

**EFFECTIVENESS OF BIO-CONTROL AGENTS IN THE MANAGEMENT OF EARLY
BLIGHT OF TOMATOES**

MATUMWABIRHI KULIMUSHI

(BSc BOTANY, UNIVERSITE OFFICIELLE DE BUKAVU)

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE IN PLANT PATHOLOGY**

DEPARTMENT OF PLANT SCIENCE AND CROP PROTECTION

FACULTY OF AGRICULTURE

UNIVERSITY OF NAIROBI

2019

DECLARATION

I, Matumwabirhi Kulimushi, hereby declare that this thesis is my original work and has never been submitted for a degree in any other University.

Matumwabirhi Kulimushi

Signature..... Date.....

This thesis has been submitted for examination with our approval as University supervisors:

Dr. William Maina Muiru

Signature..... Date.....

Department of Plant Science and Crop Protection

University of Nairobi

Prof Eunice Wanjiru Mutitu

Signature..... Date.....

Department of Plant Science and Crop Protection

University of Nairobi

DEDICATION

Dedicated to my parents Sébastien Mufungizi and Melida Byamungu; siblings, uncles, aunts, cousins, nephews and friends.

ACKNOWLEDGEMENTS

I am thankful to the almighty God for his endless grace, protection, health and guidance throughout the study period.

I thank the University of Nairobi for allowing me to pursue my Masters' in Plant Pathology at the esteemed institution and for the academic support. I wish to thank my academic supervisors; Dr William Maina Muiro and Prof Eunice Wanjiru Mutitu for their invaluable guidance and assistance from the beginning to the completion of this study. Mr Peter Mongare, my lecturer of Biometrics is also greatly appreciated for guidance on data analysis.

I also thank all the technicians and staff of the Department of Plant Science and Crop Protection, University of Nairobi for the guidance and assistance during the whole study. For my field work, I thank all the technicians and staff of Kabete Field Station and Kenya Agriculture and Livestock Research Organization (KALRO) Mwea for their great assistance. The pesticide efficacy trial unit of the Department of Plant Science and Crop Protection, University of Nairobi is also greatly appreciated for their assistance for the greenhouse experiments. I thank my fellow students for the fruitful academic discussions and the mutual support during this study. My thanks are extended to my special friends and colleagues for their company, support and encouragements in different ways during the entire study.

I am also grateful to ‘‘Bourse d'Excellence Bringmann aux Universit es Congolaises’’ (BEBUC) scholarship system for providing the financial support for my Masters' Program.

Special appreciations are expressed to all my family members for their prayers, understanding, support and encouragement throughout this Masters' program.

To anyone who in one way or another contributed to the completion of this work, I express my gratitude to you and may God bless you all.

TABLE OF CONTENTS

DECLARATION.....	i
DEDICATION	ii
ACKNOWLEDGEMENTS	iii
List of Tables.....	ix
List of Figures	xi
List of Plates.....	xii
List of Appendices.....	xiii
LIST OF ABBREVIATIONS	xiv
ABSTRACT	xvi
CHAPTER ONE: INTRODUCTION	1
1.1. Background information.....	1
1.2. Problem statement	3
1.3. Study justification.....	5
1.4. Objectives.....	6
1.4.1. Main objective.....	6
1.4.2. Specific objectives.....	6
1.5. Hypotheses	6
CHAPTER TWO: LITERATURE REVIEW	7
2.1. Origin of tomatoes.....	7

2.2. Botanical description of tomatoes	7
2.3. Requirements for growth of tomato plants.....	8
2.4. Health benefits of tomatoes	9
2.5. Economic importance of tomatoes	9
2.6. Constraints to tomato production in Kenya.....	13
2.7. Overview on tomato early blight.....	14
2.7.1. Description of tomato early blight causative agent	14
2.7.2. Infection of tomato plant by <i>Alternaria solani</i>	15
2.7.3. Symptoms caused by early blight on tomatoes	16
2.7.4. Disease cycle and source of inoculum	17
2.7.5. Environmental conditions favoring tomato early blight.....	18
2.7.6. Global distribution of tomato early blight.....	19
2.7.7. Management of tomato early blight	19
2.8. Overview on bio-control agents	21
2.8.1. Description and modes of action for <i>Bacillus subtilis</i>	21
2.8.2. Description and modes of action for <i>Pseudomonas fluorescens</i>	23
2.8.3. Description and modes of action of <i>Trichoderma</i> species	24
 CHAPTER THREE: EFFECTS OF BIO-CONTROL AGENTS ON RADIAL GROWTH OF	
<i>Alternaria solani</i>	27
3.1. Abstract	27

3.2. Introduction	28
3.3. Materials and methods.....	29
3.3.1. Isolation and identification of <i>Alternaria solani</i>	29
3.3.2. Inoculum preparation	30
3.3.3. Raising of tomato seedlings	30
3.3.4. Pathogenicity test of <i>Alternaria solani</i>	30
3.3.5. Isolation and identification of <i>Trichoderma</i> isolates.....	31
3.3.6. Isolation of <i>Pseudomonas fluorescens</i> from commercial formulation.....	31
3.3.7. <i>In vitro</i> activity of bio-control agents against <i>Alternaria solani</i>	31
3.3.8. Data analysis.....	33
3.4. Results	34
3.4.1. Isolation and identification of <i>Alternaria solani</i>	34
3.4.2. Pathogenicity test for <i>Alternaria solani</i>	34
3.4.3. Isolation and identification of <i>Trichoderma</i> isolates.....	35
3.4.4. Activity of bio-control agents against <i>Alternaria solani</i>	36
3.4.4.1. Preliminary screening of <i>Bacillus</i> isolates against <i>Alternaria solani</i>	36
3.4.4.2. <i>In vitro</i> activity of <i>Bacillus subtilis</i> isolates selected from preliminary screening and <i>Pseudomonas fluorescens</i> against <i>Alternaria solani</i>	37
3.4.4.3. Preliminary screening of <i>Trichoderma</i> isolates against <i>Alternaria solani</i>	38

