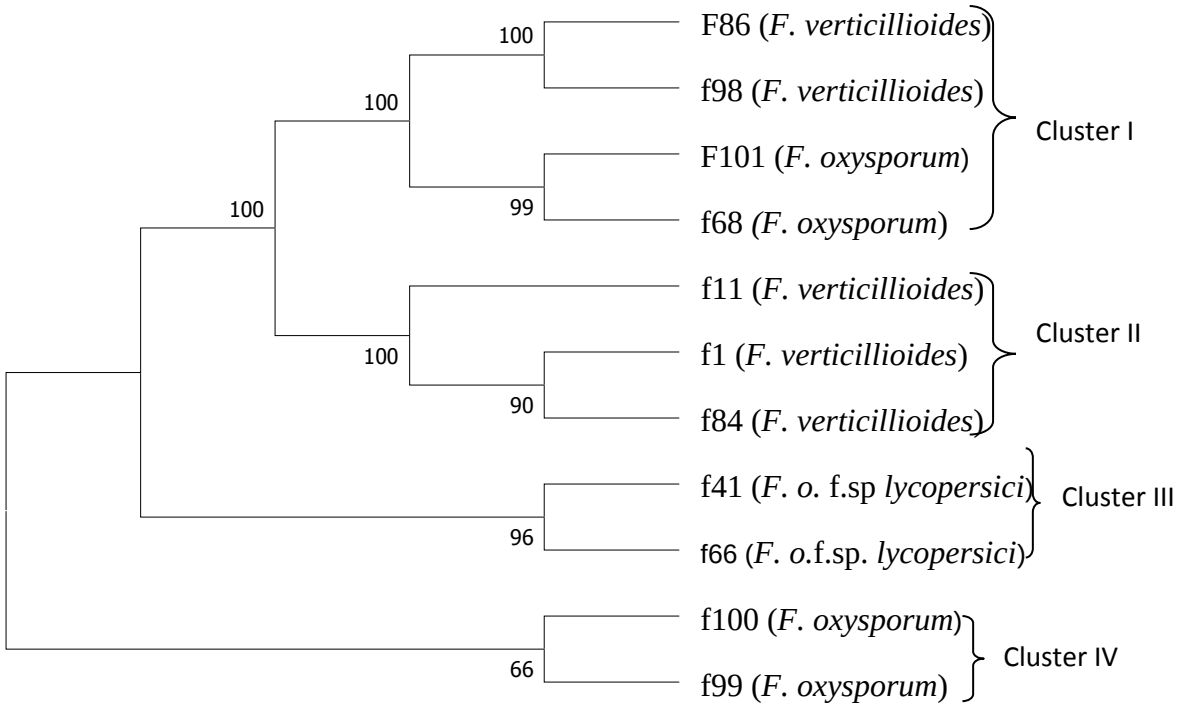


Fig. 4.1 Phylogenetic tree generated using by MEGA 7 software. Maximum Parsimony Analysis (with bootstrap analysis of 1000 replicates) for the DNA sequence of the translation elongation 1α factor gene using 11 sequences of *Fusarium* spp. The numbers at the nodes represent bootstrap support values.

4.4 Discussion

Microscopic characteristics such as presence of oval to kidney shaped, non septate microconidia and macroconidia with a slight curvature, 3-5 septate and a pointed apical cell confirm that the isolates were of *Fusarium oxysporum*, according to descriptions by Nelson *et al.* (1983) and Leslie and Summerell, (2006). However the macroconidia in some cases were abundant while in other cases were few or even absent. Some isolates produced chlamydospores while others did not. Gagkaeva (2008) reported that presence of chlamydospores as an important diagnostic feature but their absence is not informative on character. The variability in microscopic, macroscopic characteristics and pathogenicity tests may be indicative of the fact that the isolates are different physiologically. Other authors have reported the cultural, morphological and pathogenic variability among different isolates of *F. oxysporum* f. sp. *lycopersici* (Pandey and Gupta, 2012; Nirmaladevi and Srinivas, 2012). The

morphological characteristics were variable depending on the culture conditions and therefore cannot be conclusive in the identification of *Fusarium* spp.

Identification by molecular means did not correspond with the morphological characterization. Isolates of *F. verticillioides* were identified by molecular means but morphologically, were characterised as those of *F. oxysporum.* Culturally, isolates of *F. oxysporum* are indistinguishable from those of *F. verticillioides* on PDA agar. Leslie and Summerell, (2006) described isolates of *F. oxysporum* as having white to pale violet mycelia producing dark magenta pigment on PDA, while those *F. verticillioides* have white mycelia and produces violet pigments in PDA. The two species have similarities in their macro and microconidia. The maroconidia of the two species are 3-5 septate while microconidia are mono -septate or non- septate. Character of chlamydospores is distinctive in the two species as *F. verticillioides* does not form chlamydospores, though it has swollen cells in the hyphae that can easily be mistaken as chlamydospores. However formation of chlamydospores is not an informative characteristic in the identification of *F. oxysporum* as this depends on the culture conditions.

The BLAST analysis of the TEF-1α gene sequence data of the isolates was used to identify close (99–100% similarity) references in the Fusarium ID and MLST databases. The close reference of the isolates was found to be with *Fusarium* spp. *F. oxysporum, Fusarium oxysporum* f. sp. *lycopersici* and *F. Fusarium verticillioides.* Almost half of the isolates identified by molecular methods were those of *Fusarium verticillioides* (Sacc.) Nirenberg (*Syn. F. moniliforme* Sheld.). The phylogenetic tree classified the isolates into four groups with isolates of *Fusarium oxysporum* and *Fusarium Fusarium verticillioides* being clustered together showing close evolutionary relationship between the two species. *Fusarium moniliforme* has previously been isolated from tissues of infected tomato plants with no pathogenic effects on the plants (Chehri, 2016). However, Yin-dong *et al.* (2018) reported

that apart from *Fusarium oxysporum* f. sp. *lycopersici*, other *Fusarium* spp. isolated from the roots of tomato plants were pathogenic. *F. verticillioides*, *F. solani*, and *F. subglutinans* totalled to 39% of other fusarium isolates isolated from the roots pathogenic to tomato plant. Marupov *et al.* (2013) reported on a virulent isolate of *F. verticillioides* that infected the cotton crop. *Fusarium verticillioides* is a new pathogen that infects tomato plants (Yin-dong *et al.*, 2018). Rotation of the tomato crop with the maize crop which is also a major crop grown in the study area may account for the occurrence of this fungal pathogen. There is need for surveillance by all agricultural stakeholders in order to check the progress of this pathogen in tomato and other crops.

CHAPTER FIVE

EVALUATION OF REACTION OF DIFFERENT TOMATO CULTIVARS TO *MELOIDOGYNE JAVANICA* AND FUSARIUM WILT

Abstract

Root-knot nematodes (RKN) and Fusarium wilt pathogen are among the most important diseases that lower tomato production. The root-knot nematodes (RKN) and Fusarium wilt pathogen form an interaction leading to a disease complex that is far more destructive than when each of the pathogen occurs alone. Experiments were set up to determine reaction of selected tomato cultivars to Fusarium wilt pathogen and root-knot nematodes. Three week old tomato seedlings of six different tomato cultivars Prostar F1, Kilele F1, Oxly, Cal J, Rambo F1 and Roma VFN were transplanted separately into half a kilogram plastic pots and infested with 1000 J2s of *Meloidogyne javanica.* Each experiment was replicated four times and replicates arranged in a randomised complete block design in the greenhouse located at Upper Kabete Campus field station. After nine weeks, the experiment was terminated. The plants were evaluated on dry weight of shoot and root, height, number of galls and second stage juveniles per root system. A different experiment was set up with the same six tomato cultivars being infested with four different isolates of Fusarium wilt pathogen. The experiment was terminated after nine weeks and disease severity scores and incidence recorded for each of the isolates in the six tomato cultivars. An experiment was also set to test the effect of interaction between *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and *M. javanica* at different inoculum densities of second stage juveniles (J2s) in two tomato cultivars Rambo F1 and Prostar F1 in greenhouse environment. The most susceptible cultivars to *M. javanica* were Rambo F1, Prostar F1, Roma VFN, and Cal J with galling indices of 4.0, 3.5, 3.0 and 2.3 respectively, while cultivars Kilele F1 and Oxly were the least susceptible with galling indices of 2.0 and 1.5 respectively. The isolate F41 had high disease

incidence scores at 100%, 100%, 75%, in the tomato cultivars, Prostar F1, Roma VFN and Cal J, respectively. Rambo F1 was the least susceptible to FOL isolate F41 at an incidence of 16.7%. Ten weeks after inoculation, infection with FOL and *M. javanica* increased the nematode populations in the roots in both tomato cultivars. Reductions in height, shoot and dry root weight were observed with increasing nematode inoculum levels. Co-infection with FOL and *M. javanica* resulted in greater reductions in height, shoot and root dry weights compared to nematode only inoculations. Greater reductions in shoot and root dry weight was observed with increasing nematode inoculum levels. In the cultivar Prostar F1, disease severity at 3000 inoculum levels was significantly ($P < 0.05$) higher at a severity scale of 4.0 compared to 2.25 at 500 J2s inoculum level. The wilt disease in cultivar Rambo F1 was insignificant even at higher nematode inoculum levels. It is important to use tomato cultivars that are less susceptible to root-knot nematodes and Fusarium wilt in order to increase on tomato growth and production. Use of Fusarium resistant cultivars as shown in the cultivar Rambo F1 would be important in the management of root-knot nematodes and FOL disease complex.

5.1 Introduction

Root-knot nematodes, (RKN) *(Meloidogyne* spp.) affect tomato production (Sikora and Fernandez, 2005; Serfoji *et al*., 2010). Due to infection by RKN, yield losses of 30 to 40% have been reported in tropical regions (Charchar *et al*., 2003). *Fusarium oxysporum* f. sp. *lycopersici* (Snyder and Hansen) is among the most important soil-borne fungal pathogens that infect tomatoes (Schwarz and Grosch, 2003). Estimated yield losses of 30% to 40% have been reported in tomato crop due to infection by Fusarium wilt (Kirankumar *et al*., 2008).

It has been observed that tomato cultivars vary in their level of susceptibility to *Meloidogyne* spp. Several researchers evaluated degree of tomato plants susceptibility to *M. javanica* based

on reproductive factor (rate of reproduction). Reproductive factor (RF) is calculated as final population divided by the initial population (Irshad *et al*., 2012). A reproductive factor greater than one, is indicative of reproduction while RF of less than one implies no reproduction. Abbas *et al*. (2008) rated tomato cultivars with high reproductive factors as susceptible. Similarly on the basis of reproductive factor, Jaiteh *et al.* (2012) classified tomato cultivars as highly resistant, moderately resistant, and highly tolerant while Esfahani *et al*. (2012) classified them as tolerant and hypersensitive.

Formation of galls on infected roots is a primary symptom to RKN attack. Several workers based the level of susceptibility on the number of galls and galling indices. Khanzada *et al.* (2012) rated tomato cultivars into different levels of susceptibility based on galling indices. Kamran *et al*. (2012) reported different number of galls for all the tested tomato genotypes that were susceptible to *Meloidogyne* spp.

Susceptibility of tomato cultivars to FOL can be evaluated by measuring disease severity and disease incidence. Evaluation of resistance and susceptibility of Jordan tomato cultivars to FOL was determined by scoring for severity indices (Al-khatib *et al*., 2006), while Akram *et al*. (2014) determined susceptibility of tomato varieties to FOL by scoring for wilting symptoms. Characterization of FOL race 3 in Brazil was by scoring for wilting index, after isolates were inoculated onto cultivars that are race differentials having specific resistance gene loci for the three races of FOL (Reis *et al*., 2005).

Fusarium oxysporum f. sp. *lycopersici* and RKN have been known to interact forming a disease complex that causes considerable damage to crops (Bhagawati *et al.*, 2000, 2007). Disease complexes involving nematodes and Fusarium wilt pathogen have been investigated and results from such studies showed varied outcomes. Pandey (2015) reported adverse effects on growth of chickpea, due to concomitant infection with *F. oxysporum* f. sp. *ciceri* and *M. incognita* compared to when the RKN or Fusarium wilt pathogen were inoculated

alone. Other investigations reported breakdown of resistance to Fusarium wilt in tomato cultivars due to infestation with nematodes. Tomato cultivar Viradoro, resistant to FOL race 1 and *M. incognita* became susceptible to *M. incognita* at high soil temperatures as the Mi-gene resistance to *Meloidogyne incognita* was broken. This led to the cultivar becoming infected with *Meloidogyne incognita* that predisposed the host crop to infection by Fusarium wilt caused by the races 1 and 2 of *F. oxysporum* f. sp. *lycopersici* (Moura *et al*., 2007).

Tomato production relies heavily on appropriate management strategies for the control of diseases and pests. One of the methods widely used by the farmers is planting of hybrids with resistance genes to diseases and pests. Tomato cultivar with genes for resistance to Fusarium wilt, Fusarium crown and foot-rot, Verticillium wilt, RKN and Tobacco mosaic virus have been developed (Erb and Rowe, 1992). Resistant varieties are very popular because they are high yielding, despite the high cost of purchasing the seeds. There are many tomato varieties that are resistant to Fusarium wilt pathogen and RKN. These are noted in seed catalogs and variety descriptions as VF (*Verticillium*, *Fusarium*), meaning *Verticillium* and *Fusarium* resistant varieties. Some are resistant to RKN in addition to *Verticillium* and *Fusarium*, and are described as having VFN (*Verticillium, Fusarium*, Nematodes) resistance. It has been observed that tomato cultivars vary in their level of susceptibility to *Meloidogyne* sp. and FOL.

This study was done to test the susceptibility of most commonly grown tomato cultivars; Prostar F1, Kilele F1, Oxly, Cal J, Rambo F1 and Roma VFN, to *M. javanica* and four isolates of Fusarium wilt. The tomato cultivars are popular in tomato production in Mwea West Sub County, Kenya (Mwangi *et al.*, 2015).

Another experiment was carried out to test the interaction of FOL and different inoculums levels of *M. javanica* in two tomato cultivars Rambo F1 and Prostar F1. Rambo F1 was

found to be resistant to FOL isolate F41 while Prostar F1 was found to be very susceptible to it. The two cultivars were very susceptible to a root knot nematode *M. javanica*.

5.2 Materials and Methods

The study was carried out in a greenhouse located at the University of Nairobi, College of Agriculture and Veterinary Sciences (CAVS), Upper Kabete Campus field station. Ambient day temperatures ranged from 25ºC-34ºC in the greenhouse during the period of study. The ambient day temperatures ranged from 21ºC-34ºC in the greenhouse during the period of study for the experiment to test the interaction of FOL and different inoculums levels of *M. javanica* in two tomato cultivars Rambo F1 and Prostar F1.

5.2.1 Identification of *Meloidogyne* species used in the experiments

The initial single egg mass population of *M. javanica* used in these experiments was obtained from Kenyatta University Plant and Microbial Sciences Department. Confirmation of identification was by cuticular markings in the perineal area of a mature female using a modification of a method described by Kariuki *et al.* (2013). The females were teased out of galled roots by use of forceps and a fine needle taking care not to puncture the body of the female. The females were stored in 0.9% sodium chloride solution to avoid osmotic effects of water. They were stored at refrigeration temperatures of 8^0C for a period of one week. They were later taken to Nematological laboratories at International Centre for Insect Physiology and Ecology (ICIPE) Kenya where they were dissected using a modification of a procedure described by Kariuki *et al.* (2013). A pipette was used to transfer one female at a time to a drop of water on a glass slide. The female was cut half way using a sharp scalpel. The anterior containing the head was separated out from the posterior. The inner content of the posterior was removed using two pins and was transferred to another slide with a drop of

glycerine where it was trimmed, covered with a cover glass and observed under x 40 magnification and x100 magnification under oil immersion. Ten females from galled tomato roots were observed in this manner and identified using an identification guide by Eisenback *et al*. (1981).

5.2.2 Planting of tomato seeds

Certified tomato seeds of the cultivars Prostar F1, Kilele F1, Oxly, Cal J Rambo F1 and Roma VFN were sterilized in 1% solution of sodium hypochlorite and were then sown in steam sterilized and cooled soil and sand mixture. Each tomato cultivar was sown in a separate seedling tray. The procedures of preparing the soils and sowing of tomato seeds are as described in section 4.2.3 and 4.2.4.

5.2.3 Preparation and maintenance of second stage juveniles (J2s) inocula

Second stage juveniles (J2s) were extracted from tomato cultivar Cal J roots infested with *Meloidogyne javanica* using a modification of a method described by Coyne *et al.* (2007). The roots were washed and chopped into one centimetre pieces. The root pieces were weighed and water added at the rate of one gram of root to 20 ml water .and put in a domestic blender. They were blended for 15 seconds at high speed. The J2s were extracted from the blended root-water mixture using the modified Baermann tray method (Whitehead and Hemming, 1965). The mixture was placed on a sieve lined with two sheets of serviette paper placed on a plate. Water was poured at the edge, directly into the plate to wet the serviette. After 48 hours the J2s moved from the root-water mixture to into the plate below by gravitation. The suspension obtained was reduced to 10 ml by sieving through a 250 µm and 38 µm sieves. Using a compound microscope (x5 magnification) and a nematode counting

chamber, an estimate of the total J2s was made by taking the average count in one millilitre of the suspension.

In the screening experiment, the J2s concentration was adjusted approximately 1000 J2s inoculum in 30 ml of water. The J2s concentrations were adjusted approximately to 500, 1000, 2000, and 3000, per 30 ml each in the experiment to test the interaction of FOL and different inoculums levels of *M. javanica* in two tomato cultivars Rambo F1 and Prostar F1.

Tomato cultivar Cal J served as a host for maintaining nematode populations. Autoclaved soil and sand mixture, in the ratio 3:1 was put into 10 litre plastic basins. Tomato seedlings were transplanted into the plastic basins. The nematode populations were multiplied by inoculating roots of tomato cultivar Cal J with J2s. The culture of nematodes was maintained throughout the course of study by periodically uprooting the plants and planting new ones at eight week intervals.

5.2.4 Preparation of inoculum for testing the reaction of different tomato cultivars to Fusarium wilt isolates

Four (4) isolates of Fusarium wilt were first grown separately on PDA agar plate at room temperature (24± 2^{o}C) for seven days. The four isolates were coded, F41, F66, F1 and F84. Two cores of fungal mycelia on PDA agar plate were obtained by means of a cork borer (4 mm) from each isolate. These were separately used to inoculate 100 ml of czapek dox broth in a 250 ml conical flask. They were then put on a rotatory shaker (J. P. Selecta, S. B. Made in Spain 564222 S/W) at 50 revolutions per minute (rpm) for a period of one week. Afterwards the mycelium of each isolate was harvested using a sterilized tea strainer and used to separately inoculate 100 g of sterilized sand maize meal medium in 250 ml conical flasks. The media was prepared using a modification of a method described by Nene and Haware (1980). In this method 90 gm sand, 10 g maize meal, and 20 ml distilled water was put in 250 ml conical flasks which were plugged with cotton wool and were then sterilized in an

autoclave at 121^{o}C for 15 minutes. The sterilization was repeated after one day to ensure spores that are resistant germinated to vegetative form and were killed during the sterilization process. The cultures were kept at room temperature (24± 2^{o} C) for two weeks for the fungus to colonize the media in the conical flask. These cultures formed the inoculum that was used to inoculate three kilograms plastic basins measurements of soil: sand (3:1) mixture, in a procedure described by Haggag (2012), where 3% of each fungus culture was separately used to inoculate the three kilograms of sterilized soil: sand mixture. The infested soil was thoroughly mixed up to make it homogenous. Di-ammonium phosphate fertilizer was applied at the rate of six grams per basin and was also thoroughly mixed up. This infested soil was watered daily for seven days to obtain optimum and uniform growth of the fungus before planting (Haggag, 2012).

5.2.5 Preparation of *F. oxysporum f. sp. lycopersici* (FOL) inoculum

The isolate of *F. oxysporum f. sp. lycopersici* (FOL) F41 was grown on PDA for five days at room temperature (24^{o}C ±2). Two PDA cores of fungal growth were obtained by use of a cork borer (4 mm) and were used to inoculate 100 ml czapek dox growth medium put on a rotatory shaker (J. P. Selecta, S. B. made in Spain 564222 S/W) at 50 rpm at room temperature for a period of one week. After one week, the mycelia was harvested by sieving using a sterilized tea strainer and was used to inoculate one kilogram of sterilized sand-maize meal medium (900 gm sand,100 gm maize flour, 200 ml water) in a transparent three kilograms polythene bag. This was a modification of sand-maize meal medium preparation described by Nene and Haware (1980). The fungus colonized the surface within a period of two weeks. One hundred grams of the fungal growth on the surface was scraped using a sterilized spatula. This was mixed with 900 ml of water agar (0.001%) in a one litre media bottle and was shaken vigorously. The suspension was then filtered using a muslin cloth

folded four times. The spore count in the filtrate was estimated by serial dilution of the filtrate and plating. The filtrate was centrifuged at 2250 rpm for 10 min, and the top layer siphoned out by means of a pipette and then adjusted to a concentration of 1×10^7spores per ml by adding sterile distilled water.