3.4.4.4. <i>In vitro</i> activity of <i>Trichoderma</i> isolates selected from preliminary screening against <i>Alternaria solani</i>	39
3.5. Discussion	41
CHAPTER FOUR: EFFECTIVENESS OF BIO-CONTROL AGENTS IN THE MANAGEMENT OF TOMATO EARLY BLIGHT IN THE FIELD AND THE GREENHOUSE	
	45
4.1. Abstract	45
4.2. Introduction	46
4.3. Material and methods	47
4.3.1. Description of the study sites	47
4.3.2. Sampling protocol for field evaluations	48
4.3.3. Production of culture filtrates from bio-control agents	48
4.3.3.1. Production of cultures filtrates from <i>Bacillus subtilis</i> isolates.....	48
4.3.3.2. Production of culture filtrates from <i>Trichoderma</i> isolates	49
4.3.4. Application of the treatments for greenhouse and field experiments.....	50
4.3.5. Field evaluation of bio-control agents.....	50
4.3.6. Greenhouse evaluation of bio-control agents.....	52
4.3.7. Assessment of plant growth parameters	53
4.3.8. Data analysis.....	53
4.4. Results	54
4.4.1. Effects of bio-control agents on tomato early blight percent disease incidence	54

4.4.2. Effects of bio-control agents on tomato early blight percent disease severity	57
4.4.3. Effects of bio-control agents on tomato early blight percent disease index.....	60
4.4.4. Effects of bio-control agents on tomato early blight area under disease progress curve	63
4.4.5. Effects of bio-control agents on tomato plant growth parameters	66
4.4.6. Effects of bio-control agents on the quantity of marketable tomato fruits.....	67
4.4.7. Correlations between tomato early blight disease parameters and plant growth parameters.....	69
4.4.8. Site interactions between Kabete Field Station and KALRO Mwea for tomato early blight disease parameters and tomato plant growth parameters	73
4.5. Discussion	74
CHAPTER FIVE: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS	80
5.1. General discussion.....	80
5.2. Conclusion.....	82
5.3. Recommendations	83
CHAPTER SIX: REFERENCES	84
APPENDICES.....	106

List of Tables

Table 2.1: Tomato production from the major tomato producing countries in the world	10
Table 2.2: Tomato production from the major tomato producing countries in the East African Community	11
Table 3.1: <i>In vitro</i> activity of <i>Bacillus subtilis</i> isolates selected from preliminary screening and <i>Pseudomonas fluorescens</i> against <i>Alternaria solani</i>	38
Table 3.2: <i>In vitro</i> activity of <i>Trichoderma</i> isolates selected from preliminary screening against <i>Alternaria solani</i>	40
Table 4.1: Effects of bio-control agents on tomato early blight percent disease incidence at Kabete Field Station.....	54
Table 4.2: Effects of bio-control agents on tomato early blight percent disease incidence at KALRO Mwea.....	55
Table 4.3: Effects of bio-control agents on tomato early blight percent disease incidence in the greenhouse	56
Table 4.4: Effects of bio-control agents on tomato early blight percent disease index at Kabete Field Station	61
Table 4.5: Effects of bio-control agents on tomato early blight percent disease index at KALRO Mwea.....	62
Table 4.6: Effects of bio-control agents on tomato early blight percent disease index in the greenhouse	63
Table 4.7: Effects of bio-control agents on quantity of marketable tomato fruits (Tons/hectare) at Kabete Field Station.....	67

Table 4.8: Effects of bio-control agents on the quantity of marketable tomato fruits (Tons/hectare) at KALRO Mwea.....	68
Table 4.9: Effects of bio-control agents on quantity of marketable tomato fruits (Kg/Plant) in the greenhouse	69
Table 4.10: Correlations between tomato early blight parameters and plant growth parameters at Kabete Field Station.....	70
Table 4.11: Correlations between tomato early blight parameters and plant growth parameters at KALRO Mwea.....	72

List of Figures

Figure 2.1: Tomato production trends in Kenya (FAOSTAT, 2019)	12
Figure 3.1: Mean diameter of <i>Alternaria solani</i> in the presence of <i>Bacillus</i> isolates.....	37
Figure 3.2: Mean <i>Alternaria solani</i> diameter in the presence of <i>Trichoderma</i> isolates	39
Figure 4.1: Effects of bio-control agents on tomato early blight disease progress curve at Kabete Field Station.....	58
Figure 4.2: Effects of bio-control agents on tomato early blight disease progress curve at KALRO Mwea.....	59
Figure 4.3: Effects of bio-control agents on tomato early blight disease progress curve in the greenhouse	60
Figure 4.4: Effects of bio-control agents on tomato early blight area under disease progress curve at Kabete Field Station.....	64
Figure 4.5: Effects of bio-control agents on tomato early blight area under disease progress curve at KALRO Mwea	65
Figure 4.6: Effects of bio-control agents on tomato early blight area under disease progress curve in the greenhouse	66

List of Plates

Plate 2.1: Conidia of <i>Alternaria solani</i> (Kemmitt, 2002)	15
Plate 2.2: Tomato foliage affected by early blight (Kemmitt, 2002).....	17
Plate 3.1: Morphological and cultural characteristics of isolated <i>Alternaria solani</i>	34
Plate 3.2: Early blight symptoms on infected tomato leaf and healthy tomato leaf	35
Plate 3.3: Morphological and cultural characteristics of <i>Trichoderma</i> isolates.....	36
Plate 3.4: Effects of bacterial antagonists on the radial growth of <i>Alternaria solani</i>	38
Plate 3.5: Effects of <i>Trichoderma</i> isolates on the radial growth of <i>Alternaria solani</i>	40

List of Appendices

Appendix 1: Analysis of variance for the percent disease incidence at Kabete Field Station....	106
Appendix 2: Analysis of variance for the percent disease incidence at KALRO Mwea	106
Appendix 3: Analysis of variance for the percent disease incidence in the greenhouse	106
Appendix 4: Analysis of variance for the percent disease severity at Kabete Field Station	107
Appendix 5: Analysis of variance for the percent disease severity at KALRO Mwea.....	107
Appendix 6: Analysis of variance for the percent disease severity in the greenhouse	107
Appendix 7: Analysis of variance for the percent disease index at Kabete Field Station	108
Appendix 8: Analysis of variance for the percent disease index at KALRO Mwea	108
Appendix 9: Analysis of variance for the percent disease index in the greenhouse.....	108
Appendix 10: Analysis of variance for site interaction for area under disease progress curve..	109
Appendix 11: Analysis of variance for site interaction for the percent disease incidence	109
Appendix 12: Analysis of variance for site interaction for the quantity of marketable fruits	109

LIST OF ABBREVIATIONS

%	Percent
µl	Microliter
µm	Micrometer
°C	Degrees Celsius
AEZ II	Agro-ecological zone II
AEZ III	Agro-ecological zone III
ANOVA	Analysis of Variance
AUDPC	Area Under Disease Progress Curve
Cm	Centimeter
RCBD	Randomized Complete Block Design
CRD	Completely Randomized Design
FAOSTAT	Food and Agriculture Organization Statistics
Ha	Hectare
HCDA	Horticultural Crop Development Authority
KALRO	Kenya Agriculture and Livestock Research Organization
KEPHIS	Kenya Plant Health Inspectorate Service
Kg	Kilogram
KHCP	Kenya Horticulture Competitiveness Project
LM4	Lower Midland 4
LSD	Least Significant Difference
M	Meter
ml	Milliliter

Mm	Millimeter
PDA	Potato Dextrose Agar
USA	United States of America

ABSTRACT

Tomato, a major vegetable widely used in Kenya faces a number of production challenges along with diseases like late blight, early blight and bacterial wilt. In this study, bio-control agents (BCAs) which are deemed to be environmentally friendly were used for the management of early blight, a major disease of tomato. BCAs including two *Trichoderma* isolates coded Tricho 7 and Tricho 10, two *Bacillus subtilis* isolates coded CA51 and CB12 and *Pseudomonas fluorescens* (from commercial Bio-cure) were tested for their effectiveness in managing *Alternaria solani* *in vitro*. The experiments were carried out in Plant Pathology Laboratory at the Department of Plant Science and Crop Protection, University of Nairobi. The dual culture technique was used. The experimental design was a Completely Randomized Design in five replicates. Diameter of *A. solani* colony was measured and used to calculate the percent growth inhibition. Means were compared using Fisher's protected least significant difference (LSD) test at 5%. Tricho 7 and Tricho 10 were the most effective against the radial growth of *A. solani* with percent growth inhibition of 80.9 and 82.2% for Tricho 7 and Tricho 10 respectively. These were followed by CA51 and CB12 with percent growth inhibition of 56.6 and 54.1% respectively. *Pseudomonas fluorescens* also hindered *A. solani* radial growth but with a lower percent growth inhibition of 47.6%. The same BCAs were evaluated for their effectiveness in managing tomato early blight under greenhouse and field conditions. Water and Tower (Metalaxyl 8% and Mancozeb 64%) were used as control and standard check respectively. Greenhouse evaluations were carried out at Kabete Field Station. The experimental design was a Completely Randomized Design in four replicates. Data were collected on disease and plant parameters. The percent disease index by the 90th day after transplanting was significantly lower in all treatments than in the control. Isolate CB12 recorded the lowest percent disease index of 28.3% which was comparable to the standard

chemical at 30.5% and both were significantly different from the control at 61.6%. The highest mean quantity of marketable fruits of 0.21 kg/plant was recorded with Tricho 7, followed by the standard chemical with a comparable yield of 0.20 kg/plant. Control treatment recorded significantly lower marketable fruit weight of 0.06 Kg/plant. Field evaluations were carried out at Kabete Field Station and at Kenya Agricultural and Livestock Research Organization (KALRO) Mwea. A Randomized Complete Block Design in triplicate was used. At both experimental sites, on the 90th days after transplanting, the percent disease index was significantly lower in all the treatments compared to the control. The lowest percent disease index recorded for the BCAs was with Tricho 10 at 35.0% and was comparable to the standard chemical at 30.3%. The two were significantly lower than the control at 68.8%. As for yield of marketable fruits, Tricho 10 recorded significantly higher mean weight at 10.5 tons/hectare compared to the control which recorded 3.8 tons/hectare. However, the standard chemical recorded significantly higher yield at 11.7 tons/hectare compared to Tricho 10.

BCAs are effective in managing early blight *in vitro* and under greenhouse and field conditions and minimize the effects of early blight on tomato production.

Key words: bio-control agents, management, tomato and tomato early blight.

CHAPTER ONE: INTRODUCTION

1.1. Background information

Tomato (*Solanum lycopersicum* L.) a major vegetable grown worldwide (Monte *et al.*, 2013), originated in the western South America, specifically in Peru, Bolivia and Ecuador (Anonymous, 2016). In the 16th and 20th centuries, colonial settlers introduced tomato in Europe and in East Africa respectively (Wener, 2000). Currently, the vegetable is being grown in basically all countries (Abd-El-Kareem *et al.*, 2006). Tomato fruits can be used fresh in salads, prepared as vegetable, or in processed form as tomato paste, tomato sauce, Ketchup and juice. Tomato fruits are beneficial to healthy diet as they contain sufficient amounts of vitamins A, B and C. Additionally; they enclose significant amounts of potassium, ion and phosphorus (Masinde *et al.*, 2011).

Tomatoes are among the most important and commonly grown horticultural vegetables in Kenya and in other parts of East Africa (Sigei *et al.*, 2014). However, production of tomato fruits is hindered by numerous problems including physiological disorders (mainly resulting from water and nutrient stresses) (KALRO, 2005), pests and diseases (Mizubuti *et al.*, 2007; Goufo *et al.*, 2008). As an example, temperature and humidity fluctuations in long rain and short rain seasons are conducive for the development of a number of pathogens and the related diseases resulting in lower tomato yield (Engindeniz and Ozturk, 2013). Insect pests along with; cotton bollworms, whiteflies, melon thrips and tomato leaf miners (Engindeniz and Ozturk, 2013; Islam *et al.*, 2013), significantly contribute to yield losses. Diseases such as bacterial canker, bacterial spots, bacterial wilt, Fusarium wilt, early and late blights, root knot nematodes, tomato spotted virus and yellow leaf curl virus among others are major constraints in tomato production (Goufo *et al.*, 2008; Noling, 2013; Sutanu and Chakrabartty, 2014). Early and late blights are the commonest

fungal constraints in tomato gardens (Hou and Huang, 2006). When these fungi infect tomato leaves, they exhibit symptoms which can rapidly spread on entire leaf blades in conducive environments (Xie *et al.*, 2015).