5.2.6 Experimental design and application of the treatments

5.2.6.1 Reaction of tomato cultivars to *M. javanica*

Three week old tomato seedlings of the six different tomato cultivars Prostar F1, Kilele F1, Oxly, Cal J, Rambo F1 and Roma VFN F1 were transplanted separately into 0.5 kg plastic pots measuring filled with sterilized sand and soil mixture. Each pot contained one seedling which was inoculated with J2s inoculum of *M. javanica* one day after transplanting. A hole was made at two centimetres near each plant using a plastic spoon and 30 ml of suspension containing J2s inoculum was then dispensed into the hole which was then covered.

The experiment had a treatment for each tomato cultivar where each pot was inoculated with 1000 J2s of *M. javanica* and a control without inoculation. The treatments in each tomato cultivar were replicated four times. The experiment was laid out in a Completely Randomized Design (CBD) in the greenhouse. Plants were watered once every 24 hours and sprayed with fungicides and pesticides once per week to control fungal diseases and pests. The fungicide Metalaxyl-M (Ridomil Gold) was applied at the rate of five grams in two litres of water. Metalaxyl-M is a systemic fungicide against oomycete class of fungi (www.syngenta-us.com). Different pesticides were used interchangeably and these included Thiocyclam SP 50% (Evisect) at the late of one gram per litre of water, and Thiamethoxam (Actara) at the late of 0.8 g per litre of water. The experiment was terminated after nine weeks and was repeated one more time.

5.2.6.2 Susceptibility of tomato cultivars to four isolates of Fusarium wilt of tomato

The experiment was carried out to test the reaction of four Fusarium wilt isolates to tomato cultivars Kilele F1, Cal J, Oxly, Rambo F1, Roma VFN, and Prostar F1. Each of the tomato cultivar was separately inoculated with each of the four isolates. A treatment was made up of a reaction of tomato cultivar with one of the isolates. A control experiment was included for each tomato cultivar. Six seedlings of each tomato cultivar were separately transplanted into the three kilograms plastic basins infested with the four different isolates of the fungus. The control for each of the tomato cultivars were not reacted with any isolate but only sterilised media used for culturing inocula of the Fusarium wilt isolates. The treatments were arranged in a randomised complete block design in a greenhouse. Plants were watered once every 24 hours and sprayed with a fungicide and pesticides once per week to control fungal diseases and pests as in procedures 5.2.6.1. The experiment was terminated after nine weeks and was repeated one more time.

5.2.6.3 Interaction of *F. oxysporum* f. sp. *lycopersici* and different inoculum levels of *M. javanica* in two tomato cultivars Rambo F1 and Prostar F1.

Three week old tomato seedlings of the tomato cultivar Rambo F1 and Prostar F1 were transplanted separately into one kilogram plastic pots filled with sterilized sand: soil mixture (3:1). Each pot had one seedling. The seedlings were each infested with the larvae (J2s) of *M. javanica* at the time of transplanting. A hole was made in the soil near each plant using sterile plastic spoon. The suspension containing J2s was homogenised by blowing using a soda straw and were then dispensed into the holes which were then covered. Inoculation of the FOL isolate was performed by drenching each pot with the fungal suspension, at the rate of $1x10^7$ spores per gram of soil.

The experiment had a total of 10 treatments. Four pots were used as replicates for each of the treatments. The experiment was repeated twice. The treatments for each of the tomato

cultivars were; untreated control, FOL alone, 500N, 1000N, 2000N, 3000N, FOL +500N, FOL+ 1000N, FOL +2000N, FOL + 3000N where (N) represents nematode juveniles. For treatments with a combination of FOL and nematode J2s, the nematode J2s were inoculated two weeks prior to FOL inoculation. The experiment was laid out in a randomized complete block design in the greenhouse. Plants were watered once every 24 hours and sprayed with a fungicide and pesticides once per week to control fungal diseases and pests as in procedure in section 5.2.6.1. The experiment was terminated 10 weeks and was repeated once.

5.2.7 Data collection

The plant height, dry weight of shoot and root were recorded for each replicate in all the treatments where the tomato cultivars were inoculated with *M. javanica*. The height measured in centimetres was the length from the top of the shoot to the base of each plant. The root for each plant was separated from the main plant and was washed under water and dabbed dry with blotting paper. In order to obtain the dry weight of the shoots and roots, each was put in a separate envelope and oven dried to constant weight at 70^{o}C for 48 hours. The root dry weight was obtained after assessing the roots for galling and extraction of second stage juveniles.

Roots from treatments inoculated with nematodes were stained with cold eosin yellow (0.1 g L^{-1} of water) for 30 minutes to facilitate the counting of galls (Jacquet *et al*., 2005). Gall indices (GI) were measured according to a scale by Taylor and Sasser (1978) using the following values: O = zero gall; 1 =1 or 2 galls; 2 = 3 to 10 galls; 3 = 11 to 30 galls; 4 = 31 to 100 galls and 5 $\geq$ 100 galls per root system. Disease severity was assessed by modification of a wilting index by Akram *et al*. (2014). The severity scale 1 represent no symptom, 2; wilting and yellowing covered less than 25%, 3; wilting and yellowing was less than 50%, 4; wilting and yellowing was equal to or more than 50% . Disease incidence was calculated by getting

the number of infected plants divided by the total number of plants initially inoculated with FOL. Scoring for disease severity was done at the time of harvesting.

5.2.7.1 Extraction of juveniles from the roots

Second stage Juveniles (J2s) were extracted from infested tomato roots after termination of experiment using modified Baermann tray method (Whitehead and Hemming, 1965). Infected tomato roots were washed, chopped into one centimetre pieces and each was separately placed on a sieve lined with two sheets of serviette paper placed on a plate. Water was poured at the edge, directly into the plate to wet the serviette. The J2s moved from the root-water mixture into the plate below by gravitation. After seven days the extract was obtained and was reduced to 10 ml by sieving through a 250 µm and 38 µm sieves. An aliquot of one millilitre from the suspension was put in a counting dish and counting made with aid of a compound microscope (x 5 magnification). To estimate the total count of J2s per root system, the average count in one millilitre of the suspension was made and this was multiplied with the total amount of the concentrate in order to obtain the total number of J2s per root system.

5.2.7.2 Data analysis

Data collected was analysed using the GenStat statistical package (Discovery Edition 14). Where the ANOVA indicated significant treatment effects, means were compared using L.S.D at 5% level of significance. The percentage reductions in growth parameters compared to control were calculated (Irshad *et al.*, 2012).

5.3 Results

5.3.1 Identification of *Meloidogyne* species

Meloidogyne species used in the experiments were identified by the perineal patterns of the mature females. The nematode species had the characteristic lateral lines that separate the dorsal striae from the ventral striae and a low dorsal arch with a nearly flattened top and was therefore identified as *Meloidogyne javanica* (Plate 5.1)

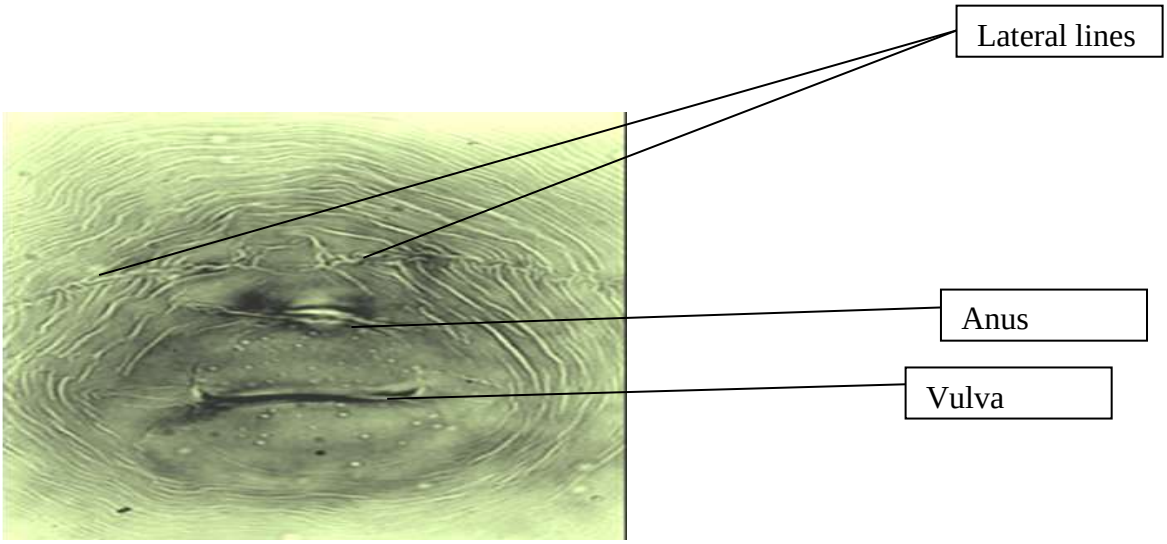


Plate 5.1 Perineal pattern of *M. javanica* x40 magnification

5.3.2 Reaction of tomato cultivars to *M. javanica* and the effect of the infestation on growth

All roots of the different tomato cultivars had galls. However, the cultivars Rambo F1, Roma VFN and Prostar F1 had significantly ($P<0.05$) higher galling indices than the cultivars Cal J, Kilele F1 and Oxly (Table 5.1). There were significance differences ($P<0.05$) in the reproductive factor of *M. javanica* on the tomato cultivars. *Meloidogyne javanica* had the highest reproductive factor in the tomato cultivars Prostar F1 and Rambo F1 at 5.98 and 5.97 respectively while in the tomato cultivar Oxly, *M. javanica* the had the least reproductive factor of 1.0 (Table 5. 1).

There were significant (P<0.05) differences in the mean dry shoot weight of tomato cultivars that were inoculated with *M. javanica* and also reductions in weight of inoculated cultivars compared to un-inoculated ones (Table 5.2). The greatest percentage reduction in the dry shoot weight was recorded in tomato cultivars Roma VFN (30.18%) and Prostar F1 (23.23 %). Meanwhile, cultivar Kilele F1 had the lowest reduction (0.54%) in the dry shoot weight, while cultivar Oxly had a slight increase (7.64%) in shoot dry weight, compared to the un-inoculated (control) tomato cultivars (Table 5.2).

There were significant differences (P<0.05) in the mean dry root weight of cultivars inoculated with *M. javanica* and also reductions in dry root weights of the cultivars inoculated with *M. javanica* compared to the un-inoculated ones (Table 5.2). The cultivar Oxly had the highest mean dry root weights and also the least reductions in the root dry weights (0.86%), due to inoculation with *M. javanica.* It was observed that there was a reduction in height of tomato cultivars inoculated with *M. javanica* compared to un-inoculated ones, with the exception of tomato cultivar Kilele F1 (Table 5.2). The height in the cultivars Kilele F1 and Oxly were least affected by the *M. javanica* inoculation.

Table 5.1 Reproduction factor, number of galls and juvenile populations per root system induced by treatment with *M. javanica* under greenhouse conditions on six tomato cultivars

Cultivar	**Galling index**	***M. javanica* J2s**	**Reproductive factor**
Cal J	2.3b	3416c	3.41c
Kilele F1	2.0b	2732b	2.73b
Oxly	1.5ab	1181a	1.18a
Prostar F1	3.5cd	5989e	5.98e
Rambo F1	4.00d	5971e	5.97e
Roma VFN	3.00bc	3844d	3.84d
L.S.D (P=0.05)	0.73	341	0.34
C.V%	14.7	5.0	5.0

Data are means of four replications. Means followed by the same letter along the same column are not significantly (P < 0.05) different according to Fisher's L.S.D test.

Table 5.2 Effect of infestation with *M. javanica* on the dry weight of shoot and root in different tomato cultivars

	Dry shoot weight		Dry root weight		Height	
Cultivar	**Un-inoculated**	**Inoculated**	**Un-inoculated**	**Inoculated**	**Un-inoculated**	**Inoculated**
Cal J	4.4a	4.11b (6.59)	3.45b	3.23b (6.47)	61.2a	53.27a (12.9)
Kilele F1	5.59b	5.56d (0.54)	3.4b	3.23b (5.0)	63.1a	65.4d (-3.65)
Oxly	4.71ab	5.07cd (-7.64)	3.48b	3.45b (0.86)	58.6a	58.2bc (0.68)
Prostar F1	5.08ab	3.90ab (23.23)	2.6a	2.4a (7.69)	62.7a	61.3cd (2.23)
Rambo F1	5.22ab	4.84c (7.3)	2.75a	2.3a (16.36)	68a	65.4d (3.82)
Roma VFN	4.87ab	3.4a (30.18)	2.45a	2.18a (11.02)	53.8a	50.3a (6.5)
L.S.D (P=0.05)	1.03	0.63	0.6	0.52	9.15	5.43
CV%	13.9	9.6	13.4	12.4	10.1	7.6

Data are means of four replications. Means followed by the same letter in the same column are not significantly (P<0.05) different according to Fisher's L.S.D test. Figures in parenthesis represent percent reductions in growth compared to the un-inoculated control

5.3.3 Reaction of different tomato cultivars to four isolates of Fusarium wilt

Isolate F41 had high disease severity and incidence scores in most of the cultivars. The severity scores of this isolate were; 3.83, 3.5, 2.67, and 2.5 for the cultivars, Prostar F1, Roma VFN, Cal J and Oxly respectively (Table 5.3). These cultivars could be considered susceptible to isolate F41, while the cultivars Kilele F1 and Rambo F1 had low severity scores of 1.67 and 1.17, respectively from the same isolate. Isolate F66 had high severity scores on cultivars Prostar F1, Roma VFN, Cal J and Kilele F1 while cultivars Oxly and Rambo F1, had low severity scores to the same isolate. Overall, isolate F41 had high disease severity and incidence scores on the tomato cultivars. Prostar F1 was the most susceptible cultivar to the isolate F41 while cultivar Rambo F1 was the least susceptible (Table 5.3).

Table 5.3 Reaction of six tomato cultivars to four Fusarium wilt isolates under greenhouse conditions

Isolate	F41		F66		F1		F84	
	D.S	D.I (%)	D.S	D.I (%)	D.S	D.I (%)	D.S	D.I (%)
Cultivar								
Prostar F1	3.83d	100e	2.5b	61.7c	1.33a	27.8b	1.00a	11.1a
Roma VFN	3.5cd	100e	2.33b	61.3c	1.00a	0a	1.67a	44.3b
Cal J	2.67bcd	77.7d	2.33b	38.7b	1.67a	41.4a	1.67a	38.8b
Kilele F1	1.67ab	38.7b	2.5b	94.4d	1.67a	27.8b	1.00a	0a
Oxly	2.5bc	55.6c	1.0a	11.3a	1.00a	0a	2.17a	38.8b
Rambo F1	1.17a	16.7a	1.00a	0a	1.17a	11.1a	1.00a	0a
L.S.D	1.21	12.17	1.13	15.67	0.83	13.97	0.95	14.9
C.V%	40	10.6	47.6	19.8	53.9	42.4	56.9	35.7

Data are mean of six replicates. Means followed by the same letter along the same column are not significantly ($P<0.05$) different according to Fisher's L.S.D test. D.I: disease incidence. D.S: disease severity.

5.3.4 Effect of *F. oxysporum* f. sp. *lycopersici* infection and different *M. javanica* inoculum levels on galling index, nematode populations in the roots and disease severity in the tomato cultivars

The higher nematode inoculum levels of 2000 and 3000 J2s produced significantly ($P < 0.05$) higher galling index and increased juvenile populations in the roots, compared to the 500 J2s inoculum level in both tomato cultivars Prostar F1 and Rambo F1 in the presence or absence of FOL (Table 5.4). In the experiment on cultivar Prostar F1, disease severity at higher nematode inoculum levels of 3000 J2s and 2000 J2s was significantly ($P<0.5$) different from the Fusarium alone (Fig. 5.2 and Table 5.5). As nematode inoculum levels increased, disease severity also progressively increased in tomato cultivar Prostar F1. The wilt resistant cultivar Rambo F1 did not show any wilt symptoms when exposed to FOL alone treatment, and even at increased nematode inoculum levels the Fusarium wilt disease was insignificant (Table 5.5).

Table 5.4 Number of galls and juvenile populations in the roots in two tomato cultivars in different treatments of *M. javanica* in combination with FOL under greenhouse conditions

Treatment	Prostar F1		Rambo F1	
	Galling index	***M. javanica* J2s**	**Gall index**	***M. javanica* J2s**
500 N	1.75a	1445 a	1.75a	1438a
1000N	2.5ab	3290 b	2.75b	2966c
2000N	3.25b	4350 de	3.0b	3781de
3000N	3.0b	4040 cd	3.0b	3659d
500N+FOL	1.75a	1576 a	1.75a	1762b
1000 +FOL	3.0b	3910 c	2.75b	3623d
2000+FOL	3.25b	4628 ef	3.0b	3872e
3000+FOL	3.25b	4874 f	3.0b	3710de
L.S.D (P=0.05)	0.77	345	0.65	203
C.V%	19.5	6.7	17.6	4.5

Data are means of four replications. Means followed by the same letter along the same column are not significantly (P < 0.05) different according to Fisher's L.S.D test

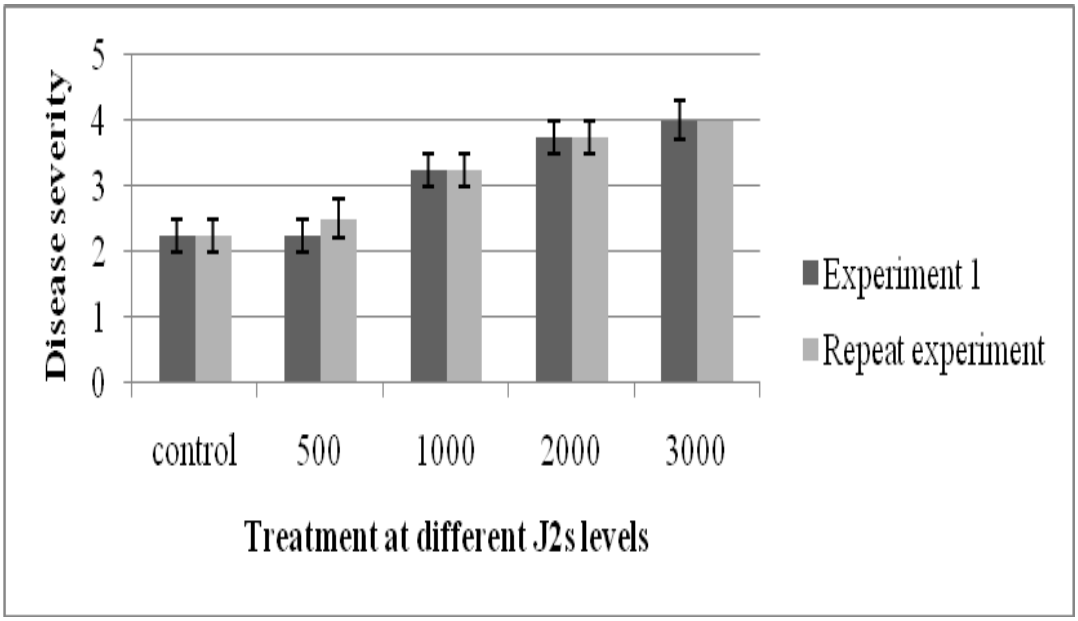


Fig .5.2 Effect of different nematode inoculum levels on disease severity in cultivar Prostar F1 under greenhouse conditions. Means were compared using LSD=0.802 for first experiment and LSD= 0.6453 in repeat experiment. The I bars signify 5% error. [Disease severity scale by Waudo *et al.*, (1995) Appendix 4].

Table 5.5 Effect of FOL and different inoculum levels of *M. javanica* on disease severity in tomato cultivars Prostar F1 and Rambo F1 under greenhouse conditions

Treatment	**Disease severity**	
	Prostar F1	**Rambo F1**
FOL	2.25a	1.0a
500 + FOL	2.25a	1.0a
1000 + FOL	3.00b	1.25a
2000 + FOL	3.5bc	1.5a
3000 + FOL	4.0c	1.5a
L.S.D (P=0.05)	0.68	0.66
C.V %	13.6	34.3

Data are means of four replications. Means followed by the same letter along the same column are not significantly (P < 0.05) different according to Fisher's L.S.D test.

5.3.5 Effect of inoculating *F. oxysporum* f. sp. *lycopersici* and different inoculum levels of *M. javanica* on plant growth parameters in cultivars Prostar F1 and Rambo F1

In tomato cultivar Prostar F1, the mean plant height was significantly (P < 0.05) reduced at all nematode levels compared to the controls (Table 5.6). The dry shoot weights were significantly reduced at all nematode levels of 1000 J2s, 2000 J2s and 3000 J2s inoculum levels, with the greatest reduction at 3000 J2s nematode inoculum combined with FOL. The dry root weights were significantly reduced at all nematode levels of 3000 J2s, but also at lower levels of 1000 J2s and 2000 J2s when combined with FOL (Table 5.6). The dry root weights at nematode inoculum levels of 1000 J2s, 2000 J2s and 3000 J2s were significantly lower in the presence of FOL than their respective treatments with nematodes in the absence of FOL.