Tomato early blight is most commonly managed by application of a limited number of chemical compounds as a result of the withdrawal of some effective fungicides reported to have detrimental effects on the environment and on human health (Singh *et al.*, 2011). Due to lack of suitable tomato germplasm, only a few varieties of tomato have been reported to be tolerant to early blight and can be integrated in the management of the disease (Sikora *et al.* 2007; Davies and Spiegel, 2011). Cultural practices are often involved in the management of early blight. These include, sanitation, rotation of tomatoes with non-host crops for at least two to three years, use of pathogen-free seeds and transplants, proper irrigation strategies and maintenance of plant vigor via adequate application of nitrogen and phosphorus fertilizers (Chaerani and Voorrips, 2006; Li, 2012). Treatment of infected seeds with hot water at 50°C for 25 minutes can prevent seedborne infections (Neils *et al.*, 2015). Control of early blight can also be achieved through use of plant extracts including; turmeric, garlic, lemon, ginger among others (Lengai, 2016).

Fluorescent *Pseudomonas* species, *Bacillus* species, *Streptomyces* species and *Trichoderma* species have been reported to have varied activities which hinder growth and development of many plant pathogens (Alabouvette *et al.*, 2006). These BCAs are friendly to the environment and have minimal effects on non-target organisms, including humans, animals and host plants. The mechanisms of action through which BCAs protect plants from pathogen attack are numerous and differ from one BCA to another (Alabouvette *et al.*, 2006). For instance, some BCAs are associated with the production of active extracellular compounds including

siderophores which acts through biological suppression of several soil borne plant pathogens (Alabouvette *et al.*, 2006). In some cases, BCAs have been associated with activities which trigger systemic resistance of host plants against pathogens. *Trichoderma* species have been reported to induce localized and/or systematic resistance to diseases through excretion of secondary metabolites that promote plant growth. These include; ethylene or terpenoid and phytoalexins among others (Alabouvette *et al.*, 2006).

1.2. Problem statement

Early blight is a common disease threatening the production of tomato fruits all over the world and can cause significant yield losses when it is not managed (Adhikari *et al.*, 2017). This may result in increased food insecurity given that tomato is an important source of nutrients and vitamins A, B and C (Giovanelli and Paradise, 2002; Masinde *et al.*, 2011). Deficiency in vitamins is associated with several health problems (Bouis, 2003; Grosso *et al.* 2013; Brescoll and Daveluy, 2015). Tomato is a high value vegetable in Kenya and is a source of livelihood for numerous families (Sigei *et al.*, 2014). Therefore, any threat to tomato production can lead to hunger and poverty among people who depend on the production of tomatoes for their livelihoods.

Synthetic chemicals are being intensively applied by most farmers to lower the intensity of early blight and the accompanying crop losses given that tomato cultivars which are resistant to early blight, are of low agronomic or commercial quality (Yadav and Dabbas, 2012). In addition, the prolonged survival of early blight pathogen in the soil (Foolad *et al.*, 2008) together with the limitation of lands for cultivation (Karuku *et al.*, 2017), have made the rotation strategy not feasible. Furthermore, because of high demand of tomato in the country and pathogen resistance,

farmers increase the rate of chemical application (Waiganjo *et al.*, 2006) and often do not observe the required pre-harvest intervals leading to not only increased chemical residues on the produce but also increased production cost (Fabro and Varca, 2011).

Regular application of synthetic chemicals has detrimental effects on the environment and on human health (Engindeniz and Ozturk, 2013; Bhattacharjee and Dey, 2014). Moreover, regular application of chemicals enhances the development of new fungal biotypes which may be resistant to chemical compounds (Rojo *et al.*, 2007). Since the introduction of systemic fungicides globally in the early 1970s, farmers are increasingly confronted with pathogen resistance to the available chemical compounds due to misuse or abuse in their usage (Sutanu and Chakrabarty, 2014). Synthetic chemicals also kill non-target organisms including pollinating insects (Rhoda *et al.*, 2006; Nderitu *et al.*, 2007).

Consequently, quality assurance standards are being implemented to minimize detrimental effects of farming operations to the environment, reducing the use of chemical inputs to ensure safety to workers, consumers as well as safe guarding animal welfare (Rhoda *et al.*, 2006; Foolad *et al.*, 2008). These concerns have led not only to restrictions or complete banning of some chemical compounds (Rhoda *et al.*, 2006) but also to interceptions of produce with excessive chemical residues at the export market (Wandati, 2014). Therefore, sustainable production of tomatoes inevitably requires the development of plant disease management strategies which are friendly to the environment and have minimal negative effects on humans (Mamgain *et al.*, 2013). Control of plant diseases using biological means and breeding for resistance are one of the most promising plant disease management approaches (Alabouvette *et al.*, 2006). Breeding for resistance strategy has not been successful in managing early blight given that tomato varieties which are tolerant to early blight do not perform well in terms of agronomic traits (Foolad *et al.*,

2002; Yadav and Dabbas, 2012). Extended use of agrochemicals for early blight management can be avoided through the integration of BCAs (Mamgain *et al.*, 2013). Most BCAs are biodegradable, friendly to the environment and have minimal effects on humans and non-targeted organisms, including host plants and beneficial insects (Alabouvette *et al.*, 2006). Antagonistic microorganisms minimize the effects of plant diseases either from microbial interactions directed against plant pathogens or from an indirect action which triggers host plant pathogen resistance (Alabouvette *et al.*, 2006). Several BCAs along with locally available formulations of microorganisms are known to be effective in the managing early blight (Zhao *et al.*, 2008). These include species of *Trichoderma*, *Pseudomonas*, *Bacillus* and *Streptomyces* genera among others. These microorganisms differ in their efficacy in managing tomato early blight (Tapwal *et al.*, 2015).

1.3. Study justification

Integration of BCAs in the management of early blight demands a better understanding of their effectiveness (Ngoc, 2013). Moreover, only a few studies have been conducted to evaluate the efficacy of BCAs in the management of early blight in the field. This study contributed to a better understanding of the effectiveness of *Bacillus* isolates, *Pseudomonas fluorescens* and *Trichoderma* isolates in managing tomato early blight *in vitro* and under greenhouse and field conditions.