In cultivar Rambo F1, the treatments at all nematode inoculum levels of 2000 to 3000 J2s, and also at lower levels of 1000 J2s in the presence of FOL caused significant (P < 0.05) reductions in mean plant height compared to the untreated controls (Table 5.6). Dry shoot

weights were significantly (P<0.05) different from the controls only at J2s levels of 1000, 2000 and 3000 in the presence of FOL, while dry root weights were significantly lower from the untreated control at all nematode inoculum levels of 500 to 3000 J2s. There were no significant differences in cultivar Rambo F1 in the plant heights, dry shoot and root weights in Fusarium only treatments compared to the control (Table 5.6).

Table 5.6 Effect of inoculation with FOL and different inoculum levels of *M. javanica* on plant growth parameters in cultivars Prostar F1 and Rambo F1

	Prostar F1			**Rambo F1**		
Treatment	**Height (cm)**	**Dsw (g)**	**Drw (g)**	**Height (cm)**	**Dsw (g)**	**Drw (g)**
Untreated	81.88e	14.82f	2.96g	77.5e	11.36cd	2.7f
FOL	78e	11.96e	2.65efg	77.75de (-	10.81cd	2.7f
	(4.7)	(19.3)	(10.4)	0.32)	(4.84)	(0)
500J2s	68.8bcd	11.5de	2.48def	82e	13e	2.28e
	(16)	(22.7)	(16.2)	(-5.8)	(14.4)	(15.6)
1000J2s	72.5d	10.69cd	2.7fg	74.38cd	10.46bc	2.2cde
	(11.5)	(27.9)	(8.7)	(4.02)	(7.29)	(18.5)
2000J2s	64.3ab	10.05bc	2.48def	71.75bc	10.72bc	2.05bcde
	(21.5)	(32.2)	(16.2)	(7.4)	(5.63)	(24.1)
3000J2s	66.6bc	9.53b	2.2bcd	67.9ab	10.84cd	1.8ab
	(18.7)	(35.7)	(25.7)	(12.4)	(4.6)	(33.3)
500J2s+ FOL	69.5cd	11.32cde	2.3de	76cd	10.63bc	2.2cde
	(15.1)	(23.6)	(22.3)	(1.93)	(6.4)	(18.5)
1000J2s+	66.88bc	10.3bcd	1.9abc	70.75bc	9.79ab	2.0abc
FOL	(18.3)	(30.5)	(35.8)	(8.7)	(13.8)	(25.9)
2000J2s+	61.3a	9.87b	1.93abc	66.5ab	8.91a	1.93abc
FOL	(25.1)	(33.4)	(34.8)	(14.2)	(21.6)	(28.5)
3000J2s+	65.12abc	7.9a	1.85a	64.88a	8.93a	1.7a
FOL	(20.5)	(46.6)	(37.5)	(16.3)	(21.3)	(37)
L.S.D(P=0.05)	4.6	1.2	0.32	5.5	1.01	0.3
C.V%	4.7	7.7	9.5	5.2	6.6	10

Data are means of four replications. Means followed by the same letter in the same column are not significantly (P < 0.05) different according to Fisher's L.S.D test. DSW =dry shoot weight, DRW=dry root weight. Figures in parenthesis represent percent reductions in growth compared to the untreated control.

5.4 Discussion

High levels of root galling and high RKN reproductive factors for the cultivars, Rambo F1, Prostar F1, Roma VFN, and Cal J are evidence that the tested tomato cultivars were good hosts for *M. javanica*, while the cultivars Kilele F1 and Oxly with low galling indices were less susceptible. The variation in the susceptibility of the six tomato cultivars to *M. javanica* might be due to genetic differences in the cultivars. Castagnone- Sereno (2006) and Jacquet *et al*. (2005) reported that the level of susceptibility to *Meloidogyne* spp. is controlled by the tomato genotype. The tomato cultivars Rambo F1, Prostar F1, Cal J, Oxly and Roma VFN were susceptible to RKN. All resistance to nematodes in *M. arenaria*, *M. javanica* and *M. incognita* is conferred by the Mi-gene and this has been the source of resistance for many years (Roberts and Thomason 1989 as cited by Verdejo-lucas *et al*., 2009). The cultivar Roma VFN was very susceptible to the nematode populations despite having the nematode resistance genes. The high temperatures (25^{o}C-34^{o}C) in the greenhouse could have rendered the Mi-gene ineffective. Kolashian *et al.* (1996) reported that temperatures above 28^{o}C make the Mi-gene ineffective. The Mi-gene for resistance to RKN is same in all cultivars. The repeated cultivation of Mi-resistant gene cultivars may lead to selection of virulent nematode populations that overcome the nematode resistance genes. Different levels of susceptibility to root-knot nematodes based on root galling indices and reproductive factor have been reported by several authors (Yazdi *et al*., 2012; Khanzada *et al*., 2012; Jaiteh *et al.,* 2012; Esfahani *et al*., 2012).

Percentage reductions in growth were observed between inoculated compared to un-inoculated plants. It was evident that cultivars with higher nematode infestations had greater reduction in growth parameters compared to least susceptible cultivars. Many authors have reported reduction in growth due to infestation by RKN (Esfahani and Pour, 2006; Okporie *et al*., 2014; Hussain *et al*., 2015). Severe root galling and arrested root development interferes

with absorption of mineral salts and water (Ahmed *et al*., 2009) and this reduces growth in plants.

All the cultivars showed different levels of disease severity and incidence in their reaction to the four different isolates of fusarium wilt pathogens. There was distinct variability in virulence of the isolates in their reaction with the six different cultivars. The isolate F41 and F66 were the most virulent, while the isolates F1 and F86 were less virulent to the tested tomato cultivars. Isolates F41 and F66 were identified as those of FOL while F1 and F84 were identified as *Fusarium Fusarium verticillioides*. Therefore in this study, though isolates of *F. Fusarium verticillioides* were identified as being pathogenic to the tomato cultivars, they were less virulent than those of FOL.

Tomato cultivar Rambo F1 was found to be most resistant against F41, F66, F1 and F86. When such resistance is available, it should be used within the context of an integrated management program to lessen the selection pressure of FOL against it. Use of resistant cultivars is effective method in controlling RKN and Fusarium wilt disease (Fesenko *et al*., 2013; Oliveira *et al*., 2013).

It was evident that in the cultivar Prostar F1, inoculation with *M. javanica* brought about greater wilt disease severity with increasing nematode inoculum levels unlike in the tomato cultivar Rambo F1, where resistant to FOL remained high or unchanged even at increasing nematode inoculum levels. This is unlike tomato cultivar Viradoro, where resistance to FOL was broken in the presence RKN nematode *Meloidogyne incognita* (Moura *et al.,* 2007). Other researchers have reported that infection with *Meloidogyne* spp. increases the severity of the Fusarium wilt in tomato plants. The severity of Fusarium wilt disease was increased in tomato by *M. incognita* (Chindo *et al*. 2010), while Jain and Jitendra (2010) observed that there was an interaction between *M. incognita* and FOL when the RKN was inoculated 10 days prior to the fungus, resulting in early expression of wilt symptoms in tomato. Bhagawati

et al. (2000) suggested that increase in severity of wilt in experiments of RKN and Fusarium wilt pathogen could be because nematodes provide entry points for fungus infection. Meena *et al.* (2016) proposed that the formation of giant cells by the host due to infection by RKN could be the reason for the nematode to predispose plants to fungal attack, as they become a source of nutrients to the fungus. The nutrient rich nematode infected cells enhance the growth of Fusarium hyphae. Population levels of *M. javanica* and number of galls increased progressively in the roots of the two tomato cultivars irrespective of infection by FOL. Okporie *et al.* (2014) also observed an increase in galling index with increase in nematode inoculum densities in two different tomato varieties.

There were greater reductions in growth of plants with increasing nematode inoculum levels in both tomato cultivars. Similarly, Okporie *et al*. (2014) reported a decrease in the mean root length, percentage dry weight, shoot dry matter and length, with increased inoculum density. Hussain *et al*. (2015) recorded minimum reduction in shoot length and shoot weight in eggplant (*Solanum melongena*) at 500 J2s inoculum, while maximum reduction was at 2000 J2s inoculum levels. Similarly, Maleita *et al*. (2012) reported that damage caused by increasing nematodes inoculum levels resulted in a decrease in plant growth in the varieties Money Maker and Easypeel irrespective of the nematode species. Animaka (2015) observed insignificant decrease in plants weights even at higher nematode inoculum levels even if the plants were resistant to nematodes. Generally, when inoculum levels of nematodes increase, greater number of juveniles are able to infect the plant roots (Okporie *et al.*, 2014) which results in reduced nutrient and water uptake by the roots and consequently reduced plant growth (Singh, 2007). It was evident in this research that greater reductions in growth parameters were higher in root-knot nematodes and FOL inoculated plants as compared to nematode only inoculations indicating, there was an interaction between RKN and FOL that brought about increased reduction in growth. These reductions in growth were greater in the

FOL susceptible Prostar F1 tomato cultivar compared to resistant Rambo F1 tomato cultivar. In this Rambo F1 cultivar though resistant to the isolate of FOL, the interaction brought about increased reduction in the dry shoot weights of FOL-RKN inoculated plants compared to RKN only inoculations at high nematode inoculum levels. Lobna *et al.* (2016) reported synergistic effects of inoculation of root-knot nematodes and Fusarium wilt pathogen by significant reduction in fresh shoot and dry shoot weight in a susceptible and resistant tomato cultivar.

Increased inoculum levels of *M. javanica* in the presence of FOL resulted in progressive reduction in the height, shoot and root dry weight in the both tomato cultivars Prostar F1 and Rambo F1. However the reduction in growth was less in the cultivar Rambo F1 which was resistant to the FOL isolate. Prostar F1 is an example of a cultivar where interaction with FOL at higher nematode inoculum levels increased the severity of the wilt disease and increased reduction in height, dry shoot and root weights, while in Rambo F1, though resistance to Fusarium wilt was not broken, there was an interaction that brought about decrease in shoot dry weights in FOL-RKN inoculated plants compared to plants treated with RKN only at higher nematode inoculum levels. There were different levels of susceptibility to *M. javanica* and isolate of FOL in the different tomato cultivars. It is important to use cultivars that are resistant to FOL in order to reduce the possibilities of formation of a disease complex.

CHAPTER SIX

MANAGEMENT OF FUSARIUM WILT AND ROOT-KNOT NEMATODES DISEASE COMPLEX BY USE OF ANTAGONISTIC FUNGI, PLANT RESISTANCE AND ORGANIC AMENDMENTS

Abstract

Simultaneous crop infestation by root-knot nematodes (RKN) and infection by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) in tomato leads to formation of a disease complex that increases yield losses that are higher than the effect of either RKN or FOL alone. In this study effectiveness of an integrated management programme involving plant resistance, *Purpureocillium lilacinum* (PL), *Trichoderma harzianum* (TH) and neem an organic amendment, was carried out to manage RKN and Fusarium wilt disease complex and was compared to single applications of each of the components. *In vitro* dual culture plates were set up to test the interaction of biological control agents and their antagonism towards FOL. Greenhouse experiments were conducted using two tomato cultivars Rambo F1 and Prostar F1 where each was inoculated with 2000 *M. javanica* J2s and FOL and the following treatments applied; PL, TH, PL-TH, PL neem, TH neem, neem, PL-TH neem, a positive control where a systematic fungicide carbendazim was applied and a negative control where only *M. javanica* J2s and FOL were inoculated. The treatments PL neem, TH neem, PL-TH neem, neem, and the controls were also done in non sterile soils in cultivar Prostar F1. This was to test the efficacy of biological control agents in non sterile soil conditions. Each treatment was replicated four times and the treatments set up in a randomized complete block design in the greenhouse. The experiments were terminated after 11 weeks. Inhibition of FOL mycelia growth by *T. harzianum* and *P. lilacinum* was 51.9%, and 44% respectively by the ninth day in the *in vitro* culture plates. In the cultivar Prostar F1, the treatments PL-TH, PL, and TH had an FOL disease severity score and gall index that was significantly lower than

the untreated control. The resistance of the cultivar Rambo F1 sufficed to prevent infection by FOL. There was evidence that the treatments PL-TH, PL and TH reduced FOL propagules in the roots and soil and performed even better when combined with neem in both tomato cultivars. Results showed that a resistant host combined with biological control agents and organic amendments can effectively control RKN and FOL. Neem significantly decreased FOL disease severity and reduced the total population of *Meloidogyne javanica* J2s when applied combined with the biological control agents. There were no significant ($P<0.05$) differences in Fusarium wilt control in sterile and non sterile soils in the treatments, PL neem, TH neem and PL-TH neem, but there was significantly ($P<0.05$) higher control of *M. javanica* in the same treatments in sterile soils compared to non sterile soils. Therefore the biological control agents and neem have potential to be used in the control of FOL and RKN in non sterile, natural soil conditions although they were found to be less effective in the control of the RKN *M. javanica*.

6.1 Introduction

Vascular wilt of tomato causes yield losses in tomato crop and the losses are further increased by mixed infections with root-knot nematodes (RKN). The interaction of the two pathogens lead to formation of a disease complex that requires a well planned management strategy. Resistance of pests and pathogens to chemical control, environmental pollution and hazardous effects on human beings has brought a shift in the control of pests and plant pathogens from reliance on chemicals to alternative safer methods (Singh, 2002). The approach in this study involves incorporating compatible management strategies with different modes of actions on the pathogens. Such management options include biological control, organic amendments, and host resistance. In the management of Fusarium wilt and RKN disease complex, some of the most researched management options include organic

amendments, biological control and improved or resistant host varieties (Nagesh *et al*., 2006 ; Srivastava and Tewari, 2011; Fesenko *et al*., 2013; Mantelin *et al*., 2013). Biological control agents have been found to be inconsistent and their effect do not persist for a long time (Agbenin, 2011). To overcome the problem of persistence and inconsistencies, application of two or more biological control agents has been recommended (Meyers and Roberts, 2002). The application of different compatible disease suppressive microorganisms to mimic the natural scenario may improve on biological control.

Among the most researched biological control agents are fungal agents like *Paecilomyces lilacinus* (Thom) Samson syn. *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson (Luangsa-ard *et al*., 2011). *Paecilomyces lilacinus* a saprophytic fungus found in soil has good potential for the control of RKN by parasitizing their eggs (Jatala, 1986). It parasitizes female nematodes, reduces hatching of nematode eggs and reduces the number of juveniles in the soil (Pau *et al.,* 2012). It has been widely used in greenhouses to control nematodes in tomato (Schenck, 2004).

Trichoderma harzianum has also been widely used in the control of plant pathogens and nematodes. The effect of *Trichoderma harzianum* in parasitizing eggs (Khattak and Stephen, 2008; Naserinasab *et al.,* 2011) and J2s of root-knot nematodes (Dababat and Sikora, 2007), and inhibiting fungal pathogens have been reported by several authors (Mwangi, *et al*., 2011; Rehman *et al.,* 2012; El-mohamedy *et al.,* 2014; Selvakumar *et al*., 2014; Fatima *et al*., 2015).

Animal manures and other organic amendments such as composts have been known to increase agricultural productivity and improve plant health (Bonilla *et al.*, 2012). It has been hypothesized that organic amendments provide substrates for growth of beneficial microorganisms that in turn assist in rapid decomposition of the amendments, releasing chemical substances that are suppressive to plant parasitic nematodes and disease causing

pathogens, at the same time releasing nutrients for the plants (Renčo, 2013). Neem seed cake is known to have anti-nematicidal, anti-bacterial and anti-fungal properties and also provides macro nutrients essential for general plant growth (Lokanadhan *et al*., 2012).

Use of resistant cultivars is the most effective method of controlling Fusarium wilt disease (Fesenko *et al*., 2013; Oliveira *et al*., 2013). Tomato cultivars that are resistant to RKN have the Mi gene, that confers resistance to *Meloidogyne incognita, M. javanica* and *M. arenaria* (Roberts and Thomason 1989 as cited by Verdejo-lucas *et al*., 2009). Host resistance should be used together with other management options to slow down the rate at which resistance to Fusarium wilt pathogen and RKN develop.

This study was carried out to investigate the effect of using disease resistance, biological control and neem in the management of RKN (*Meloidogyne javanica*) and FOL wilt pathogen.

6.2 Materials and Methods

The study was carried out in a greenhouse located at the University of Nairobi, College of Agriculture and Veterinary Sciences (CAVS), Upper Kabete Campus field station. Ambient day temperatures ranged from 21^{o}C-34^{o}C in the greenhouse during the period of study. *In vitro* tests were carried out in the Health and Applied Sciences (HAS) laboratories at Nairobi Technical Training Institute.

6.2.1 Interaction of biocontrol fungi and *F. oxysporum* f. sp. *lycopersici* in dual culture plates

The interaction between the fungi *Fusarium oxysporum* f. sp. *lycopersici* (FOL), *Purpureocillium lilacinum* (PL) and *Trichoderma harzianum* (TH) was studied by dual culture techniques under *in vitro* conditions. Five day old culture plates of FOL, PL and TH were used in these experiments. To study the antagonistic activity between FOL and PL; a

plate of PDA was inoculated with five millimeter mycelia disc of FOL. The fungal disc was placed 10 mm from the edge of a 90 mm petri-dish. A five millimeter mycelia disc of PL was placed at the opposite side of the petri-dish. To study antagonism between FOL fungi and TH, a five millimeter mycelia plug of FOL was placed 10 mm from the end of a petri-dish while a five millimeter mycelia plug of TH was placed on the opposite end. The latter were placed after three days because of the very high growth rate of the fungus. The FOL fungus was then placed separately on a PDA agar plate as control. Each set was then replicated four times and were set in a completely randomized design on the laboratory bench. The culture plates were incubated at 24± 2^{o}C for 12 days. Colony growth of FOL fungi was observed and radial growth of FOL towards the biocontrol fungi in the dual culture plates recorded every day.

To test for compatibilities between PL and TH, a five millimeter mycelia plug of PL was placed 10 mm from the edge of a Petri-dish while a five millimeter mycelia plug of TH was placed on the opposite end. Mycelia discs of TH were placed after three days because of its very high growth rate. A five millimeter mycelia plug of PL and TH was then placed separately on a PDA plate as controls. Each set was then replicated four times and were set in a completely randomized design on the laboratory bench. The culture plates were incubated at 24± 2^{o}C for 12 days. Data was collected by measuring radial growth of biocontrol fungi in the dual culture plates and in the controls just as in the FOL/biocontrol fungi experiment.

6.2. 2 Planting of tomato seeds

Certified tomato seeds of the cultivars Prostar F1 and Rambo F1 were planted separately in seedling trays. The procedure for planting of the seeds is as described in procedure 4.2.4. The two cultivars here selected because Rambo F1 was found to be very resistant to FOL isolate F41 in a previous experiment, while Prostar F1 was found to be very susceptible.

6.2.3 Preparation of *F. oxysporum f. sp. lycopersici* (FOL) inoculum

The isolate of FOL, F41 was grown on PDA for five days at room temperature (24^{o}C ±2). Two PDA cores of fungal growth were obtained by use of a cork borer (4 mm) and were used to inoculate 100 ml czapek dox growth media put on a rotatory shaker (J. P. Selecta, S. B. Made in Spain 564222 S/W) at 50 rpm, and at room temperature for a period of one week. After one week, the mycelium were harvested by sieving using a sterilized tea strainer and was used to inoculate one kilogram of sterilized sand-maize meal medium (900 g sand, 100 g maize flour, 200 ml water) in a transparent three kilograms polythene bag. This was a modification of sand-maize meal medium preparation described by Nene and Haware (1980). The fungus colonized the surface within a period of two weeks. One hundred grams of FOL fungal growth from the surface was scraped using a sterilized spatula and was suspended in 900 ml of water agar (0.001%) as described in procedure 5.2.5.

6.2.4 Source and production of *P. lilacinum*, *T. harzianum* inocula

Paecilomyces lilacinus was obtained from a commercial product (Bio-Nematon 1.15WP by Osho chemical industries Ltd.). One gram of the product was dissolved in nine milliliter of dilution media (0.001% of water agar). The 10^{-4} dilution was cultured on a PDA agar plate and was incubated for seven days at room temperature (24^{0}C ±2). A colony of the fungus was purified by the single spore technique. Using a sterilized needle, a portion of the fungus was put on a slide and emulsified using sterile distilled water. A pure colony of the fungus was obtained by streaking a suspension of spores on a water agar plate and after incubation for 48 hrs a hyphal thread of the fungus was subcultured on a PDA plate for one week at room temperature. Two PDA cores of fungal growth were obtained by use of a cork borer (4 mm) and were used to inoculate 100 ml czapek dox growth media put in a rotatory shaker (J. P. Selecta, S. B. made in Spain 564222 S/W) at 50 rpm and at room temperature for a period of

one week. After one week, the mycelia were harvested by sieving using a sterilized tea strainer and were used to inoculate one kilogram of sterilized sand-maize meal medium (900 gm sand, 100 gm maize flour, 200 ml water) in a transparent three kilograms polythene bag (Appendix 6). This was a modification of sand-maize meal medium preparation described by Nene and Haware (1980). The fungus colonized the surface within a period of two weeks (Appendix 6).