Integration of effective BCAs in early blight management will contribute to a sustainable production of tomatoes through reduction of the dependence on synthetic chemicals (Mizubuti *et al.*, 2007; Engindeniz and Ozturk, 2013). This will help farmers to minimize the losses caused by tomato early blight and still meet the quality standards which require the agriculture products to

be safe for consumers given that BCAs are biodegradable and leave no residues on the produce (Gupta *et al.*, 2014). This will result in reduced interceptions of tomato produce at the export market. Integration of BCAs in the management of tomato early blight will also contribute to a better conservation of the environment given that BCAs do not pollute the environment as they are biodegradable. BCAs isolated in this study are beneficial to biopesticide processing companies and biopesticide resellers.

1.4. Objectives

1.4.1. Main objective

The main objective of this study is to integrate BCAs in managing early blight for sustainable production of tomatoes.

1.4.2. Specific objectives

- ❖ To evaluate the antagonistic effects of BCAs namely; *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas fluorescens* on *in vitro* growth of *A. solani*.
- ❖ To evaluate the effectiveness of BCAs (*Trichoderma* spp., *Bacillus* spp. and *Pseudomonas fluorescens*) in managing tomato early blight under field and greenhouse conditions.

1.5. Hypotheses

- ❖ BCAs namely; *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas fluorescens* have significant antagonistic effects on *in vitro* growth of *A. solani*.
- ❖ BCAs (*Trichoderma* spp., *Bacillus* spp. and *Pseudomonas fluorescens*) are effective in managing early blight of tomatoes under field and greenhouse conditions.

CHAPTER TWO: LITERATURE REVIEW

2.1. Origin of tomatoes

Tomatoes originated in the western South America; specifically in Peru, Bolivia and Ecuador from where it was spread to Europe in the 16th century and later to East Africa in 20th century through colonial settlers (Wener, 2000). Currently, tomato is a major vegetable worldwide (Monte *et al.*, 2013) being grown in essentially all countries (Abd-El-Kareem *et al.*, 2006).

2.2. Botanical description of tomatoes

The cultivated tomato belongs to the family *Solanaceae*, genus *Solanum*, section *Lycopersicon* which includes 12 tomato wild relatives endemic to western South America (Peralta *et al.*, 2008). The *Solanum* genus additionally includes 4 species of *Juglandifolia* and *Lycopersicoides* sections which are also considered as wild relatives to tomato (Knapp and Peralta, 2016).

The cultivated tomato was firstly named *Solanum lycopersicum* by Carolus Linnaeus in 1753. However, one year later, Philip Miller placed the cultivated tomato in a new genus *Lycopersicon*; segregated from *Solanum* based on the anther morphology (Hunziker, 2001; Kimura and Sinha, 2008). Recently tomato was moved into the original genus *Solanum* L. based on morphological and molecular evidence (Bohs, 2005). The genus *Solanum* is classified in the *Solanaceae* referred to as nightshade family (Knapp, 2002). The solanaceous family contains many species along with food crops (tomato, potatoes, peppers and egg plants), medicinal plants (deadly nightshade, henbane, datura), ornamental plants (petunias) among many others (Kimura and Sinha, 2008; Peralta *et al.*, 2008). Although tomato is botanically a berry or a subset of fruits, it is nutritionally categorized as a vegetable. Tomatoes are naturally short-lived perennial dicots but are grown as annual plants (Peralta *et al.*, 2008). They comprise of a branching stem (Peralta *et al.*, 2008) that has a terminal bud at its tip involved in the actual growth (Acquaah, 2002).

Stem of tomato plant typically grows to a height of 2-3 m, with a growth habit ranging from erect to prostrate. Tomato plant is held into the soil by a tough tap root system which can reach a depth of 50 cm or slightly above (Naika *et al.*, 2005). Leaves are pinnate and arranged spirally (Piazza *et al.*, 2005).

2.3. Requirements for growth of tomato plants

Environmental conditions are known to significantly affect tomato plant growth (Grey, 2010). Light, carbon dioxide, adequate temperature, water and nutrient availability are key requirements for growth of tomato plants (Hendricks, 2012). Tomato plants grow better under conditions of high light but their growth is not affected by day length that they rapidly grow under conditions of short or long days and complete their life cycle between 90 to 150 days (Grey, 2010). On the other hand, growth of tomato plant requires stable and suitable narrow temperature ranges. Thus, huge temperature variations can result in poor fruit quality and reduced yields. An optimum daily mean temperature reaching 18 to 26⁰C with night temperatures averaging 18 to 21⁰C favors tomato plant growth (Hendricks, 2012). A controlled water supply throughout the growing period is required for a high quantity and quality production of tomatoes (Papadopoulos, 1991). In greenhouses, tomato production requires a drip system and fertigation system (Hendricks, 2012). Adequate nutrient supply has been reported to enhance tomato yield, nutrient content and taste of tomato fruits. Additionally, adequate application of nutrients improves the quality of storage of tomato fruits. On the other hand, excessive nutrient application often interferes with tomato yield, compromises fertilizer-use efficiency and significantly contributes to environmental degradation.

Although tomato plants generally can grow on essentially all types of soils, they thrive on well drained, light, loam soils with pH ranging from 5 to 7 and can negatively be affected by soil salinity (Jaria, 2012).

2.4. Health benefits of tomatoes

Tomato fruits have been reported to contain adequate amounts of vitamins A, B and C. Additionally, tomato fruits harbor reasonable amounts of potassium, iron, and phosphorus (Masinde *at al.*, 2011). The consumption of lycopene, the substance which gives the reddish coloration to tomato, by human beings has been reported to minimize the incidences of prostate, lung and digestive tract cancers (Wilkerson *et al.*, 2007). The antioxidant properties of tomatoes also have health benefits (Giovannucci, 2002).

2.5. Economic importance of tomatoes

Tomatoes are grown and produced for consumption and exportation worldwide. Tomatoes are highly consumed vegetables worldwide (Foolad, 2007). Global annual tomato production was estimated at 177.0 million tons accounting for approximately \$88 billion in 2016 (FAOSTAT, 2019). Globally, 4.8 million ha of land were estimated to be under tomato production. China is the world largest tomato producing country and contributes to approximately one third of global production of tomatoes (FAOSTAT, 2019). World major tomato producing countries are illustrated in Table 2.1. Africa total tomato production was estimated at 21.5 million tons in 2017 (FAOSTAT, 2019). Major tomato producing countries in the Eastern African Community are mentioned in Table 2.2.

Table 2.1: Tomato production from the major tomato producing countries in the world

Country Year	China		USA		Turkey		India		Egypt	
	Harvested area (10 ³ ha)	Production (10 ³ tons)	Harvested area (10 ³ ha)	Production (10 ³ tons)	Harvested area (10 ³ ha)	Production (10 ³ tons)	Harvested area (10 ³ ha)	Production (10 ³ tons)	Harvested area (10 ³ ha)	Production (10 ³ tons)
2008	850.9	39,938.7	161.9	13,700.7	195.2	10,985.4	566.0	10,303.0	240.2	9,204.1
2009	920.8	45,365.5	176.2	15,457.5	186.9	10,745.6	599.1	11,148.8	251.8	10,278.5
2010	951.7	46,876.1	158.7	14,053.0	179.1	10,052.0	634.4	12,433.2	216.4	8,545.0
2011	959.7	49,323.2	148.9	13,761.7	181.0	11,003.4	865.0	16,526.0	212.4	8,105.3
2012	954.0	48,168.6	152.7	14,478.8	189.2	11,350.0	907.0	18,653.0	216.4	8,625.2
2013	985.7	50,694.1	152.4	13,828.6	189.1	11,820.0	880.0	18,227.0	205.3	8,290.6
2014	994.1	52,614.0	163.4	15,875.0	183.0	11,850.0	882.0	18,735.9	214.0	8,288.0
2015	1,015.3	55,891.8	163.0	14,580.4	192.8	12,615.0	767.0	16,385.0	196.9	7,737.8
2016	1,020.5	57,583.0	142.3	12,936.4	189.1	12,600.0	774.0	18,732.0	185.0	7,320.7
2017	1,033.3	59,626.9	126.1	10,911.0	187.1	12,750.0	797.0	20,708.0	182.4	7,297.1
Total	9,686	506,081.9	1,545.6	139,583.1	1,872.5	115,771.4	7,671.5	161,851.9	2,120.8	83,692.3
Mean	968.6	50,608.2	154.6	13,958.3	187.3	11,577.1	767.2	16,185.2	212.1	8,369.2
Yield (tons/ha)	52.2		90.3		61.8		21.1		39.5	

Source: FAOSTAT, 2019

Table 2.2: Tomato production from the major tomato producing countries in the East African Community

Country Year	Kenya		Rwanda		Uganda		Tanzania	
	Harvested area (ha)	Production (tons)	Harvested area (ha)	Production (tons)	Harvested area (ha)	Production (tons)	Harvested area (ha)	Production (tons)
2008	16,400	402,070	5,586	41,035	4,175	24,500	21,888	195,000
2009	17,230	526,922	5,500	129,751	4,828	28,005	25,952	250,000
2010	18,477	539,151	6,500	135,000	5,500	31,000	28,000	300,000
2011	20,584	396,544	7,568	122,167	5,178	30,000	30,000	350,000
2012	21,874	444,862	6,800	115,000	6,000	35,000	32,000	390,000
2013	23,866	494,037	7,861	116,083	5,916	34,953	34,713	423,323
2014	24,531	443,271	8,396	117,732	5,933	35,714	36,939	458,117
2015	19,027	402,513	8,974	118,517	6,178	37,176	36,600	485,378
2016	21,921	410,033	10,439	118,774	6,424	38,650	38,067	528,034
2017	14,595	283,000	11,329	97,426	6,671	40,124	39,251	565,441
Total	198,505	4,342,403	78,953	1,111,485	56,803	335,122	323,410	3,945,293
Mean	19,850.5	434,240.3	7,895.3	111,148.5	5,680.3	3,3512.2	32,341	394,529.3
Yield (tons/ha)	21.9		14.1		5.9		12.2	

Source: FAOSTAT, 2019

Kenya is one of the major tomato producing countries in Africa (FAOSTAT, 2019). Tomato is a highly consumed vegetable in Kenya (Wachira *et al.*, 2014) where it is being produced for both local and international markets (Koenig *et al.*, 2008). The production of tomatoes in the country offers employment opportunities and increases incomes for many households (Koenig *et al.*, 2008). Major tomato producing areas in Kenya include; Kirinyaga (accounting for 14% of the total production), Kajiado (9 %), Taita Taveta (7 %), Meru (6 %), Bungoma and Kiambu (5 %), Migori and Makueni (4 %), Homa Bay and Nakuru (3 %) and Machakos (2 %) (HCDA, 2013). Varieties grown for processed tomato production include Cal-J, Rio Grande, Roma VF, Parma VF, Rubino, Nema 1400 among others while fresh market varieties include Anna F1, Mavuno F1, Money maker, Marglobe, Capitan, Kentom 1 and Beauty among others (Monsanto, 2013). The trends in tomato production in Kenya are mentioned in Figure 2.1.