One hundred (100) grams of PL fungal growth on the surface was scraped using a sterilized spatula. This was mixed with 900 ml of dilution media (0.001% of water agar) in a one litre media bottle and was shaken vigorously. The suspension was then filtered using a muslin cloth folded four times. The count of active propagules in the filtrate was estimated by serial dilution of the filtrate and plating. The filtrate was centrifuged at 2250 rpm for 10 min, and the top layer siphoned out by means of a pipette and then adjusted to a concentration of 1×10^7spores per ml by adding sterile distilled water. Inoculation was performed by drenching each pot with the fungal suspension, at the rate of $1x10^7$ spores per gram of soil. The *T. harzianum (*Trianum, $1x10^9$ by Koppert Biological Systems) was applied as a drench by dissolving 2.5 g / in 250 ml of sterile distilled water per treatment of *T. harzianum.*

6.2.5 Preparation of the J2s and inoculation of tomato plants

Second stage juveniles (J2s) were extracted from infested tomato roots of *M. javanica* using a modification of method of extraction described by Coyne *et al.* (2007). The inoculum of the J2s was prepared as in the procedure described in section 5.2.3. The seedlings were each infested with J2s of *M. javanica* one day after transplanting. A hole was made two centimetres near each plant using a plastic table spoon. Thirty millilitres of suspension contained 2000 juveniles was dispensed into the holes which were then covered.

6.2.6 Experimental design and treatment application

Three week old tomato seedlings of the tomato cultivars Rambo F1 and Prostar F1 were transplanted separately into one kilogram plastic pots filled with sterilized sand and soil mixture. Each pot had one plant. The soil sand mixture was prepared as in procedure described in section 4.2.3. The FOL pathogen and *M. javanica* J2s were inoculated as described in section 6.2.3 and 6.2.5 respectively. The treatments were applied four days after inoculating with FOL inoculum and *M. javanica* juveniles (Kamali, *et al.*, 2015).

The treatments were; PL, TH, PL-TH, PL neem, TH neem, neem, PL-TH neem. The neem cake powder had ingredients as indicated in appendix 5. It had a Carbon/Nitrogen ratio greater than 19 (>19) and was applied at the rate of 20 g per plant. The negative control was co-infected plants (inoculated with 2000 J2s and FOL but untreated) while the positive control was co-infected + Carbendazim. The treatments PL neem, TH neem, neem, positive control and negative control were also done in non sterile soils in cultivar Prostar F1. In the positive control treatment, 40 ml of 0.25% Carbendazim 500 WP (Bavistin) Carbendazim was applied as per manufacturer's instructions at 20 ml/ 20 L. Carbendazim, was also found to control nematodes in this investigation. Each treatment was replicated four times and the experiment was laid out in a randomized complete block design (RCBD) in the greenhouse and was repeated once.

Plants were watered once every 24 hours and sprayed with a fungicides and pesticides once per week to control fungal diseases and pests. The fungicide Metalaxyl-M (Ridomil Gold) was applied at the rate of five grams per two litres of water. The fungicide Metalaxyl-M is a systemic fungicide against oomycete class of fungi (www.syngenta-us.com). Different pesticides were used interchangeably and these included Thiocyclam SP 50% (Evisect) at one gram per litre, Thiamethoxam (Actara) at the late of 0.8 g/litre of water, and Chlorantraniliprole (coragen'20 SC) at 2 ml per litre of water.

6.2.7 Data collection

Data on *in vitro* culture plates was collected by measuring the radial growth in mm using a ruler of FOL fungi in the direction of biocontrol fungi in the dual cultures. Radial growth of FOL in the controls was also measured and growth inhibition calculated as; (A-B) / A x100 where A is the control measurement while B is treatment. Data on *in vitro* culture tests of *T. harzianum* versus *P. lilacinum* was collected by measuring radial growth in mm of biocontrol fungi in the dual culture plates and in the controls just as in the FOL/biocontrol fungi experiment.

The test plants were harvested 11 weeks after transplanting. Data on plant height, dry shoot weight, were taken and recorded according to procedure in section 5.2.7. Yield data was obtained by taking total weight of the fruits in each replicate treatment. Roots from treatments inoculated with nematodes were stained with cold eosin yellow (0.1 g L^{-1} of water) for 30 minutes to facilitate the counting of galls (Jacquet *et al*., 2005). Data was also taken on galling according to a scale by Taylor and Sasser (1978) as in section 5.2.7. Disease severity was assessed by modification of a wilting index by Akram *et al*. (2014) as in section 5.2.7 at the time of harvesting the plants.

6.2.7.1 Extraction of juveniles from the roots

Second stage Juveniles (J2s) were extracted from infested tomato roots after termination of the experiment. They were extracted from the roots in a procedure described in 5.2.7.1

6.2.7.2 Estimation of *F. oxysporum* f. sp. *lycopersici* fungal propagules in the soil and roots of tomato plants

Data on number of fungal propagules of FOL from the roots was taken after termination of the experiment. The number of fungal propagules was obtained in terms of colony forming

units (CFU) of FOL per gram of root and soil by the dilution plate technique. Roots from each replicate treatment were washed in tap water and were then cut into one centimeter pieces. Nine milliliter of water agar was added to one gram of the root sample and was crushed with mortar and pestle. One milliliter suspension from the crushed roots was serially diluted to 10^{-1} and 10^{-2} using dilution media (0.001% of water agar). From the dilution of 10^{-1}, one milliliter was plated on PDA agar in duplicate and incubated at 25^{o}c for 14 days. After incubation the colonies of the FOL fungus were counted and the counts were used to calculate the number of CFU per gram of root.

One gram of the soil from each replicate was suspended in nine milliliter of dilution media (0.001% of water agar) and was diluted to 10^{-1} and 10^{-2}. From the dilution, 10^{-1}, one milliliter was cultured in duplicate on PDA plates and incubated at 25^{o}c for 14 days. After incubation, the colonies of the FOL fungus were counted and were estimated as the number of fungal propagules per gram of soil.

6.2.7.3. Data analysis

Data was analysed using GenStat statistical package (Discovery Edition 14). The Data on *in vitro* culture plates obtained by measuring mycelia growth was analysed by paired T-tests. Data on effect of treatments on plant growth in terms of mean height, mean dry shoot and yield was analysed by one way ANOVA. Means were compared using Least Significant Difference (L.S.D) at 5% level of significance. The means were separated by using Fisher's Protected Least Significant Difference test.

6.3 Results

6.3.1 Antagonism of *T. harzianum* and *P. lilacinum* against *F. oxysporum* f. sp. *lycopersici in vitro*

Trichoderma harzianum and *P. lilacinum* showed antagonistic activity on FOL. Paired T sample tests results showed there were significant differences between mycelia growth of FOL in dual culture plate of FOL and TH, versus FOL control plate (Table 6.1). There were also significant differences in FOL mycelia growth between a dual culture plate of FOL and PL versus FOL control plate. The two biological control agents *T. harzianum* and *P. lilacinum* were not significantly different in antagonism to each other *in vitro* conditions (Table 6.1, Plate 6.1 c). Antagonistic activity on FOL by *T. harzianum* was 44%, 51.9% and 58.3% on day 6, 9 and 11 respectively while that by *P. lilacinum* was 27.53%, 44% and 44% respectively for the same days (Fig.6.1, Plate 6.1 a, b).

Table 6.1 Fungal mycelia growth in dual culture plates compared with controls and results of paired T sample tests.

Dual culture vs control	**Mean mycelia growth in mm**			
	Dual culture	**Control**	**T-test statistic**	**P-value (0.05)**
FOL/TH vs FOL	1.86	3.9	6.69	< 0.05
FOL/PL vs FOL	2.3	3.9	5.01	< 0.05
TH/PL vs TH	3.9	4.5	.99	>0.05
PL/TH vs PL	1.55	1.58	0.45	>0.05

FOL= *Fusarium oxysporum* f. sp. *lycopersici* PL= *P. lilacinum*, TH= *T. harzianum*

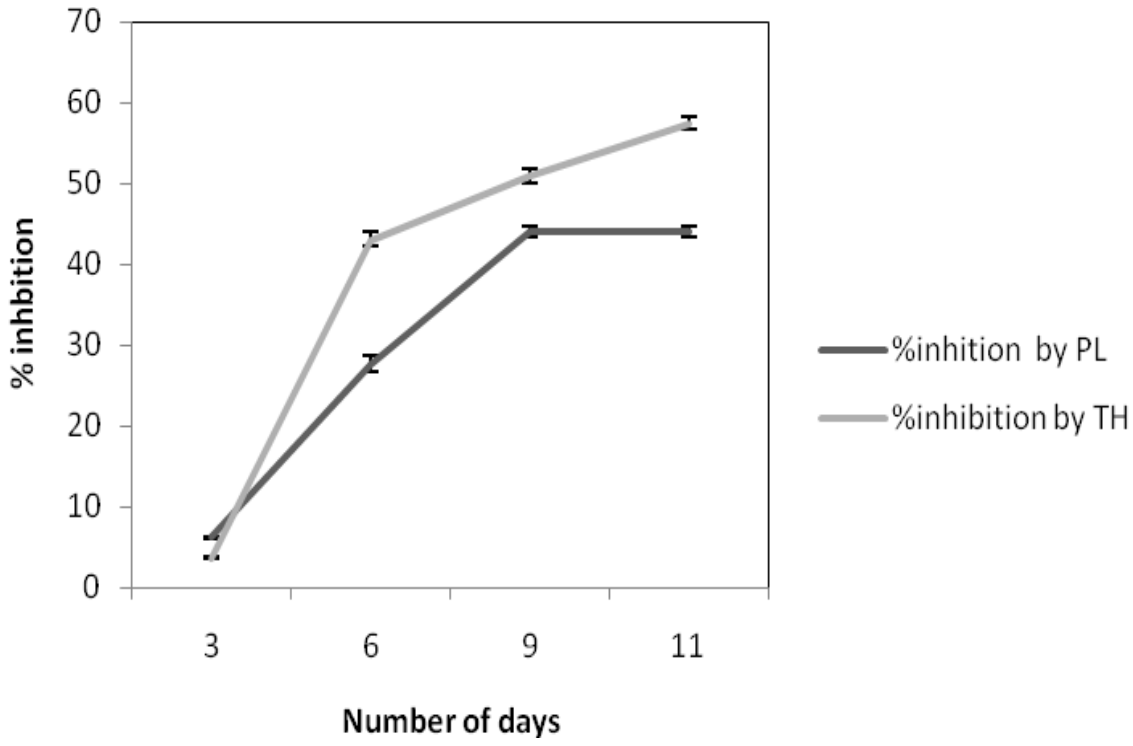


Fig. 6.1 *In vitro* percentage inhibition of FOL by TH and PL.The I bars signify 5% error. FOL= *F. oxysporum* f. sp. *lycopersici* PL= *P. lilacinum*, TH= *T. harzianum*

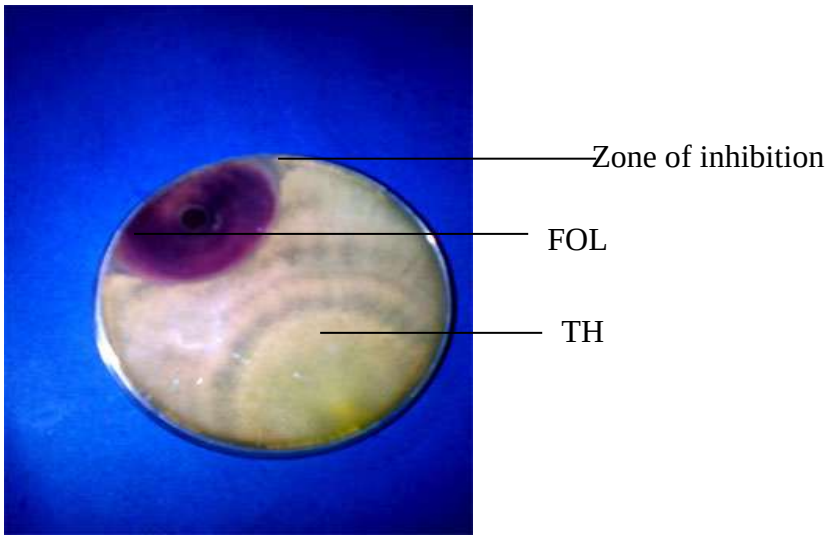


Plate 6.1a Antagonism between *F. oxysporum* f. sp. *lycopersici* (FOL) and *T. harzianum* (TH)

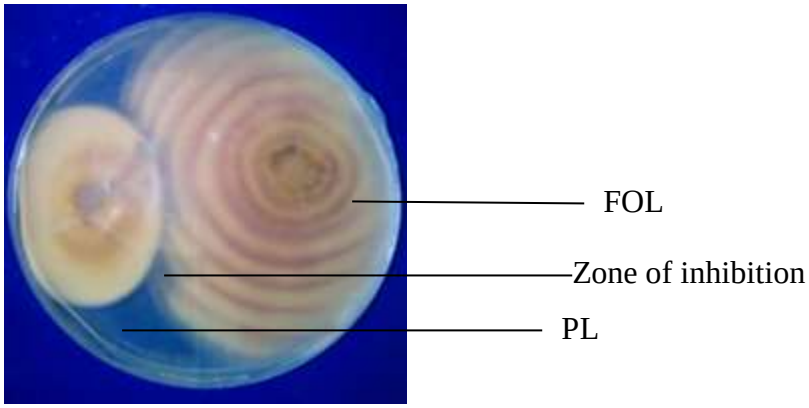


Plate 6.1b Antagonism between *F. oxysporum* f. sp. *lycopersici* (FOL) and *P. lilacinum* (PL)

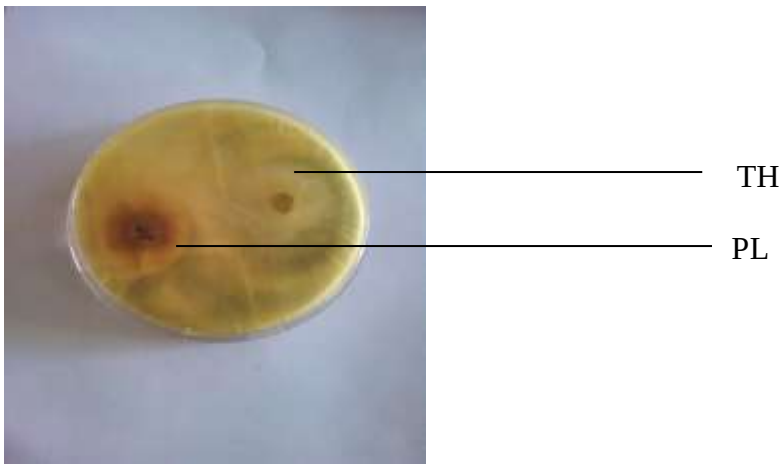


Plate 6.1c Dual culture of *P. lilacinum* (PL) and *T. harzianum* (TH)

6.3.2 Effect of *T. harzianum, P. lilacinum* and neem treatment in controlling *Meloidogyne javanica* and *F. oxysporum* f. sp. *lycopersici* in tomato cultivars

In the cultivar Prostar F1, the disease severity in the treatments PL, TH and PL-TH was significantly ($P<0.05$) lower than the untreated control (Fig 6.2). Disease severity in the treatments, TH, PL and PL-TH was not significantly ($P<0.05$) diferent from each other in cultivar Prostar F1. Disease severity was not significantly ($P<0.05$) diferent from the control in all treatments in cultivar Rambo F1 (Fig. 6.2). When neem was added to the biological control agents the performance of the biological agents was enhanced such that the treatments, PL neem, TH neem and PL-TH neem, had a lower FOL disease severity score than their respective treatments without neem in the cultivars Prostar F1 and Rambo F1.

However, there were no significant differences in disease severity score in the treatments, PL neem, TH neem and PL-TH neem in both tomato cultivars (Fig. 6.2).

The treatments, PL, TH, and PL-TH treatment had significantly ($P<0.05$) lower number of *M. javanica* J2s compared to the control in both tomato cultivars (Table 6.2 and 6.3). Neem, when combined with biological control agents enhanced their biological control ability such that the treatments, PL neem, TH neem and PL-TH neem had a significantly higher control of *M. javanica* J2s and lowered galling indices compared, to their respective treatments without neem, in both tomato cultivars Prostar F1 and Rambo F1 (Table 6.2 and 6.3).

The treatments, PL, TH, and PL-TH significantly reduced the FOL propagules in the roots this reduction was further enhanced when neem was added to the biological control agents. The treatments, PL, TH and PL-TH had significantly higher dry shoot weight and total weight of fruits compared to the control. The dry shoot weight and total weight of fruits were significantly higher in the PL neem, TH neem and PL-TH neem treatment compared to the respective treatments, PL, TH and PL- TH treatment in both tomato cultivars (Tables 6.2 and 6.3).

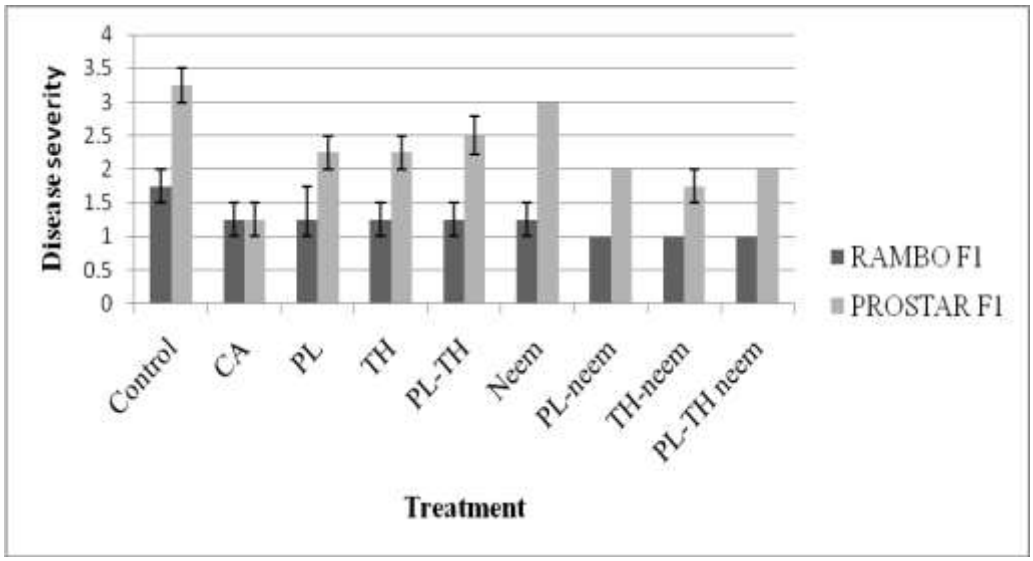


Fig. 6.2 Effect of different treatments on FOL and root-knot disease complex in greenhouse environment. Means were compared using LSD=0.59 for Rambo F1and 0.61 for Prostar F1.The I bars signify 5% error. FOL= *Fusarium oxysporum* f. sp. *lycopersici*, PL= *P. lilacinum*, TH= *T. harzianum*

Table 6.2 Effect of different treatments on FOL propagules, *M. javanica* juveniles and growth in cultivar Prostar F1

Treatment	*M. javanica* J2s	Galling index	CFU FOL/ gm of root	CFU FOL/ gm of soil	Height (cm)	DSW	Fruit weight in (g)
Control	4663f	3.5d	400c	127.7d	81.33a	15.4a	85.8a
Carbendazim	1167a	1.5ab	150.3ab	1.3a	88.67a	20.73c	102.7b
PL	2753cd	2.3bc	161b	40bc	83.3a	19.5bc	114.7cd
TH	3290de	2.3bc	14.3a	30abc	83.2a	18.93bc	115.3d
PL-TH	2270b	2.3bc	7.7a	10ab	82.2a	17.23ab	104.7bc
Neem	3619e	3.0cd	453.3c	64c	90a	25d	113.0cd
PL neem	1720ab	1.5ab	40ab	17.7ab	91ab	27de	136.7e
TH neem	2253bc	2.0ab	10a	20ab	101b	25d	133.0e
PL-TH neem	1503a	1.5ab	11a	1.3a	100ab	28.6e	138.7e
L.S.D (0.05)	683	0.93	146	36.6	10.2	3.06	10.25
C.V%	15.4	27.3	61.2	61.5	6.7	8.1	5.1

Data are mean of four replicates. Means followed by the same letter in the same column are not significantly ($P < 0.05$) different according to Fisher's L.S.D test. J2s= stage two juveniles, FOL= *Fusarium oxysporum* f. sp. *lycopersici* PL= *Purpureocillium lilacinum,* TH= *Trichoderma harzianum,* control= inoculated with *M. javanica* and FOL but not treated

Table 6.3 Effect of different treatments on FOL propagules, *M. javanica* juveniles and growth in cultivar Rambo F1

Treatment	*M. javanica* J2s	Galling index	CFU FOL/ gm of root	CFU FOL/ gm of soil	Height (cm)	DSW (g)	Fruit weight in (g)
Control	4435d	3.5d	300d	87.7f	84.33a	18.3a	91.1a
Carbendazim	1328a	2.0a	153c	2.5a	93.7bc	23.8b	116.8ab
PL	2783c	2.3ab	116b	51.7e	92.7bc	24b	119.6ab
TH	3028c	2.3ab	13a	24d	88ab	23.3b	122.2b
PL-TH	3010bc	2.75bc	10a	17cd	85.3a	24b	119.6b
Neem	2952c	3.0cd	286d	56.7e	96.7c	27bc	205.9c
PL neem	1953b	2.0a	14a	10bc	106.7d	32.2d	202.4c
TH neem	2041b	2.0a	2.5a	3.5ab	107d	28.5bc	197.3c
PL-TH neem	1800b	2.0a	1.9a	1.3a	110.7d	29.7cd	180c
L.S.D	330	0.66	32	7	6.6	3.9	28
C.V%	7.4	15.1	18.5	15.1	3.3	8.6	10.3

Data are mean of four replicates. Means followed by the same letter in the same column are not significantly ($P < 0.05$) different according to Fisher's L.S.D test. J2s= stage two juveniles, FOL= *Fusarium oxysporum* f. sp. *lycopersici,* PL= *Purpureocillium lilacinum,* TH= *Trichoderma harzianum,* control= inoculated with *M. javanica* and FOL but not treated

6.3.3 Effect of biological control agents on *F. oxysporum* f. sp. *lycopersici* disease severity, propagules and *M. javanica* J2s in sterile and non-sterile soils

There were no significant (P<0.05) differences in Fusarium wilt control and number of FOL propagules in the roots and soil in the treatments PL neem, TH neem and PL-TH neem in sterile soils compared to their respective treatments in non sterile soils. Disease severity and FOL propagules was higher in sterile soils compared to non-sterile soils for the treatments PL neem, TH neem and PL-TH neem, though the differences were not significant (Fig 6.3, Table 6.4). There was also significantly higher control of J2s in sterile soils than in non sterile soils for the same treatments (Table 6.4).