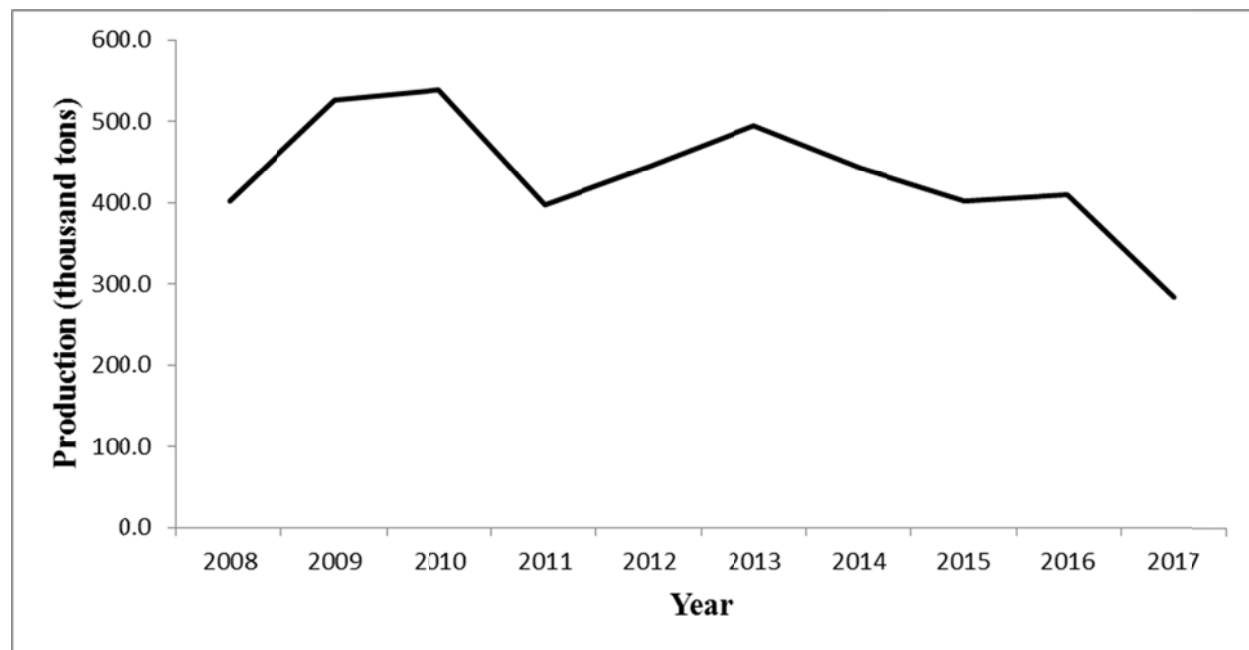


Figure 2.1: Tomato production trends in Kenya (FAOSTAT, 2019)

2.6. Constraints to tomato production in Kenya

The production of tomato in the country has been reported to be hindered by several challenges including physiological disorders (KALRO, 2005), pests and diseases (Singh *et al.*, 2014a) along with postharvest diseases (KHCP, 2011). The most common pests include; African bollworms, aphids, leaf miners, spider mites, thrips, whiteflies and weeds (Waiganjo *et al.*, 2006). Whiteflies and red spider mites mainly affect tomato plants during the dry season by sucking the plant sap resulting in reduced growth rate and productivity (Onduso, 2014). Weeds (including nightshade and black jack) compete for light, space and nutrients with tomato plants and thus interfere with tomato production by affecting colour, flavour and consistency of the produce. Physiological disorders are mainly caused by water and nutrient stresses (KALRO, 2005). For instance, low levels of calcium in the soil result in blossom end rot causing black spots at fruit bottoms; low levels of potassium in the soil result in uneven ripening of tomato fruits; excessive levels of nitrogen impede the production of tomato fruits (KALRO, 2005).

Diseases are the major constraints to tomato production and contribute to economic losses of 15-95% (Jones, 2008; Tahat and Sijam, 2010). Although, several disease causing pathogens have been reported to hinder the production of tomatoes in Kenya, the most devastating include late blight, early blight, bacterial wilt, bacterial canker, bacterial spots, Fusarium wilt, yellow leaf curl virus and tomato spotted virus (KALRO, 2005; Singh *et al.*, 2014b).

Early blight is known to be an important constraint to production of tomato fruits in Kenya (Waiganjo *et al.*, 2006). The disease is difficult to manage and can lead to significant reduction in yield or render tomato fruit unmarketable (Foolad *et al.*, 2008).

2.7. Overview on tomato early blight

2.7.1. Description of tomato early blight causative agent

First early blight report on tomatoes occurred in 1882 in New Jersey, in the USA (Galloway, 1891) and next on potato in Australia (Bose and Som, 1986). The causative agent was originally known as *Alternaria porii* f.sp. *solani* and classified under Eukaryota domain, Kingdom Fungi, phylum Deuteromycota, class Hyphomycetes, order Hyphales (Neergaard, 1945; Van der Waals *et al.*, 2001). Deuteromycota encompasses organisms described as fungi imperfecti or Deuteromycetes since their sexual stage is not well known. Some research work has claimed the ascomycete *Pleospora solani* as the teleomorph stage of *A. solani* (Chaerani and Voorrips, 2006). *Alternaria solani* hyphae are septate, branched, light brown turning darker as they mature. Raising individually or in tiny groups, *A. solani* conidiophores are septate, straight or flexuous, dark colored and measure 50 to 90µm (Ganie *et al.*, 2013). *Alternaria solani* pertains to the large spored group (Woudenberg, 2015) and its conidia are 120-296 x 12-20 µm in size, beaked, muriform, dark in colour and mostly arising individually. Both longitudinal and transverse septa are present in mature conidia. *Alternaria solani* conidia are illustrated in Plate 2.1.

Several strains of *A. solani* have been identified and reported to have high morphological and physiological variation in addition to their dissimilarity in genetic make-up and in pathogenicity (Martinez *et al.*, 2004; Van der Waals *et al.*, 2004). *Alternaria solani* isolates arising from different germ tube tips forming on the same conidium can exhibit differences in their pathogenicity (Woudenberg, 2015).

The genus *Alternaria* comprises of various saprophytic and pathogenic species (Thomma, 2003). Besides causing severe damage to crops, *Alternaria* species have been recorded to be food spoiling, mycotoxigenic and generating mycosis in animals and humans (Rotem, 1994; Thomma,

2003). The genus *Alternaria* comprises approximately 60 species of which *A. solani* is the most devastating to tomato production areas worldwide (Pelletier and Fry, 1989). First described by Ellis and Martin in (1882), *A. solani* was originally named *Macrosporium solani*. Based on the fact that the disease is more prevalent in early maturing crops than in medium or late maturing ones, Jones (1893) suggested the name early blight to the disease caused by *A. solani* in order to differentiate it from late blight which on the other hand is more severe in late maturing crops.