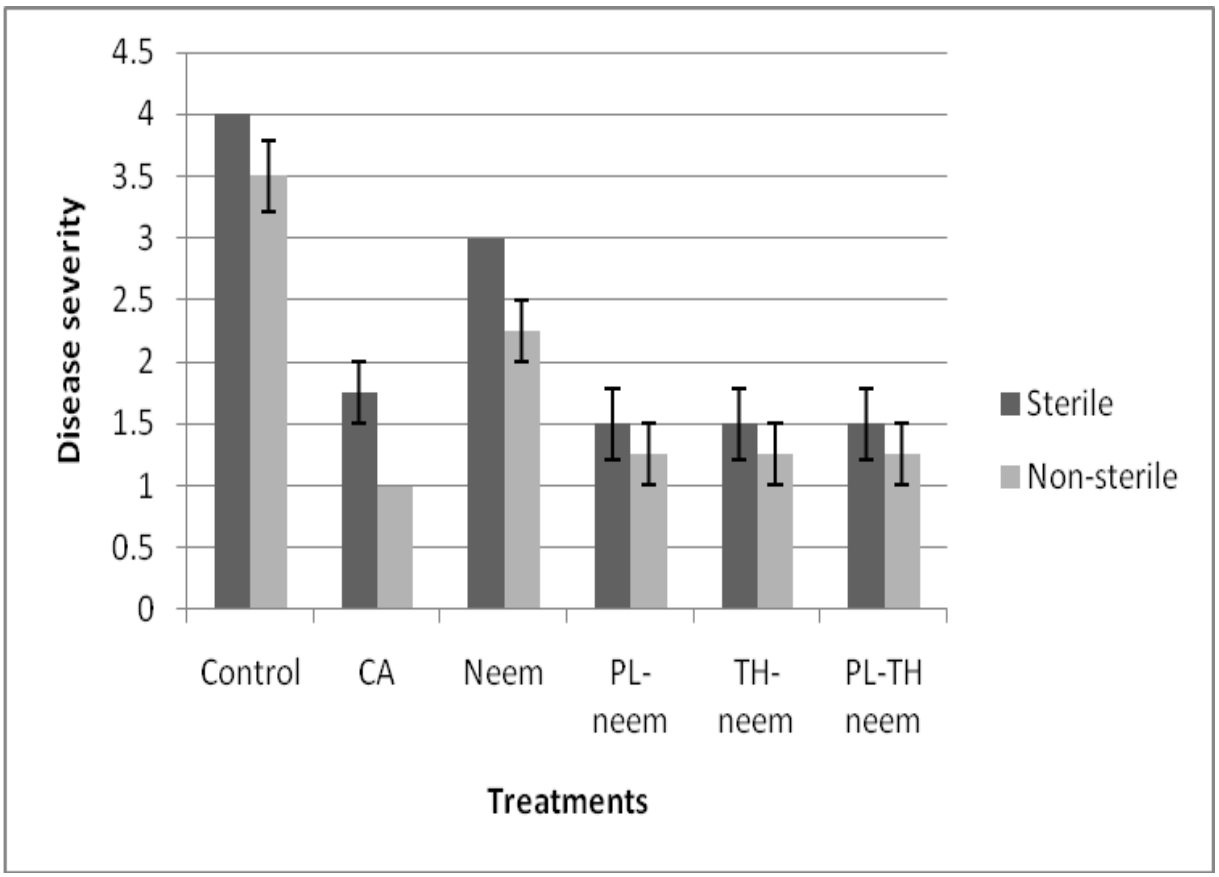


Fig. 6.3 Effect of TH neem, PL neem and a combined application (PL-TH) neem in controlling FOL in Fusarium wilt and root-knot disease complex in greenhouse environment in sterile and non sterile soils in the cultivar Prostar F1. Means were compared using LSD=0.68 for sterile and LSD=0.7 for non-sterile soils.The I bars signify 5% error.

Table 6.4 Effect of fungal antagonists and organic amendments on FOL propagules and *M. javanica* J2s in sterile and non sterile soils in cultivar Prostar F1

	Treatment	[1]Total J2s	Galling index	FOL [2]g/root	FOL [2]g/soil
Non-sterile	Control	5021e	3.5c	157d	40bcd
	Carbendazim	1446a	2.0ab	133bcd	1.3a
	Neem	4010d	3.0c	296e	51cd
	PL neem	2387c	2.0ab	30.67abc	24abc
	TH neem	3440d	2.3b	3.3a	3a
	PL-TH neem	2600c	2.0ab	10.3ab	4a
Sterile	Control	4663e	3.5c	400ef	127e
	Carbendazim	1167a	1.5a	150.3cd	1.3a
	Neem	3619d	3.00c	453f	64d
	PL neem	1720ab	1.5a	40.0 abcd	17.7ab
	TH neem	2253bc	2.0ab	10ab	20 abc
	PL-TH neem	1503a	1.5a	11ab	1.3a
	L.S.D	583	0.57	125	33
	C.V%	12.3	17.3	52.3	63

Data are mean of four replicates. Means followed by the same letter within the same column are not significantly ($P < 0.05$) different according to Fisher's L.S.D test. J2s= stage two juveniles, FOL= *Fusarium oxysporum* f. sp. *lycopersici* PL= *Purpureocillium lilacinum,* TH= *Trichoderma harzianum,* control= inoculated with *M. javanica* and FOL but not treated

Table 6.5 Effect of TH neem, PL neem and PL-TH neem on growth of tomato cultivar Prostar F1 inoculated with *M. javanica* and FOL pathogen in sterile and non sterile soils

	Treatment	Height (cm)	Dsw (g)
Non -Sterile	Control	74.7a	14.63a
	Carbendazim	86abc	21.97bc
	Neem	96cd	23.83bcd
	PL neem	89.7bcd	26.7de
	TH neem	97bcd	24.7bcde
	PL-TH neem	86.2a	24.9bc
Sterile	Control	81.33ab	15.4a
	Carbendazim	86.67abc	20.73b
	Neem	90bcd	25.07cde
	PL neem	91bcd	27.60e
	TH neem	101d	25.77de
	PL-TH neem	100d	28.17e
	L.S.D	13.0	3.7
	C.V%	8.6	9.4

Data are mean of four replicates. Means followed by the same letter are within the same column are not significantly ($P < 0.05$) different according to Fisher's L.S.D test. DSW=Dry Shoot weight, PL= *Purpureocillium lilacinum,* TH= *Trichoderma harzianum* control= inoculated with *M. javanica* and FOL but not treated

6.4 Discussion

Trichoderma harzianum was found to be very effective in inhibiting the radial growth of FOL mycelia under *in vitro* conditions. Occurrence of an inhibition zone may be due to production of diffusible volatile and non volatile fungal metabolites that prevent pathogen mycelia growth (Rini and Sulochana, 2007). Other researchers, have reported inhibition of FOL *in vitro* by *T. harzianum* (Nagesh *et al*., 2006; Anil and Garampalli, 2013; Kamali *et al.*, 2015; Javaid *et al*., 2014).

T. harzianum reduced the severity of FOL disease and decreased the number of J2s in the soil. *T. harzianum* in greenhouse experiments has been reported to be effective in suppressing Fusarium wilt pathogen in tomato plants (Selvakumar *et al*., 2014). Sharon *et al*., (2001) reported that *T. harzianum* reduced galling of root-knot nematode *M. javanica* on tomato plants, while Dababat and Sikora, (2007) observed reduction in root galls in tomato plants. Application of *T. harzianum* significantly reduced population density of *Meloidogyne* spp. and the severity of Fusarium wilt (Kamali *et al.*, 2015).

In vitro tests showed that *P. lilacinum* was antagonistic towards FOL and inhibited mycelia growth, the evidence being a zone of inhibition between PL and FOL. The mode of antagonism was likely by production of fungal metabolites and not by a competitively high growth rate since radial mycelia growth of PL was less than that of FOL. There are very few reports of antagonism of *P. lilacinum* towards fungal pathogens; however Lan *et al.* (2017) found it to have high inhibitory activity against *Rhizoctonia solani*, and *Verticillium dahlia in vitro*. Under *in vivo* conditions, the number of FOL fungal propagules in the soil was reduced in both tomato cultivars Rambo F1 and Prostar F1. Application of *P. lilacinum* lowered total *M. javanica* J2s populations in the roots and soil, and reduced galling indices. There are many reports on *P. lilacinum* parasitizing nematode eggs (Nagesh *et al*., 2006; Kannan and Veeravel, 2012), resulting in reduction of nematode multiplication in the soil.

The two biological control agents, TH and PL were not found to have an inhibitory effect on each other under *in vitro* conditions. The PL-TH treatment did not perform better than TH in the control of FOL wilt in tomato cultivar Prostar F1 and in the control FOL propagules in the roots for both tomato cultivars. The PL-TH treatment performed better than PL and TH in the cultivar Prostar F1 in the control of *M. javanica* J2s but not in the cultivar Rambo F1. The control of *M. javanica* juveniles by PL-TH treatment in the cultivar Prostar F1 was cultivar dependant. The effectiveness of a combined treatment of *P. lilacinum* and *T. harzianum* in the control of FOL and RKN has been reported (Nagesh *et al.,* 2006). So far, there are no reports of antagonism between *P. lilacinum* and *T. harzianum.* The lack of synergism or additive effect when the two were combined in the control of FOL and RKN might suggest that in soil, one fungus inhibited the other. Boer *et al*. (1998) reported that antagonism between introduced biological control agents can be detected if effect of application of a combination is zero, or similar to one of the biological control agents. It is possible that a conducive environment for antagonism between the two was created in the soil that did not exist under *in vitro* laboratory conditions. Bardin *et al*. (2015) suggested that biological control fungi should only be combined if they have different modes of action. In this study the two fungi had a similar mode of action in that both were antagonistic to FOL and produced zones of inhibition in dual culture agar plates. However this was over looked because compatibility tests of the two fungi in dual agar plates showed they were compatible and did not cause any significant inhibition against each other.

Neem treatment controlled *M. javanica* J2s in both tomato cultivars. Neem cake powder has also been used in the greenhouse and field conditions in order to control nematodes by producing azadirachtin and other chemicals which inhibit larval growth (Lokanadhan *et al.,* 2012). Neem has also been reported to control FOL (Nagesh *et al.,* 2006; Kimaru *et al*., 2004). In this experiment, application of neem treatment alone did not control the FOL wilt

pathogen or reduce the FOL propagules in the roots of both tomato cultivars. The mode of action by neem could have been provision of nutrients and in the absence of antagonistic fungi, FOL fungal pathogen multiplied because of the available nutrients. Neem action in promoting growth in plants is by providing nutrients in the soil (Lokanadhan *et al.*, 2012). The implication of lack of control of FOL by neem is that, if it is to be used alone in the control of any fungal pathogen, then pretesting has to be done to elucidate its mode of action. Neem increased the biological control of Fusarium wilt and *M. javanica* when applied together with the biological control agents. Neem as an organic amendment could have enhanced the activity of biological control agents by providing a high Carbon to Nitrogen ratio that is optimal for fungal growth. A high ratio of Carbon to Nitrogen is important for fungal growth (Lamour *et al*., 2000). Gao *et al.* (2007) reported extensive fungal growth for different biocontrol fungi when provided with a Carbon/ Nitrogen ratio between 10:1 and 80: 1 with optimum growth for two different strains of *P. lilacinus* at a ratio of 10:1 and 20:1. Nagesh *et al.* (2006) used neem cake powder to boost the establishment of fungal antagonists in the soil. Similarly, Khan and Saxena (1997), reported that combining *P. lilacinus* with oil cakes of neem, castor, groundnut, mustard and sesame enhanced the control of RKN. Sikora (1992), in his review suggested incorporation of soil amendments among many other options, to increase the activity of naturally occurring biological control agents in the control of plant parasitic nematodes.

Addition of neem in the presence or absence of fungal antagonists increased growth in both tomato cultivars. Organic amendments improve plant health by providing nutrients (Malik *et al*., 2013). In this investigation addition of neem enhanced plant growth that eventually masked out the effects of biological control agents.

The control of FOL propagules was not different in sterile soil compared to non sterile one. Similarly, there were no differences in dry shoot weights and plant heights in sterile and non

sterile soils. However, there was higher control of *M. javanica* J2s in sterile soils compared to non sterile. The establishment of biological control agents may have been higher in the absence of competition in sterile soils. Wang *et al*. (2004) reported that nematophagous fungi establish better in sterile soils than in non-sterile, and this may have made them more effective against the *M. javanica* J2s. Heydari and Pessarakli (2010) reported that in order for bio control agents to be effective, they must first establish in the soil while Thomashow *et al.* (2007) indicated the importance of rhizosphere competence for biocontrol agents.

This study has demonstrated that, the two biological control agents' *P. lilacinum* and *T. harzianum* applied separately showed antagonistic activity to FOL under *in vitro* conditions. In the greenhouse environment, the two biological control agents, reduced the severity of the wilt, decreased the number of FOL propagules and the total *M. javanica* J2s in the roots and the soil. It is important to note that a combination of PL-TH was not consistently more effective than either PL or TH applied separately in the control of FOL and *M. javanica* juveniles and therefore, if a combination of biological control agents is recommended, this should be done only if tests prove that there is no antagonism between them. Future research should endeavour to establish conditions that can lead to possible antagonisms of biological control agents in *in vitro*.

Host resistance as seen in the cultivar Rambo F1 in this experiment, application of biological control agents and organic amendments are an important strategy for the control of RKN and FOL in tomato production.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

7.1 GENERAL DISCUSSION

The results of this study showed that wilting of tomatoes and infestation by nematodes was observed by farmers in their tomato farms. There was no evidence that farmers applied any fungicides into the soil for controlling Fusarium wilt as there was low level of awareness among the farmers on the existence of the disease. Another study (Mugo, 2012) in Mwea (Kirinyaga County) also indicated that the farmers had poor understanding of Fusarium wilt. There was also lower number of farmers who applied chemicals in order to control nematodes. This also can be attributed to low levels of awareness on threat of nematodes to tomato production. Oruko and Ndung'u (2001) also reported that only 2.5% of farmers recognized nematodes as an important disease constraint in tomato production in Mwea, Kirinyaga County. Farmers had a high of awareness on early and late blight diseases by *Alternaria solani* and *Phytophthora infestans* and applied chemicals to control them.

The field study confirmed that plant parasitic nematodes were the most predominant trophic group comprising of *Meloidogyne* spp., *Tylenchus* spp., and *Pratylenchus* spp. *Meloidogyne* spp. is the most damaging of the plant parasitic nematodes as they form galls that interfere with water and mineral uptake. Charchar *et al*. (2003) reported that root-knot nematodes cause 30 to 40% yield losses in tropical regions.

It was evident that susceptible tomato cultivars Rambo F1, Roma VFN, Prostar F1 and Cal J had higher nematode infestations and galling indices compared to the less susceptible cultivars, Oxly and Kilele F1. Susceptibility to *M. javanica* resulted in greater reduction in growth in the tomato cultivars. It has been reported that root galling interferes with absorption of mineral salts and water and this reduces growth in plants (Ahmed *et al*., 2009).

Isolates of *F. verticillioides* were identified together with those of *F. oxysporum* and *F. oxysporum lycopersici* by (TEF)-1α marker gene, as causing infection in the tomato crop. This pathogen will normally infect cereals, however there are reports on *F. verticillioides* being pathogenic to cotton (Marupov *et al*., 2013). It has been observed that other *Fusarium* spp. such as *F. verticillioides, F. solani*, and *F. subglutinans* can infect the tomato crop resulting in symptoms very similar to those caused by fusarium wilt (Yin-dong *et al.*, 2018).

In the cultivar Prostar F1, presence of RKN predisposed it to increased FOL disease severity. This may be attributed to possible formation of giant cells as reported by Meena *et al*. (2016). This might be the reason for increase in FOL disease severity in tomato cultivar Prostar F1. Though RKN has been reported to cause breakdown of tomato resistance to FOL disease (Hadavi and Sahebani, 2008), the cultivar Rambo F1 remained resistant in presence of *M. javanica*.

Infection with Fusarium wilt pathogen and RKN increased nematode populations in both tomato cultivars. Infection with Fusarium wilt pathogen modifies the physiology of the plant roots enabling multiplication and increase of the nematode populations. Meena *et al.* (2016) suggested that there is a modification that takes place in the plant roots that favour the growth and development of Fusarium wilt.

It was evident in this research that greater reductions in growth parameters were higher in *M. javanica* and FOL inoculated plants as compared to nematode only inoculations. Maheshwari *et al*. (1995) reported co-infection by both *Fusarium oxysporum* f. sp. *circeri* and root-knot nematode in wilt resistant chickpea causing a greater reduction in shoot weights in four genotypes, compared to that of either pathogen inoculated alone, or untreated control. Similarly, Lobna *et al.* (2016) reported synergistic effects of inoculation of root-knot nematodes and Fusarium wilt pathogen by causing significant reduction in fresh shoot and dry shoot weight, in a susceptible and resistant tomato cultivar. Increased inoculum levels of

M. javanica combined with FOL resulted in greater reduction in the height, shoot and root dry weight in the susceptible tomato cultivar Prostar F1. The reduction in growth was due to effect of infection by FOL pathogen and RKN. Growth reduction also happened in wilt resistant Rambo F1 but to a much lesser extent. The reduction in growth could be attributed only to infection by the RKN since the cultivar remained resistant to FOL even in the presence of *M. javanica*.

Trichoderma harzianum was found to be very effective in reducing the radial growth of FOL under *in vitro* conditions. Similar results were obtained by other researchers where *T. harzianum* was found to inhibit the growth of the FOL *in vitro* (Nagesh *et al*., 2006; Anil Kumar and Garampalli, 2013; Javaid *et al*., 2014; Kamali *et al*., 2015). *Trichoderma harzianum* inhibits fungal pathogen growth by production of volatile and non volatile fungal metabolites such as harzianic acids, tricholin, viridian and gliovir (Rini and Sulochana, 2007). *Trichoderma harzianum* in greenhouse environment decreased the severity of the wilt and at the same time reduced the number of J2s in the soil, and this resulted in significant increase in height, shoot and root dry weight in both tomato cultivars Rambo F1 and Prostar F1. *T. harzianum* parasitizes the RKN eggs and J2s ((Naserinasab *et al.*, 2011). Other researchers also reported on effectiveness of *T. harzianum* in reducing population density of *Meloidogyne* spp. and the severity of Fusarium wilt (Kamali *et al*., 2015). Sharon *et al*. (2001) also reported that *T. harzianum* reduced galling of *M. javanica* on tomato plants, while Dababat and Sikora (2007), observed that it reduced root galls and egg masses in tomato plants.

Application of *P. lilacinus* lowered galling indices and *M. javanica* J2s in the roots and soil. *Paecilomyces lilacinus* reduces the number of juveniles in the soil and parasitizes nematode eggs such that multiplication of the nematode is significantly reduced (Nagesh *et al.,* 2006; Kannan and Veeravel, 2012). *Paecilomyces lilacinus* also produces chitinases that destroy the nematode eggs (Chan *et al.,* 2010). *Paecilomyces lilacinus* inhibited FOL growth *in vitro*

conditions and reduced the severity of the wilt. The occurrence of a zone of inhibition may suggest production of fungal metabolites that prevent pathogen growth. There are very few reports of *P. lilacinum* antagonism towards fungal pathogens. However *P. lilacinium* was found to have high inhibitory activity to *Rhizoctonia solani*, and *Verticillium dahlia in vitro* (Lan *et al*., 2017).

It has been advised that biological control agents should be used when combined in two or more, as long as those biological control agents are compatible, in order to overcome inconsistencies that are common in the application of biological control agents (Ojiambo and Scherm, 2006). However in this study the PL-TH treatment did not consistently perform better than PL or TH when applied separately in the control of Fusarium wilt and *M. javanica.* It is possible that other mechanisms such as mycoparasitism which were not investigated in this study were at work in this PL-TH combination. It might be that the complex soil environment would have created conditions that were conducive for antagonism between the two biological control agents even though this was not evident in the *in vitro* tests. An experiment on effectiveness of application of *P. lilacinus* when combined with *T. harzianum* in the control of FOL and RKN has been reported (Nagesh *et al*., 2006). There are no experiments that have been reported on antagonism of *P. lilacinum* and *T. harzianum.*

Neem enhanced the activity of biological control agents in the PL, TH, and PL-TH treatments possibly by providing the fungal antagonists with nutrients, and a Carbon to Nitrogen ratio that is optimum for fungal growth. Nagesh *et al.* (2006) included neem cake in biocontrol experiments of PL and TH to provide them with nutrients. Gao *et al*. (2007) reported extensive growth for fungal biological control agents in media containing C/N ratios of between 10.1 and 80.1. In this study, neem with a C/N ratio greater than 19 (≤ than 19) (Appendix 4), may have provided optimal growth conditions for biological control fungi making them to perform better in the presence of neem.

It was evident that the performance of introduced fungal antagonists and neem in the treatments, PL neem, TH neem and PL-TH neem was as effective in sterile as in non sterile soils in the control of FOL wilt and propagules in the soil, while being less effective in the control of *M. javanica* J2s in non sterile soil. The effectiveness of biocontrol fungi in the control of *M. javanica* in sterile soils could have been due to better establishment of the antagonists in the absence of competition from naturally occurring microflora. *Purpureocillium lilacinum* has been reported to be more effective in sterile than in non sterile soils in the control of RKN (Wang *et al.,* 2004).

7.2 CONCLUSION

The survey established that farmers had observed wilting and infestation with nematodes in the tomato crop in their farms, but only a few of them applied chemicals into the soil in order to control the nematodes. Farmers had little awareness of fusarium wilt disease though they had observed wilting on their farms and did not apply any chemicals to control this disease. The field survey identified important nematode genera associated with the tomato crop. Plant parasitic nematodes and specifically *Meloidogyne* spp. are prevalent in the study area and therefore, there is potential of nematode damage on the tomato crop.

There were different levels of susceptibility to *M. javanica* in the different tomato cultivars. The study identified that cultivars Roma VFN, Rambo F1, Prostar F1 and Cal J as being very susceptible to infestation by the RKN *M. javanica*, while cultivars Oxly, Kilele F1 had low susceptibility levels. The study established that high infestation of tomato plants with RKN results in high galling of the roots and brings about reduction in plant growth.

The most susceptible cultivars to the isolate of FOL F41 were Prostar F1 and Roma VFN, while Rambo F1 was the most resistant. In the FOL susceptible cultivar Prostar F1, an interaction with *M. javanica* increased the disease severity by FOL pathogen and reduced

growth measured in terms of height, shoot and root dry weight. In the FOL resistant cultivar Rambo F1, the interaction with *M. javanica* at increased inoculum levels brought about increased reduction in dry shoot weights even though host resistance to FOL was not broken.

The study established that *T. harzianum* and *P. lilacinum* effectively inhibited FOL mycelia growth under *in vitro* conditions. Under greenhouse environment, TH and PL each applied separately significantly reduced disease severity in Prostar F1 and root galling indices. The two, *P. lilacinum* and *T. harzianum* applied separately reduced the negative effects of FOL disease and RKN on the growth of the tomato plants, thereby increasing growth. The study has also shown that the combined treatment of PL-TH was not as efficacious as each of them applied separately.

Use of neem as an organic amendment combined with biological control agents have great potential in enhancing the control of RKN and at the same time increasing nutrients for plant growth.

Plant resistance is a very important strategy in the control of Fusarium wilt and root-knot disease complex especially when integrated with biological control agents and neem.

The fact that biological control agents and neem controlled Fusarium wilt in non sterile soils as in sterile soils in the cultivar Prostar F1 although not as effective in the control of RKN in non sterile soils, suggest that there is potential to use them in non sterile, natural soil conditions to control FOL pathogen and RKN as an alternative to use of chemicals.

7.3 Recommendations

1. The study established the need to create awareness on RKN and FOL
2. *Fusarium verticillioides* was identified as a new pathogen that infects tomato plants. There is need of surveillance by all agriculture stake holders to check on the progress of *Fusarium verticillioides* as a pathogen that can infect tomato and other crops.

3. It is important to use tomato cultivars that are resistant to FOL in order to reduce the possibility of formation of disease complexes that can drastically reduce growth.
4. The possible effects of RKN and FOL interaction in an FOL susceptible cultivar and FOL resistant cultivar and at different inocula levels was established, and therefore the need to manage RKN to prevent their build up to unmanageable proportions.
5. The management of FOL and RKN by host resistance, biological control agents and organic amendments is a viable option. However the use of combined biological control agents should be used with caution due to possibility of antagonism in soil conditions, which might not be easily detected under *in vitro* conditions.
6. Experiments on compatibility of biocontrol agents by dual culture plate techniques are a prerequisite in the application of combined biological control agents. *In vitro* mycoparasitism tests should also be an important consideration before application of more than one biological control agents.
7. Future research should endeavour to establish possible conditions that can lead to possible antagonisms of biological control agents in *in vitro*.
8. *Purpureocillium lilacinum* inhibited FOL fungal pathogen. Possibility that PL inhibits fungal pathogens should be investigated in view of integrating it with other strategies in the control of fungal pathogens and RKN in tomato.

REFERENCES

Abbas, W., Anwar S. A., Zia, A. and Javed, N. 2008. Response of four tomato cultivars to *Meloidogyne incognita* infection and its chemical management. *Pakistan Journal of Nematology* 26 (1); 37-43.

Agbenin, N. O. 2011. Biological Control of Plant Parasitic Nematodes : Prospects and challenges for the poor africa farmer. *Plant Protection Science* 47(2); 62–67.

Agricultural Research Service Culture Collection (accessed 2017 April 30). NRRL, http://nrrl.ncaur.usda.gov/cgi-bin/usda

Agrios, G. N. 2005. Plant Pathology. Fifth Edition. Academic Press, New york.

Ahmad, S. F. and Khan, T. A. 2004. Management of root knot nematode, *Meloidogyne incognita* by integration of *Paecilomyces lilacinus* with organic materials in chilli. *Archives Phytopathology and Plant Protection* 37(1); 35-40.

Ahmed, N., Abbasi, M. W., Shaukat, S. S., and Zaki, M. J. 2009. Physiological changes in leaves of mungbean plants infected with *Meloidogyne javanica. Phytopathololologia. Mediterranea* 48; 262–268.

Akram, W., Anjum, T. and Ahmad, A. 2014. Basal Susceptibility of tomato varieties against different isolates of *Fusarium oxysporum* f. sp. *lycopersici*. *International Journal of Agriculture and Biology* 16; 171–176.

Akyazi, F., Yildiz, S. and Felek, A. F., 2013. Soil nematode communities associated with hazelnut orchards in Turkey. *Journal of Entomology and Nematology* 6(8);112–121.

Alam, M. M., Ahmad, M. and Khan A. M. 1980. Effect of organic amendments on the growth and chemical composition of tomato, eggplant and chilli, and their susceptibility to attack by *Meloidogyne incognita*. *Plant and Soil* 57; 231–236.

Al-khatib, M., Abu-blan, H. and Masoud, S., 2006. Determination of resistance of locally grown tomato varieties to *Fusarium oxysporum* f. sp. *lycopersici* in Jordan under greenhouse conditions. *Jordan Journal of Agricultural Sciences* 2(3); 251–256.

Amin, N., 2014. The use of fungal endophytes *Gliocladium* spp. in different concentration to control of root-knot nematode *Meloidogyne* spp. *Academic Research International* 5(2); 91–95.

Amin, A. W., Anter, A. A., Ashoub, A. H. and El-Nuby, A. S. 2014. Evaluation of rhizobacteria as resistance inducers or bio-control agents for the control of *Meloidogyne incognita* in tomato. *Pakistan Journal of Nematology* 211-221.

Anang, B. T., Zulkarnain, A. Z. and Yusif, S. 2013. Production constraints and measures to enhance the competitiveness of tomato industry in Wenchi Municipal District of Ghana. *American Journal of Experimental Agriculture* 3 (4); 824-838.

Anamika, A. 2015. Study on pathogenicity test of *Meloidogyne incognita* on different vegetable crops. *Journal of Agricultural Science* 7(3); 105–10.

Anastacia, M. A. O., Thomas, K. K. and Hilda, W. N. 2011. Evaluation of tomato (*Lycopersicon esculentum*) variety tolerance to foliar diseases at Kenya Agricultural Research Institute Centre-Kitale in North west Kenya. *African Journal of Plant Science* 5(11); 676–681.

Anil, K. R. and Garampalli, R. H. 2013. Screening of indigenous potential antagonistic *Trichoderma* species from tomato rhizospheric soil against *Fusarium oxysporum* f. sp. *lycopersici*. *Journal of Agriculture and Veterinary Science* 4(3); 42–47.

Asare-Bediako, E., Showemimo, F. A., Buah, J. N. and Ushawu, Y. 2007. Tomato production constraints at Bontaga Irrigation Project in the Northern Region of Ghana. *Journal of Applied Sciences* 7; 459-461.

Atkins, S. D., Hidalgo-Diaz, L., Kalisz, H., Mauchline, T. H., Hirsch, P. R. and Kerry, B. R. 2003. Development of a new management strategy for the control of root-knot nematodes (*Meloidogyne* spp.) in organic vegetable production. *Pest Management Science* 59(2); 183-189.

Awan, S. M., Hussain, Azhar, A. T. and Karamu, R. 2012. Assessment of production practices of small scale farm holders of tomato in Bagrote Valley, CKNP region of Gilgit-Baltistan, Pakistan. *Acta Agriculture Slovenica* 99(2); 191-199.

Badri, D. V. and Vivanco, J. M. 2009. Regulation and function of root exudates. *Plant, Cell and Environment* 32; 666–681.

Baleiras Couto, M. M., Vogels, J. T., Hofstra, H., Huis in't Veld, J. H and Vossen, J. M 1995. Random amplified polymorphic DNA and restriction enzyme analysis of PCR amplified rDNA in taxonomy: two identification techniques for food-borne yeasts. *Journal of Applied Bacteriology* 79; 525–535.

Barakat, M. and AL-Masri, M. I. 2009. *Trichoderma harzianum* in combination with sheep manure amendment enhances soil suppressiveness of Fusarium wilt of tomato. *Phytopathologia Mediterranea* 48; 385–395.

Bardin, M., Ajouz, S., Comby, M., Lopez-Ferber, M., Graillot, B., Siegwart, M. and Nicot P. C. 2015. Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides. *Frontiers in Plant Science* 6;566.

Beecher, G. R. 1998. Nutrient content of tomatoes and tomato products. *Proceeding of the Society for Experimental Biology and Medicine.* 218(2); 98-100.

Bhagawati, B., Das, B. C. and Sinha, A. K. 2007. Interaction of *Meloidogyne incognita* and *Rhizoctonia solani* on okra. *Annals of Plant Protection Sciences.* 15; 533-535.

Bhagawati, B., Goswami, B. K. and Singh, C. S. 2000. Management of disease complex of tomato caused by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* through bioagent. *Indian Journal of Nematology* 30; 16-22.

Boer, M., Van der Sluis, I., Van Loon, L. C. and Bakker, P. A. H. M. 1998. *In vitro* compatibillity between fluorescent *Pseudomonas* spp. strains can increase effectivity of Fusarium wilt control by combination of these strains. *IOBC Bulletin* 21(9); 237-261.

Bogale, M. 2006. Molecular characterization of Fusarium isolates of Ethiopia. PHD Thesis University of Pretoria, South Africa.

Bogner, C. W., Kariuki, G. M., Elashry, A., Sichtermann, G., Buch, A., Mishra, B., Thines, M., Grundler, F. M. W. and Schouten, S. 2016. Fungal root endophytes of tomato from Kenya and their nematode biocontrol potential. *Mycological Progress*, 15, 30

Bonilla, N., Gutiérrez-barranquero, J., Vicente, A. and Cazorla, F. M. 2012. Enhancing soil quality and plant health through suppressive organic amendments. *Diversity* 4; 475-491.

Briar, S. S. 2007. Nematodes as bioindicators of soil food web health in agroecosystems: a critical analysis. PhD Dissertation, Ohio State University. Ohio, U.S.A.

Bruns, T. D. 2001. ITS reality. *Inoculum* 52; 2–3.

Burgess, L, W., Summerell, B. A., Bullock, S., Gott, K. P. and Backhouse, D. 1994. Laboratory manual for Fusarium research. 3rd edition. Department of Crop Sciences, University of Sydney.

Cai, G., Gale, L., Rosewich, S. R. W., Kistler, H. C., Davis, R. M., Elias, K. S. and Miyao, E. M. 2003. Origin of Race 3 of *Fusarium oxysporum* f. sp. *lycopersici* at a single site in California. *Phytopathology* 93; 1014-1022.

Castagnone-Sereno, P. 2006. Genetic variability and adaptive evolution in parthenogenetic root-knot nematodes. *Heredity* 96; 282–289.

CBS-KNAW Fungal Biodiversity Centre's Fusarium MLST website (accessed 2017 April 30). http://www.cbs.knaw.nl/Fusarium

Chan, Y. L., Cai, D., Taylord, P. W. J., Chan, M-T., and Yeha, K. W. 2010. Adverse effect of the chitinolytic enzyme PjCHI-1 in transgenic tomato on egg mass production and embryonic development of *Meloidogyne incognita*. *Plant Pathology* 59(5); 922–930.

Charchar, A. U., Gonzaga, J. M., Giordano, V., Boiteuy, L. D. and Reis, L. S. 2003. Reaction of tomato cultivars to infection by a mixed population of *M. incognita* race and *M. javanica* in the field. *Nematologia Brasileira* 27; 49-54.

Chehri K. 2016. Molecular identification of pathogenic *Fusarium* species, the causal agents of tomato wilt in western Iran. *Journal of Plant Protection Research* 56(2); 143-148.

Chindo, P. S., Shabayan, J. A. Y. and Marley, P. S. 2010. Effect of pre-emergence herbicides on *Meloidogyne* sp. and Fusarium wilt of tomato in Samaru, Zaria, Nigeria. *Journal of Agricultural Research* 48; 489–95.

Chitwood, D. J. 2002. Phytochemical based strategies for nematode control. *Annual Review of Phytopathology* 40; 221–249.

Coyne, D. L., Nicol, J. M., and Claudius-Cole, B. 2007. Practical Plant Nematology: A field and laboratory guide. IITA, Ibadan, Nigeria.

Dababat, A. A., Selim, M. E., Saleh, A. A. and Sikora, R. A. 2008. Fusarium wilt resistant tomato cultivars on root colonization of the mutualistic endophyte *Fusarium*

oxysporum strain 162 and its biological control efficacy. *Journal of Plant Diseases and Protection* 115(6); 273–278.

Dababat, A. A. and Sikora, R. A. 2007. Use of *Trichoderma harzianum* and *Trichoderma viride* for the Biological Control of *Meloidogyne incognita* on Tomato. *Jordan Journal of Agricultural Sciences* 3(3); 297–309.

Davis, R. F. and Webster, T. M. 2005. Relative host status of selected weeds and crops for *Meloidogyne incognita* and *Rotylenchulus reniformis. Journal of Cotton Science* 9; 41-46.

Di Pietro, A., García-Maceira, F. I., Huertas-González, M. D., Ruíz-Roldan, M. C., Caracuel, Z., Barbieri, A. S. and Roncero, M. I. G. 1998. Endopolygalacturonase PG1 in different formae speciales of *Fusarium oxysporum*. *Applied and Environmental Microbiology* 64(5); 1967–1971

Di Pietro, A., Madrid, M. P., Caracuel, Z., Delgado-Jarana, J., Roncero, M. I. G. 2003. *Fusarium oxysporum*: Exploring the molecular arsenal of a vascular wilt fungus. *Molecular Plant Pathology* 4; 315–325.

.

Djigal, D., Chabrier, C., Duyck, P. Achard, R., Quénéhervé, P. and Tixier, P. 2012. Cover crops alter the soil nematode food web in banana agroecosystems. *Soil Biology and Biochemistry* 48; 142–150.

Eisenback, J. D., Hirschmann, H., Sasser, J. N. and Triantaphyllou, A. C. 1981. A Guide to the Four Most Common Species of Root-Knot Nematodes (*Meloidogyne* spp.), with a pictorial key. International Meloidogyne Project.

Elanchezhiyan, K. Keerthana, U. Nagendran, U. Prabhukarthikeyan, S. R. Prabakar, K. Raguchander,T. G. Karthikeyan G. 2018. Multifaceted benefits of *Bacillus amyloliquefaciens* strain FBZ24 in the management of wilt disease in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. *Physiological and Molecular Plant Pathology* Doi 10.1016/j.pmpp.2018.05.008.

El-Fadly, G. B., El-Kazzaz, M. K., Hassan, M. A. A. and El-Kot Gan 2008. Identification of some *Fusarium* spp. using RAPD-PCR Technique. *Egyptian Journal of Phytopathology* 36; 71-80.

El-Mohamedy, R. S. R., Abdel-Kareem, F., Jabnoun-Khiareddine, H. and Daami- Remadi, M. 2014. Chitosan and *Trichoderma harzianum* as fungicide alternatives for controlling Fusarium crown and root rot of tomato. *Tunisian Journal of Plant Protection* 9; 31-43.

Esfahani, M. N., Ahmadi, A. R. and Shirazi, K. 2012. Susceptibility assessments of tomato genotypes to root-knot nematodes *Meloidogyne javanica. Journal of Ornamental and Horticultural Plants 2*(2); 113–121.

Esfahani, M. N. and Pour, B. A. 2006. The effects of *Paecilomyces lilacinus* on the pathogenesis of *Meloidogyne javanica* and tomato plant growth parameters. *Iran Agricultural Research* 24; 68-76.

Erb, W. A. and Rowe, R. C. 1992. Screening tomato seedlings for multiple disease resistance. *Journal of American Society for Horticultural Science* 117(4); 622-627.

FAO 2000. Available online: (accessed; 2014 December 4) http://www.fao.org/hortivar.

FAO 2005. FAOSTAT. Available online: (accessed; 2014 December 4) http://faostat.fao.org.

Fatima, K., Noureddine, K., Jamal, E., Henni, J. E. and Mabrouk, K. 2015. Antagonistic effect of *Trichoderma harzianum* against *Phytophthora infestans* in the North-West of Algeria. *International Journal of Agronomy and Agricultural Research* 6 (4); 44-53.

Ferris, H. and Matute, M. M. 2003. Structural and functional succession in the nematode fauna of a soil food web. *Applied Soil Ecology.* 23(2); 93 – 110.

Fesenko, I. A., Kuklev, M.Y. and Karlov, G. I. 2013. Development of a breeder-friendly functional codominant DNA marker for the I2 locus covering resistance to *Fusarium oxysporum* f. sp. *lycopersici*. *Molecular Breeding* 31(2); 495–499.

Foroutan, A. 2013. Evaluation of *Trichoderma* isolates for biological control of wheat fusarium foot and root rot. *Romanian Agricultural Research* 30; 334-342.

Freckman, D. W. 1988. Bacterivorous nematodes and organic matter decomposition. *Agriculture, Ecosystems and Environment* 24; 195-21.

Freckman, D.W. and Virginia, R. A. 1997. Low-diversity Antarctic soil nematode communities: distribution and response to disturbance. *Ecology* 78; 363–369.

Fufa, F., Hanson, P., Dagnoko, S. and Dhaliwal, M. 2009. AVRDC-the world vegetable center. Tomato breeding in sub-saharan africa: Lessons from the past, present work, and future prospects. *www.actahort.org. Acta Horticulturae*.

Fusarium-ID databases(acessed 2017 January 30). http://isolate.fusariumdb.org

Fusarium Research Centre (accessed 2017 April 30). http://plantpath.psu.edu/ facilities/fusarium-research-center

Gagkaeva, T., 2008. Introduction to Fusarium taxonomy, Fusarium genus. Tatiana Gagkaeva Laboratory of Mycology and Phytopathology. All-Russian Institute of Plant Protection St. Petersburg, Russia.

Gao, L., Sun, M. H., Liu, X. Z. and Che, Y. S. 2007. Effects of carbon concentration and carbon nitrogen ration the growth and sporulation of several biocontrol fungi. Mycology Research (1); 87–92.

Gazis, R., Rehner, S. and Chaverri, P. 2011. Species delimitation of fungal endophyte diversity studies and its implications in ecological and biogeographic inferences. *Molecular Ecology* 20; 3001–3013.

Gebremikael, M. T., Steel, H., Buchan, D. Bert, W. and De Neve, S. 2016. Nematodes enhance plant growth and nutrient uptake under C and N-rich conditions. *Scientific Reports* 6; doi: 10.1038/srep32862.

Geiser, D. M., del Mar Jiménez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T. J., Zhang, N., Kuldau, G. A. and O'Donnell, K. 2004. FUSARIUM-ID v.

1.0: a DNA sequence database for identifying Fusarium. *European Journal of Plant Pathology* 110; 473–479.

Giannakou, I. O. and Anastasiadis, I. 2005. Evaluation of chemical strategies as alternatives to methyl bromide for the control of root-knot nematodes in greenhouse cultivated crops. *Crop Protection* 24 (6); 499-506.

Goswami, B. K., Singh, N. and Bhattacharya, C. 2013. Solar assisted integrated approach for the management of soil borne fungus and root-knot nematode diseases on tomato at nursery level. *International Journal of Pure and Applied Sciences and Technology* 19(1); 75–81.

Guetsky, R., Shtienberg, D., Elad, Y. and Dinoor, A. 2001. Combining biocontrol agents to reduce the variability of biological control. *Phytopathology* 91; 621–627.

Gutpta, V. K., Misra, A. K., Gaur, R. K., Jain, P. K. and Sharma, S. 2010. Current status of *Fusarium* wilt disease of Guava (*Psidium guajava* L.) in India. *Biotechnology* 9(2); 176- 195.

Hadavi, N. and Sahebani, N. 2008. Study on the systematic effect of RKN (*Meloidogyne javanica*) on phenhlalanine Ammonia lyase enzyme activity on tomato root in the interaction between root-knot nematode and tomato Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici). Journal of Science and Technology of Agriculture and Natural Resource Water and Soil Science* 12(43); 217-225.

Haggag, H. E. 2012. *In vitro* study on *Fusarium solani* and *Rhizoctonia solani* isolates causing the damping off and root rot diseases in tomatoes. *Nature and Science* 10(11); 16-25.

Harman, G. E., Björkman, T., Ondik, K. and Shoresh, M. 2008. Changing paradigms on the mode of action and uses of *Trichoderma* spp. for biocontrol. *Outlooks on Pest Management;* 1–6.

Heydari, A. and Pessarakli, M. 2010. A review on biological control of fungal plant pathogens using microbial antagonists. *Journal of Biologial Sciences* 10 (4):273 – 290.

Hirooka, T. and Ishii, H. 2013. Chemical control of plant diseases. *Journal of General Plant Pathology* 79(6); 390-401.

Hussain, M. A. Fatima, I. Mukhtar, T. Aslam, M. N. and Kayani, M. Z. 2015. Effect of inoculum density of root-knot nematode *Meloidogyne incognita* on damage potential in eggplant. *Mycopathologia* 13(1); 33-36.

Hussey R. S. and McGuire, J. M. 1987. Interactions with other organisms. In: Principles and Practice of Nematode Control in Crops (Brown R. H. and Kerry B. R., eds). pp. 294-320. Academic Press, Marrickville, Australia.

Inami, K.,Yoshioka-Akiyama, C., Morita, Y., Yamasaki, M., Teraoka, T. and Arie, T. 2012. A genetic mechanism for emergence of races in *Fusarium oxysporum* f. sp. *lycopersici*: Inactivation of avirulence gene AVR1 by transposon insertion. *PLoS ONE* 7; 1-10.

Irshad, U., Mukhtar, T., Ashfaq, M., Kayani , Z., Kayani, S. B., Hanif, M. and Aslam, S.

2012. Pathogenicity of citrus nematode *Tylenchulus semipenetrans* on *Citrus jambhiri. The Journal of Animal and Plant Sciences* 22(4); 1014-1018.

Ito S. I., Nagata, A., Kai, T., Takahara, H. and Tanaka, S. 2005. Symptomless infection of tomato plants by tomatinase producing *Fusarium oxysporum* formae speciales non pathogenic on tomato plants. *Physiological and Molecular Plant Pathology* 66;183-191.

Jacobs, A., Govender, R., van Heerden, S.W. 2013. *Fusarium oxysporum* f. sp. *lycopersici* race 3 causing tomato wilt in South Africa. *Australasian Plant Disease Notes* 8; 145-147

Jacquet, M., Bongiovanni, M., Martinez, M., Verschave, P., Wajnberg, E., and Castagnone-Sereno, P. 2005. Variation in resistance to the root-knot nematode *Meloidogyne incognita* in tomato genotypes bearing the Mi gene. *Plant Pathology 54*; 93–99.

Jafri, I., Shahid, A. A., Ibrahim, A. and Atif, M. 2015. Efficacy of different bacterial strains against *Meloidogyne incognita. Pakistan Journal of Nematology* 33(1); 39–46.

Jain, A. and Jitendra, M. 2010. Pathogenicity of *Fusarium oxysporum* and *Meloidogyne incognita* and cumulative effects on tomato. *Annals of Plant Protection Sciences* 18(1); 197-20.

Jaiteh, F., Kwoseh, C. and Akromah, R. 2012. Evaluation of tomato genotypes for resistance to root-knot nematodes. *African Crop Science Journal* 20(1); 41-49.

Jatala, P. 1986. Biological control plant parasitic nematodes. *Annual Review of Phytopathology* 24; 491-453.

Javaid, A., Afzal, L., Bashir, A. and Shoaib, A. 2014. *In vitro* screening of *Trichoderma* species against *Macrophomina*. *Pakistan Journal of Phytopathology* 26(1); 37–41.

Jegathambigai, V., Wijeratnam, R. S. W. and Wijesundera, R. L. C. 2011. Effect of *Trichoderma viride* strain NRRL 6418 and *Trichoderma harzianum* (Hypocrea lixiii TWC1) on *Livistona rotundifolia* root knot nematode *M. incognita*. *Journal of Entomology* 8(4); 229.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter* 48; 692.

Jiskani, M. M., Pathan, M. A., Nizamani, S. M., Khuhro, R. D. and Rustamani, M. A. 2008. Effect of different inoculum levels of *Meloidogyne incognita* on nematode reproduction, plant growth and disease severity in tomato. *Pakistan Journal of Phytopathology* 20; 200-203.

Jordão do Amaral, D. O., Alves de Almeida, C. M., Malafaia, C. B., Bastos da Silva, M. L. R., Correia, M. T., Lima, V. L. and Vanusa da, S. M. 2013. Identification of races 1, 2 and 3 of *Fusarium oxysporum* f. sp. *lycopersici* by molecular markers. *African Journal of Microbiology Research* 7(20); 2324-2331.

Kamali, N., Pourjam, E. and Sahebani, N., 2015. Elicitation of defense responses in tomato against *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *lycopersici* wilt complex. *Journal of Crop Protection* 4(1); 29–38.

Kamran, M., Anwar, S. A., Javed, N., Khan, S. A., Haq, I. and Ullah, I. 2012. Field evaluation of tomato genotypes for resistance to *Meloidogyne incognita*. *Pakistan Journal of Zoology 44*(5); 1355–1359.

Kannan, R. and Veeravel, R., 2012. Effect of different dose and application methods of *Paecilomyces lilacinus* (Thom.) Samson against RKN, *Meloidogyne incognita* (Kofoid and White) Chitwood in Okra. *Journal of Agricultural Science* 4(11); 119–127.

Kariuki, G. M., Coyne, D. L., Kinyua, Z. M., Mweke, A. and Onkendi, E. M. 2013. Standard operating procedures for root-knot nematodes. The International Plant Diagnostics Network.

Kariuki, G. M., Kariuki, F. W., Birgen, J. K. 1 and Gathaara, V. 2010. Participatory development, testing and validation of concepts and technologies for site-specific detection and control of plant parasitic nematodes infecting tomatoes in Mwea, Kenya. Second RUFORUM Biennial Meeting 20 - 24 September 2010, Entebbe, Uganda.

Karssen, G. and Moens, M. 2006. Root-knot nematodes. In: Perry, R. N. and Moens, M. (Eds.) pp. 59-90. *Plant Nematology*. CABI publishing, Wallingford, UK.

Katsantonis, D., Hillocks, R. J. and Gowen, S. 2003. The comparative effect of root-knot nematode on severity of Verticillium and Fusarium wilt in Cotton. *Phytoparasitica* 31(2); 154-162.

Kenya Ministry of Agriculture and Livestock development. 2003. Fruits and vegetables technical hand book.

Kewnick, S. and Sikora, R. A. 2006. Biological control of the root knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. *Biological Control* 38(2); 179-187.

Khan, M. R. and Akram, M. 2000. Effects of certain antagonistic fungi and Rhizobacteria on wilt disease complex of tomato caused by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici. Nematologia Mediterranean* 28; 139-144.

Khan, T. A. and Saxena. S. K. 1997. Effect of root-dip treatment with fungal filtrates on root penetration, development and reproduction of *M. javanica* on tomato. *International Journal of Nematology* 7(1); 85-88.

Khanzada, S., Jiskani, M. M., Khanzada, S. R., Khanzada, M. S., Ali, S., Khanzada, K. A., Saeed, N., Anwar, S. and Khalid, M. 2012. Response of some tomato cultivars against root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood. *The journal of Animal and Plant Sciences* 22(4); 1076–1080.

Khattak, B. and Stephen, M. 2008. Effect of some indigenous isolates of *Trichoderma harzianum* on RKN, *Meloidogyne javanica* (Treub) Chitwood. *Sarhad Journal of Agriculture* 24(2); 1–3.

Kimaru, S. K., Waudo, S. W., Monda, E., Seif, A. A. and Birgen, J. K. 2004. Effect of Neem Kernel Cake Powder (NKCP) on Fusarium wilt of tomato when used as soil

amendment. *Journal of Agriculture and Rural Development in the Tropics and Subtropics* 105(1); 63–69.

Kimenju, J., Kagundu, A., Mutua, G. and Kariuki, G. 2008. Incorporation of green manure plants into bean cropping systems contribute to root-knot nematode suppression. *Asian Journal of Plant Sciences* 7; 404-408.

Kirankumar, R., Jagadeesh, K. S., Krishnaraj P. U. and Patil M. S. 2008. Enhanced growth promotion of tomato and nutrient uptake by plant growth promoting Rhizobacterial isolates in presence of tobacco mosaic virus pathogen. *Karnataka Journal of Agricultural Sciences* 21; 309-311.

Kolashian, I.,Williamson, V. M., Miyao, G., Lawn, D. A. and Westerdan, B. B. 1996. Resistance breaking nematodes identified in California tomatoes. *California Agriculture* 50; 18-9.

Krejcie, R. V. and Morgan, D. W. 1970. Determining sample size for research activities. *Educational and Psychological Measurement* 30; 607-610.

Lairini, K. and Ruiz-Rubio, M. 1997. Detection of tomatinase from *Fusarium oxysporum* f. sp. *lycopersici* in infected tomato plants. *Phytochemistry* 45; 1371–1376.

Lamour, A., Van den Bosch, F., Termorshuizen, A. J., and Jeger, M. J. 2000. Modelling the growth of soil-borne fungi in response to carbon and nitrogen. *IMA Journal of Mathematics Applied in Medicine and Biology* 17(4); 329–46.

Lan, X., Zhang, J., Zong, Z., Ma, Q. and Wang, Y. 2017. Evaluation of the Biocontrol Potential of *Purpureocillium lilacinum* QLP12 against *Verticillium dahliae* in Eggplant. *BioMed Research International* ; 1-9.

Leslie, J. F. and Summerell, B. A. 2006. The Fusarium Laboratory Manual (1st ed.) Blackwell Publishing Co., Ames, Iowa, USA.

Li, G., Zhang, K., Xu, J., Dong, J. and Liu. Y. 2007. Nematicidal substances from fungi. *Recent Patents on Biotechnology* 1; 1-22.

Liang, W., Zhang, X. Li, Q., Jiang, Y. Ou, W. and Neher, D. A. 2005. Vertical distribution of bacteriovorous nematodes under different land uses. *Journal of Nematology* 37(3); 1-5.

Lievens, B., Baarlen, P. V., Verreth, C., Kerckhove, S. V., Rep, M. and Thomma, B. P. H. J. 2009. Evolutionary relationships between *Fusarium oxysporum* f. sp. *lycopersici a*nd *F. oxysporum* f. sp. *radicis-lycopersici* isolates inferred from mating type, elongation factor-1α and exopolygalacturonase sequences. *Mycological research* 113(10); 1180-1191.

Lievens, B., Rep, M., and Thomma, B. P. H. J. 2008. Recent developments in the molecular discrimination of formae speciales of *Fusarium oxysporum*. *Pest Management Science* 64; 781–788.

Lobna, H., Hajer, R. Naima, M. and Najet, H. 2016. Studies on disease complex incidence of *Meloidogyne Javanica* and *Fusarium oxysporum* f. sp. *lycopersici* on resistant and susceptible tomato cultivars. *Journal of Agricultural Science and Food Technology* 2(4); 41–48.

Lokanadhan, S., Muthukrishnan, P. and Jeyaraman, S. 2012. Neem products and their agricultural applications. *Journal of Biopesticides* 5 ; 72-76.

Luambano, N., Kimenju, J. W., Narla, R. D. and Waceke, J. W. 2011. Colonisation of the rhizosphere of plants which are poor host to root-knot nematodes by the biological agent *Pochonia chlamydosporia*. Proceedings of African Crop Science Conference, Uganda 10; 195 – 197.

Luangsa-ard, J., Houbraken, J., van Doorn, T., Hong, S., Borman, A. M., Hywel-Jones, N. L., and Samson, R. A. (2011). *Purpureocillium,* a new genus for the medically important *Paecilomyces lilacinus*. *FEMS Microbiology Letters* 321(2); 141–149.

Maerere, A. P., Sibuga, K. P. and Mwajombe, K. K. 2006. Baseline survey report of tomato production in Mvomero district-Morogoro region, Tanzania. Sokoine University of Agriculture Faculty of Agriculture, Morogoro 1-31.

Maheshwari, T. U., Sharma, S. B., Reddy, D. D. R. and Haware, M. P. 1995. Co-infection of wilt-resistant chickpeas by *Fusarium oxysporum* f. sp. *ciceri* and *Meloidogyne Javanica. Journal of Nematology* 27; 649–653.

Malafaia, C. B., Silva, T. D., Amaral, D. O. J., Almeida, C. M. A., Silva, M. L. R. B., Correia, M. T. dos S., and Silva, M. V. 2013. Evaluation of the resistance and differential induction of chitinases in tomato in response to inoculation with *Fusarium oxysporum* f. sp. *lycopersici. Journal of Plant Physiology and Pathology* 1; 1–6.

Maleita, M. N. C., Curtis, H. C. R., Powers, J. S. and Abrantes, O. M. I. 2012. Inoculum levels of *Meloidogyne hispanica* and *Meloidogyne javanica* affect nematode reproduction, and growth of tomato genotypes. *Phytopathologia Mediterranea* 51(3); 566-576.

Malik, M. A., Khan, K. S., Marschner, P. and Ali, S. 2013. Organic amendments differ in their effect on microbial biomass and activity and on P pools in alkaline soils. Biology and Fertility of Soils 49(4); 415-425.

Mantelin, S. Bhattarai, K. K., Jhaveri, T. Z. and Kaloshian, I. 2013. Mi-1-Mediated resistance to *Meloidogyne incognita* in tomato may not rely on ethylene but hormone perception through ETR3 participates in limiting nematode infection in a susceptible host. PLOS ONE 8(5); 63281.

Marupov, A. Stipanovic, R. D., Turamuratova, G. H., Mambetnazarov, A. B. and Marupova, M. A. 2013. *Fusarium verticillioides*: A new cotton wilt pathogen in Uzbekistan. *International Open Journal of Plant Disease and Pathology* 1(1); 1 – 5.

Meena, K. S., Ramyabharathi, S. A., Raguchander, T. and Jonathan, E. I. 2016. Interaction of *Meloidogyne incognita* and *Fusarium oxysporum* in carnation and physiological changes induced in plants due to the interaction. *SAARC Journal of Agriculture* 14(1): 59–69.

Mekete, T., Dababat, A. A., Sikora, N., Akyazi, F. and Ababe E. 2012. Identification key for agriculturally important plant-parasitic nematodes prepared for the International Nematode Diagnosis and Identification Course 2012-A manual for nematology. Mexico, D. F. CIMMYT.

Meyers, S. L. and Roberts D. P. 2002. Combinations of biocontrol agents for management of plant-parasitic nematodes and soilborne plant-pathogenic fungi. *Journal of Nematology* 34; 1–8.

Michielse, C. B. and Rep, M. 2009. Pathogen profile update: *Fusarium oxysporum*. *Molecular plant pathology* 10; 311–324.

Mishra, K. K., Kumar, A. and Pandey, K. K. 2010. RAPD based genetic diversity among different isolates of *Fusarium oxysporum* f. sp. *lycopersici* and their comparative biocontrol. *World Journal of Microbiology and Biotechnology* 26; 1079–1085.

Ministry of Agriculture 2006. Annual report.

Mohammed, A. S., Kadar, N. H., Kihal, M. 2016. Characterization of Fusarium oxysporum isolates from tomato plants in Algeria. *African Journal of Microbiology* 10(30); 1156-1163

Moura, R. M., Rosa, R. C. T. and Pedrosa, E. M. R. 2007. Study of *Meloidogyne-Fusarium* interaction on tomato containing MI resistance gene under high soil temperature. *Nematologia-Brasileira* 25(2); 229-233.

Mudawi, H. I. and Idris, M. O. 2015. Efficacy of the bioagents Bacillus isolates and Trichoderma spp. in the control of wilt/root-rot disease in chickpea, *World Journal of Science, Technology and Sustainable Development* 12 (4); 303-314

Mugo, D. N. 2012. Integrated management of Fusarium wilt of tomato using fungicides, organic matter and neem extract. Msc. Thesis, Kenyatta University, Kenya.

Musyoki, R., Omari, F. and Mwangi, T. 2005. Evaluation of Elite Tomato Varieties in the Semi-arid regions of Eastern Kenya (KARI Publication).

Mwangi, M. W., Kimenju, J. W., Narla, R. D., Kariuki, G. M. and Muiru, W. M. 2015. Tomato management practices and diseases occurrence in Mwea West Sub County. *Journal of Natural Sciences Research* 5(20); 119–124.

Mwangi, M. W., Monda, E. O., Okoth, S. A. and Jefwa, J. M. 2011. Inoculation of tomato seedlings with *Trichoderma harzianum* and Arbuscular mycorrhizal fungi and their effect on growth and control of wilt in tomato seedlings. *Brazilian journal of Microbiology* 42(2); 508-518.

Nagesh, M., Hussaini, S. S., Ramanujam, B. and Chidanandaswamy, B. S. 2006. Management of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* wilt complex using antagonistic fungi in tomato [*Lycopersicon esculentum* Mill.]. *Nematologia Mediterranea* 34(1); 63-68.

Naserinasab, F., Sahebani, N. and Etebarian, H. R. 2011. Biological control of *Meloidogyne javanica* by *Trichoderma harzianum* BI and salicylic acid on tomato. *African Journal of Food Science* 5(3); 276 - 280.

Nawar, L. S. 2015. Studies on the Efficacy of non-pathogenic *Fusarium oxysporum* isolate to control Fusarium wilt of tomato plants in Saudi Arabia. *International Journal of Science and Research* 4(1); 1949–1954.

Ndalo, V., Kahuthia-Gathu, R., Maina, M. 2015. Chemical management of *Tuta absoluta* (meyreck) in Loitoktok, Kajiado County, Kenya. Proceedings, Sustainable Horticultural Production in the Tropics. Murang'a County Kenya.

Nelson, P. E., Toussoun, T. A. and Marascs, W. F. O. 1983. *Fusarium* species. An illustrated manual for identification. Pennsylvania State University. University Press, Pennsylvania.

Nene, Y. L. and Haware, M. P. 1980. Screening chickpea for resistance to wilt. *Plant Disease* 64; 379-380.

Nirmaladevi, D. and Srinivas, C. 2012. Cultural, morphological, and pathogenicity variation in *Fusarium oxysporum* f. sp. *lycopersici* causing wilt of tomato. *Batman University Journal of Life Sciences* 2(1); 1–16.

Noling, J. W. 2010. Nematode management in tomatoes, peppers and egg plant. (accessed: November 2012) http;//edis.ifas.ufl.edu/topic. Pepper-Ipm.

O'Brien, P. A. 2017. Biological control of plant diseases. *Australasian Plant Pathology* 1-12.

Odame, P. S. 2009. Manual on Greenhouse Technology. Agriculture Information Resource Centre. Essensho Company Ltd. Nairobi, Kenya.

O'Donnell, K., Kistler, H. C., Cigelnik, E. and Ploetz. R. C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences* 95; 2044–2049.

O'Donnell, K. O., Ward, T. J., Robert, V. A. R. G., Crous, P. W., Geiser, D. M. and Kang, S. 2015. DNA sequence-based identification of Fusarium: Current status and future directions. *Phytoparasitica* 1-13.

Ojiambo, P. S. and Scherm, H. 2006. Biological and application-oriented factors influencing plant disease suppression by biological control. A meta-analytical review. *Phytopathology* 96; 1168-1174.

Okporie, E. O., Chukwu, S. C. and Onyishi, G. C. 2014. Influence of plant age ,tomato variety and nematode inoculum density on pathogenicity of *Meloidogyne incognita* on tomato in Abakaliki Agro-Ecology. *IOSR Journal of Agriculture and Veterinary Science* 7(1); 45–50.

Oliveira, D., Maria, C., Almeida, A., Barbosa, C., Luiza, M., Bastos, R., Tereza, M. and Lucia, V. 2013. Identification of races 1, 2 and 3 of *Fusarium oxysporum* f. sp. *lycopersici* by molecular markers. *African Journal of Microbiology Research* 7(20); 2324–2331.

Onkendia, E. M., Kariuki, G. M.,Marais, M. and Moleleki, L. N.2014. The threat of root-knot nematodes *Meloidogyne* spp. in Africa. a review: *Plant Pathology* 63, 727-737.

Oostendrop, M. and Sikora, R. A. 1989. Utilization of antagonistic rhizobacteria as a seed treatment for the biological control of *Heterodera schachtii* in sugarbeet. *Nematology* 12; 77–83.

Oruko, L. and Ndungu, B. 2001. Final social-economic report of the peri-urban vegetable IPM cluster. CABI/KARI/HRI/ University of Reading/IACR Rothamsted Collaborative Project.

Paminondas, E. and Paplomatas, K. 2004. Molecular diagnostics for soilborne fungal pathogens. *Phytopathology Mediterrenean* 43 (2); 213 – 220.

Pandey, K. K. and Gupta, R. C. 2011. Management of Fusarium wilt of tomato by application of bioagents and salicylic acid. *Indian Phytopathology* 65(1); 94–96.0

Pandey, V. K. 2015. Studies on interaction between root knot nematode (*Meloidogyne incognita*) and wilt fungus (*Fusarium oxysporum* f. sp. *ciceri*) in Chickpea (*Cicer arietinum).* MSc. Thesis, College of Agriculture, JNKVV, Jabalpur, Madhya Pradesh.

Pareja-Jaime, Y., Roncero, M. I. G. and Ruiz-Roldán, M. C. 2008. Tomatinase from *Fusarium oxysporum* f. sp. *lycopersici* is required for full virulence on tomato plants. *Molecular plant-microbe interactions* 21; 728–736.

Parmar, P., Oza, V. P. and Subramanian, R. B. 2010. Optimization of fusaric acid production by *Fusarium oxysporum* f. sp. *lycopersici* using response surface methodology. *Indian Journal of Science and Technology* 3(4); 411–416.

Pau, C. G., Leong, C. T. S., Wong, S. K., Eng, L., Jiwan, M., Kundat, F. R., Aziz, Z. F. B. A., Ahmed, O. H. and Majid, N. M. 2012. Isolation of indigenous strains of *Paecilomyces lilacinus* with antagonistic activity against *Meloidogyne incognita*. *International Journal of Agriculture and Biology* 14; 197–203.

Pest Control Products Board. 2017. Full List of Registered Products.www.pcp.or.ke.accessed on 20th Jan 2018.

Philip, R. G. Robertson and Diana W. F.1995. The spatial distribution of nematode trophic groups across a cultivated ecosystem *Ecology*, 76(5);1425-1432.

Pritesh, P. and Subramanian, R. B. 2011. PCR based method for testing Fusarium wilt resistance of tomato. *African Journal of Basic and Applied Sciences* 3(5); 222.

Regaieg, H. A., Ciancio, N. H., Horrigue, G. B. and Rosso. L. 2010. Effects of culture filtrates from the nematyophagous fungus *Verticillium leptobactrum* on viability of the root-knot nematode *Meloidogyne incognita. World Journal of Microbiology and Biotechnology* 26; 2285-2289.

Rehman, S., Lawrence, R., Kumar, E. J. and Badri, Z. A. 2012. Comparative efficacy of *Trichoderma viride*, *T. harzianum* and carbendazim against damping-off disease of cauliflower caused by *Rhizoctonia solani* kuehn. *Journal of Biopesticides* 5(1); 23-27.

Reis, A., Hélcio C., Leonardo, S. B. and Carlos, A. L. 2005. First Report of *Fusarium oxysporum* f. sp. *lycopersici* Race 3 on tomato in Brazil. *Fitopatologia Brasilia* 30(4); 426–428.

Renčo, M. 2013. Organic amendments of soil as useful tools of plant parasitic nematodes control. *Helminthologia* 50; (1): 3.

Ridomil Gold EC EPA-EPA-Syngenta-US. (accessed 2018 June 2).www.syngenta-us.com

Rini, C. R. and Sulochana, R. K. 2007. Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato. *Journal of Tropical Agriculture* 45: 21-28.

Roberts, P. A. and Thomason, I. J. 1989. A review of variability in four *Meloidogyne* spp. measured by reproduction on several hosts including Lycopersicon. *Agricultural Zoology Review* 3; 225–52.

Rumbos, C. I., Khah, E. M. and Sabir, N. 2011. Response of local and commercial tomato cultivars and rootstocks to *Meloidogyne javanica* infestation. *Australian Journal of Crop Science* 5(11); 1388–1395.

Saikia, R. and Kadoo, C. N. 2010. Molecular detection and identification of *Fusarium oxysporum.* In: Gherbawy, Y. and Voigt, K. (eds.). Molecular Identification of Fungi pp.1-29.

Sally, A. M., Randal, C. R. and Richard, M. R. 2006. Fusarium and Verticillium wilts of Tomato, Potato, Pepper and Egg plant. The Ohio State University Extension.

Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, Vol. 3. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Schenck, S. 2004. Control of nematodes in tomato with *Paecilomyces lilacinus* Strain 251. *Vegetable Report* 5;1-5.

Schwarz, D. and Grosch, R. 2003. Influence of nutrient solution concentration and a root pathogen on tomato root growth and morphology. *Scientia Horticulturae* 97; 109–120.

Selvakumar, V., Ramamurti, A., Vijayakumar, N., Gerold, A., Kumar, K. R. and Panneerselvam, A. 2014. Biological suppression of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Synder and Hans using antagonistic fungus *Trichoderma harzianum* (Rifai) in tomato (*Lycopersicon esculentum* (Mill). *International Journal of Current Research* 6(2); 4962-4964.

Semagn, K., Bjornstad, A. and Ndjiondjop, M. N. 2006. An overview of molecular marker methods in plants. *African Journal of Biotechnology* 5 (25); 2540 – 2568.

Serfoji, P., Rajeshkumar, S. and Selvaraj, T. 2010. Management of root-knot nematode, *Meloidogyne incognita* on tomato cv Pusa Ruby by using vermicompost, AM fungus, *Glomus aggregatum* and mycorrhiza helper bacterium, *Bacillus coagulans*. *Journal of Agricultural Technology* 6(1); 37-45.

Sharon, E., Bar-Eyal, M., Chet, I., Herrera- Estrella, A., Kleifeld, O. and Spiegel, Y. 2001. Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. *Phytopathology* 91; 687-693.

Sheu, Z. M. and Wang, T. C. 2006. First report of race 2 of *Fusarium oxysporum* f. sp. *lycopersici*, the causal agent of Fusarium wilt on tomato in Taiwan. *The American Phytopathological Society* 90 (1); 111.

Sikora, R. A. 1992. Management of the antagonistic potential in agricultural ecosystems for the biological control of plant-parasitic nematodes. *Annual Review of Phytopathology* 30; 245-270.

Sikora, R. A. and Fernandez, E. 2005. Nematode parasites of vegetables, pp. 319-392. In: Luc, M., Sikora, R.A, Bridge, J. (Eds.). Plant Parasitic Nematodes in Subtropical and Tropical Agriculture, CABI Publishing, Wallingford, UK.

Singh, S. K. 2007. Susceptibility of six tomato cultivars to the root-knot nematode, *Meloidogyne incognita*, *The South Pacific Journal of Natural Science* 72-75.

Singh, K. P. 2002. Eco-friendly approaches for plant disease management-a report. *Journal of Scientific and Industrial Research*; 239-240.

Singh, R., Biswas, S. K., Nagar, D., Singh, J., Singh, M. and Mishra, Y. K. 2015. Sustainable integrated approach for management of Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Synder and Hansen. *Sustainable Agriculture Research* 4(1); 138-147.

Smith, S. N. 2007. An overview of ecological and habitat aspects in the Genus Fusarium with special emphasis on the soil borne pathogenic forms. *Plant Pathology Bulletin* 16; 97-120.

Srivastava, N. and Tewari, J. P. 2011. Study and management of disease complex caused by root knot nema- tode and root wilt fungus on tomato. *International Referred Reseach Journal* (19); 2009–2011.

Staniaszek, M., Kozik, E. U. and Marczewski, W. 2007. A CAPS marker TAO1902 diagnostic for the I-2 gene conferring resistance to *Fusarium oxysporum* f. sp. *lycopersici* race 2 in tomato. *Plant Breeding* 126; 331–333.

Suhardi, A., Dede, R. and Lukman, H. 1980. The persistence of Ohio MR. 9 tomato resistance to Fusarium wilt in nematode infested soil. *Bulletin Penelitian Hortikultura* 8(4); 19-27.

Tanyolaç, B. and Akkale, C. 2010. Screening of resistance genes to Fusarium root rot and Fusarium wilt diseases in F3 family lines of tomato (*Lycopersicon esculentum*) using RAPD and CAPs markers. *African Journal of Biotechnology* 9(19); 2727-2730.

Taylor, A. L. and Sasser, J. N. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* spp). North Carolina State University Graphics,

Cooperative Publication of Department of Plant Pathology, North Carolina State University and USAID, Washington.

Thomashow, L. S., Weller, D. M., Mavrodi, O. V. and Mavrodi, D. V. 2007. Selecting monitoring, and enhancing the performance of bacterial biocontrol principles, pitfalls, and progress. In: Vurro M, Gressel J, editors. Novel biotechnologies for biocontrol agent enhancement and management. New York: Springer; p. 87–105.

Tinyami, E. T., Choi, J. W., Tumenta, T. S., Eko, A. and Chick, O. A. 2014. Small-Scale tomato cultivators' perception on pesticides usage and practices in Buea Cameroon. *Health* 6; 2945-2958.

Tusiime, S. M. 2014. Evaluating horticultural practices for sustainable tomato production in Kamuli, Uganda. MSc. Thesis, Lowa State University, Ames Iowa.

Tzortzakakis, E. A., Adam, M. A. M., Blok, V. C., Paraskevopoulos, C. and Bourtzis, K. 2005. Occurrence of resistance-breaking populations of root-knot nematodes on tomato in Greece. *European Journal of Plant Pathology* 113; 101–105.

UNEP. 2001. United Nations Environment Program, Ozone Action Programme, Source Book of Technologies for Protecting the Ozone Layer: Alternatives to Methyl Bromide **(**www.uneptie. org/ozonaction.html).

Vakalounakis, D. J. and Fragkiadakis, G. A. 2008. Genetic variation among *Fusarium oxysporum* isolates from cucumber. *EPPO Bull* 30; 175-177.

Van der Does, H. C., Lievens, B., Claes, L., Houterman, P. M., Cornelissen, B. J. C. and Rep, M. 2008. The presence of a virulence locus discriminates *Fusarium oxysporum* isolates causing tomato wilt from other isolates. *Environmental Microbiology* 10; 1475–1485.

Varela, A. M., Seif, A. and Lohr, B. 2003. A guide to Integrated Pest Management (IPM) in Tomato Production in Eastern and Southern Africa. ICIPE Science Press, Nairobi.

Vauramo and Setälä. H.2010. Urban belowground food-web responses to plant community manipulation – Impacts on nutrient dynamics, *Landscape and Urban Planning* 97; 1-10

Verdejo-lucas, S., Cortada, L., Sorribasb, F. J. and Ornat, C. 2009. Selection of virulent populations of *Meloidogyne javanica* by repeated cultivation of Mi resistance gene tomato rootstocks under field conditions. *Plant Pathology* 58; 990–998.

Verdejo-Lucas, S. Talavera,M. M. F. Andrés, M. F. 2012. Virulence response to the Mi.1 gene of *Meloidogyne* populations from tomato in greenhouses. *Crop Protection* 39; 97-105.

Waiganjo, M. M., Wabule, N. M., Nyongesa, D., Kibaki, J. M., Onyango, I., Webukhulu, S. B. and Muthoka, N. M. 2006. Tomato production in Kiriyanga District, Kenya. A baseline survey report. KARI/IPM-CRSP Collaborative project.

Walid, N., Jim, M., Steve, W. 2010.The efficiency of *Trichoderma harzianum* and *Aneurinobacillus migulanus* in the control of gladiolus corm rot in soil-less culture system. *American Journal of Agricultural and Biological Science* 5 (4); 436-445.

Wang, K. H, McSorey, R. and Gallaher, R. N. 2004. Effect of *Crotalaria juncea* amendment on squash infected with *M. incognita*. *Journal of Nematology* 36(3); 290–296.

Wanjohi, J. W., Wafula, G., O., Macharia, C. M. 2018. Integrated Management Fusarium wilt-root knot nematode complex on tomato in central highlands of Kenya. *Sustainable Agriculture Research* 7(2); 8-18.

Wasilewska, L. 1979. The structure and function of soil nematode communities in natural ecosystems and agrocenoses. *Polish Ecological Studies* 5; 97-145.

Waudo, S. W., Owino, P. O. and Kuria, M. 1995. Control of Fusarium wilt of tomatoes using soil amendments. *East African Agriculture and Forestry Journal* 60; 207-217.

Whitehead, A. G. and Hemming, J. R. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* 55; 25-38.

Windham, G. L., Windham, M. T. and William, W. P. 1989. Effect of *Trichoderma* spp. on maize growth and *Meloidogyne arenaria* reproduction. *Plant Disease* 73; 493-495.

Yang, K., Lule, U. and Xiao-Lin, D. 2006. Lycopene: Its properties and relationship to human health. *Food Review International* 22; 309–333.

Yazdi, A., Gharabadiyan, F., Jamali, S. and Eskandari, A. 2012. Source of resistance to root-knot nematode *Meloidogyne javanica* in tomato cultivars. *Journal of Agricultural Technology* 8(6); 2011-2021.

Yeates, G. W. Bongers, T., De Goede, R. G. M., Freckman, D. W. and Georgieva, S. S. 1993. Feeding-habits in soil nematode families and genera - an outline for soil ecologists. *Journal of Nematology* 25(3); 315–331.

Yeates, G. W., Ferris, H., Moens, T. and Putten, V. 2009. The role of nematodes in ecosystems. In: Wilson, M. J. and Kakouli-Duarte, T.eds. Nematodes as environmental indicators. CAB International, Cambridge, U.S.A.

Yildiz, Ş., 2012. Nematode biodiversity in a semi-arid pasture under different grazing regimes. *African Journal of Agricultural Research* 7(3); 414–417.

Yin-dong, C., Bin, D., Ling, W., Pei, J., Yu-jie, X., Xin-feng, L., Zhi-gang, L. and Jianming, W. 2018. A study on the pathogen species and physiological races of tomato Fusarium wilt in Shanxi. *Journal of Integrative Agriculture* 17(0); 60345-7.

Zareen, A., Javed, N. and Zaki, J. M. 2002. Field evaluation of *Pasteuria* isolates for the control of root-knot nematodes, *Meloidogyne javanica* on tomato. *Plant Pathology Journal* 1(1); 14-16.

Zhang, X. K., Li, Q., Liang, W. J., Zhang, M., Bao, X. L. and Xie, Z. B. 2013. Soil nematode response to biochar addition in a Chinese wheat field. *Pedosphere* 23(1); 98–103.

Zheng, G. D., Shi, L.B., Wu, H.Y., Peng, D. L. 2011. Nematode communities in continuous tomato- cropping field soil infested by root-knot nematodes, *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science* 62(3); 216-223.

APPENDICES

APPENDIX 1 QUESTIONNAIRE

CONSTRAINTS ENCOUNTERED IN THE FIELD DURING TOMATO CULTIVATION IN MWEA WEST SUB COUNTY KIRINYAGA COUNTY, KENYA.

1.0 HOUSEHOLD CHARACTERISTICS

1.1 Name of the farmer ……Location/Zone……

1.2 Division.....

1.3 Gender of the head of household 1= Male……2=Female

1.4 Age of farmer in years: 1= 20-30 2= 31-40 3= 41-50

4= 51- 60 5= ≥ 61

1.5 Highest level of education for the head of the household

1 = None, 2 = Primary, 3 = Secondary,

4 = Tertiary, 5 = University

2.0 Land use Practices

2.1 What is the size of your farm……Acres

2.2 What is the type of land ownership?

1= Own 2= Family owned 3= Communal

4= Rented/Hired 5= Others ……

2.3 What is the main farm use? Tick appropriately:

1= Crops 2= Livestock – (cattle, poultry etc)

3= Woodlot//agro-forestry 4= Other (specify)……..

2.4 What reasons influence the size of land allocated to the different uses?

1=Food security 2= Income generation 3= Size of family

4= Social status 5= Others (specify)……

2.5 What are the major crops grown on your farm?

Crop	Acreage	Main use 1=household, 2=Sale	Rank1=Most important, 2 = 2nd most important, 3 =3rd in importance
1			
2			
3			
4			
5			

2.6 If growing tomatoes, what cropping patterns do you practice?

1= Mono cropping 2= Inter-cropping 3= Relay cropping

4= Others (specify)…….

Give a reason for your answer.....

2.7 Do you rotate your tomato crop with any other crop?

1=Yes 2=No

Give a reason for your answer……..

If yes, what crop(s) do you rotate tomato with…….

2.8 which varieties of tomato do you grow on your farm? _____ Why do you prefer the varieties? _____ _____

3.0 Diseases/pests of tomatoes

3.1 Are there any diseases/pests that attack tomatoes in the field? 1= Yes 2= No

3.2 If yes, name the diseases or disease symptoms and rank them based on the losses that they cause

Disease/symptoms/pests	Ranking	*Control measures*
	1. 2. 3.	*1.* *2.* *3*
	1. 2. 3.	*1.* *2.* *3*
	1. 2. 3.	*1.* *2.* *3*
	1. *2.* *3.*	*1.* *2.* *3*

Disease ranking-1-Heavy loss; 2-Moderate loss; 3-Slight loss

3.3 Have you observed wilting of tomato plants 1=Yes 2=No

If yes, what do you attribute the wilt to…….....

3.4 What proportion of plants are affected in the field ……..

Propotion of plants	Farmers response
1-10%	
11-20%	
21-30%	
31-40%	
41-50%	

3.6 What are the main sources of seedlings and what proportion do you obtain from each source?

Source	Proportion (half, quarter etc)	Reasons
Own farm		
Commercial seedling nurseries		
Local market		

4.0 Farm inputs

4.1Do you apply any pesticides into the soil.1= Yes 2= No

If yes, what is the target disease?…… and what is the pesticide applied?.....

5.0 Agricultural information

5.1 In the past one year has the household had access to agricultural information?

1 = Yes, 2 = No

5.2 What are main sources of agricultural information in order of preference?

Source of information	***Rank the 3 most important sources using the scores*** 1= Most preferred, 2 = somehow preferred, 3 = Least preferred)
Government extension staff	
Private extension staff	
NGOs	
Field day	
Other farmers	
Mass media (Radio/TV)	
Print media	
Family and friends	
Agrovet shops	
Neighbors	
Internet	
Cellphones	

Appendix 2 Composition of Different Media

Media	Composition
Potato Dextrose Agar (PDA)	39 g in a litre of disilled water
Water Agar	10 g agar, 50 mg streptomycin, 50 mg chloramphenical
Dilution media	One gram agar in a one litre distilled water
Czapek Dox broth	35.01g in one litre of distilled water

Appendix 3 Physico- chemical properties of soil used in greenhouse experiments

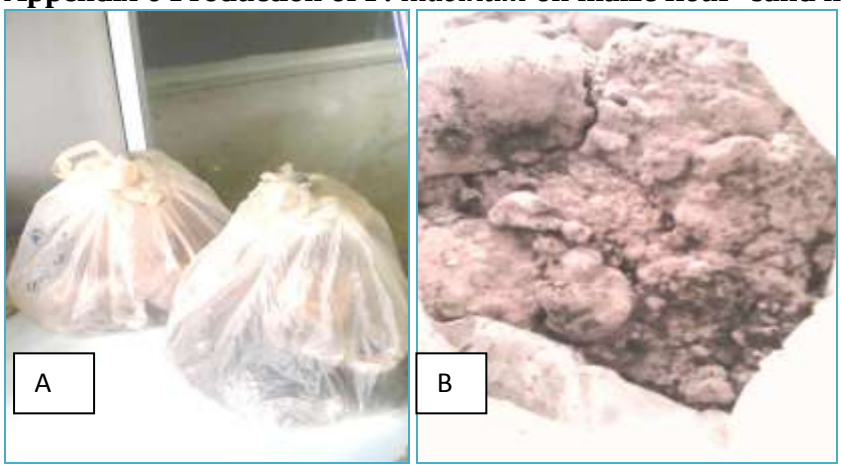
Property	Content
pH	6.45
% Organic Carbon	2.21
%Total Nitrogen	0.28
ppm P	79
cmol/kg CEC	18.6
cmol/kg K	1.03
Texture class	sandy clay loam

Appendix 4 Disease severity scale done based on length of discoloured vascular tissue Using a scale of 0-4, where 0= no discoloured tissue, 1=1-25%, 2=26-49%, 3=50-74%, 4=75-100% (Waudo *et al.*, 1995).

Appendix 5 Ingredients and their approximate amount in neem

Property	Approximate content
pH	5.7 -8.7
Carbon Nitrogen Ratio	≤19:1
Nitrogen	1-3%
Moisture	8-18%
Organic Matter % by weight	70%
Organic Carbon by weight	10-15%
Calcium	1%
Magnesium	0.5%
Sulphur	1%
Boron	0.015%
Cobalt	0.02%
Copper	0.15%
Iron	0.15%
Magnesium	0.03%

Appendix 6 Production of *P. lilacinum* on maize flour- sand media



Production of *P. lilacinum* inoculum. A. 1kg (maize flour, sand media) inoculated with mycelia of *P.lilacinum.* B. Surface growth of *P. lilacinum* on the media