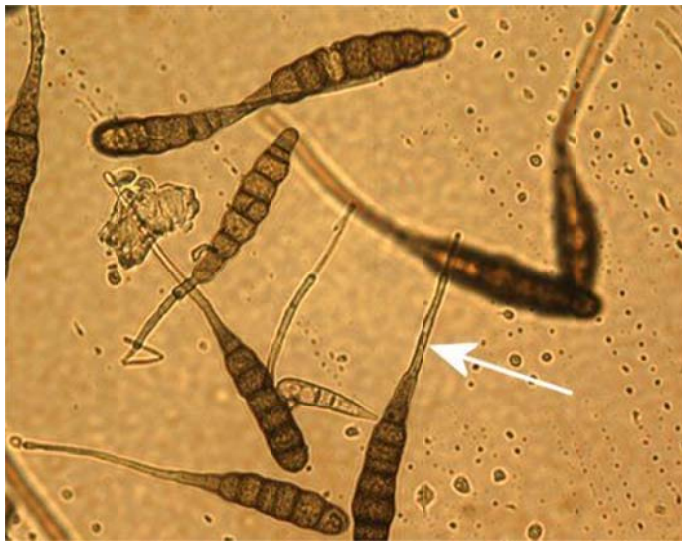


Plate 2.1: Conidia of *Alternaria solani* (Kemmitt, 2002)

2.7.2. Infection of tomato plant by *Alternaria solani*

The production of *A. solani* spores is induced by daylight and large number of spores can be formed during alternation of moist and dry conditions (Singh *et al.*, 2015). The germination of *A. solani* spores is favored by free moisture or relative humidity close to saturation. Varying levels of minimum, optimum and maximum temperatures for germination of *A. solani* spores have been reported. Singh *et al.* (2015) reported that *A. solani* spore could germinate after less than two-hour wetting period when temperature is high (20°C). *Alternaria solani* produces a germ tube that in turn forms an appressorium and penetrates the epidermis directly or through wounds or

stomata. Rotem (1994) reported that in moist conditions, a minimum temperature of 10⁰C and a maximum temperature of 35⁰C, are required for the germination of *A. solani* spores. The incubation time varies with age and the susceptibility of plants (Rotem, 1994).

To date, the molecular basis of *A. solani* infection is not well known (Adhikari *et al.*, 2017). However, it has been reported that *A. solani* secretes diverse types of phytotoxic compounds which include alternariol, altersolanol A, altertoxin, macrosporin, solanapyrone A, B, C and alternaric acid among others (Montemurro and Visconti, 1992; Anderson *et al.*, 2008). Although, it is known that development of necrotic and chlorotic symptoms is caused by alternaric acid and solanapyrone A, B, C; the role in disease development of most of these metabolites is not well documented (Adhikari *et al.*, 2017). Moreover, *A. solani* secretes extracellularly a serine protease and metalloprotease which may be related to pathogenicity (Chandrasekaran *et al.*, 2014; Chandrasekaran *et al.*, 2016).

2.7.3. Symptoms caused by early blight on tomatoes

Early blight induces an array of symptoms that appear at any stage of plant development. Early blight symptoms include; damping-off, collar rot, tuber cankers, leaf blight and fruit rot. Leaf blight is most frequent and destructive stage of *A. solani* infection. Symptoms firstly appear on lower leaves and extend to upper leaves with time (Rottem, 1994). Symptoms start as small, dark, papery spots which expand to form brown-black lesions displaying concentric rings surrounded by a yellow halo as illustrated in Plate 2.2. Signs of the pathogen (spores) can be present in the center of lesions, giving them a dark fuzzy appearance (Neils *et al.*, 2015). As the disease develops, the photosynthetic rate lowers resulting in poor fruit quality and significant yield loss (Foolad *et al.*, 2008). Persistent periods of leaf wetness and high temperatures can lead to complete defoliation of tomato plants (Ashour, 2009). Lesions produced on tomato leaves by

Septoria lycopersici are often mistaken for early blight. Nonetheless, *Septoria* lesions appear lighter tan coloured and form in their center, slight pepper shaped fruiting bodies. In addition, *A. solani* thrives in warmer temperatures than with the case of *S. lycopersici*. Infection on tomato fruits firstly establishes on the tuber-end and can occur during the green or ripe stage exhibiting dark colored, sunken, leathery and purple lesions with concentric rings (Chaerani and Voorrips, 2006; Junior *et al.*, 2011). Exposed fruit also become vulnerable to sunburn damage (Foolad *et al.*, 2008). Symptoms on tubers and petioles also appear as small, dark, slightly hollow spots that expand and form circular or elliptical concentric lesions which can extremely weaken the plant and may eventually girdle and kill the infected plants. A desiccated rot usually form internally under the skin. The pathogen can also damage the vascular system of newly emerged seedlings by inducing a collar rot at the ground level (Foolad *et al.*, 2008).



Plate 2.2: Tomato foliage affected by early blight (Kemmitt, 2002).

2.7.4. Disease cycle and source of inoculum

Although *A. solani* mainly infects potato and tomato plants, other solanaceous plants (such as eggplant and pepper), wild cabbage and cucumber can also be infected by the fungus (Schultz and Ronald, 2009).

Alternaria solani reproduces asexually through the production of dark to black spores called conidia. The sexual stage of this pathogen is not well documented (Foolad *et al.*, 2008). In cool

and humid environment, germination of *A. solani* conidia occurs at temperature of 8-32⁰C and leads to the formation of germ tubes (Kemmitt, 2002). Using their pressing appressoria, *A. solani* germ tubes directly penetrate host plant epidermis. *Alternaria solani* can also penetrate plant tissues through stomatal pores, wounds, or moist induced swelling of lenticels on tubers, thereafter causing the disease. Depending on leaf age and cultivar susceptibility, symptoms can appear within a week of infection if environmental conditions are conducive (Kemmitt, 2002). Although, prolonged periods of wetness are needed for spore production in most cases, sporulation of the fungus can occur when moist and dry conditions alternate. Thus, conidiophores formed in a moist night can bear conidia in the following moist night after a period of dry day. The so formed conidia can readily be disseminated by wind, rain splashes, insects and other animals including man through machinery and the disease cycle goes on in other healthy host plants. Early blight pathogen can complete several cycles within the same cropping season (Kemmitt, 2002).

The fungus can survive one to several years in the soil, plant debris, seed, infected seedlings, alternate hosts and weeds in its conidial or mycelial form, which can therefore serve as primary sources of inoculum. Conidial cells of the fungus are protected by a thick cell wall which enables them to withstand adverse environmental conditions (Foolad *et al.*, 2008).

2.7.5. Environmental conditions favoring tomato early blight

A. solani thrives in warm temperature (20-25⁰C) and prolonged periods of leaf wetness resulting from high humidity or overhead irrigation (Neils *et al.*, 2015). These conditions are conducive for spore germination and dissemination of spores from diseased plants. Tomato early blight has often been associated with plants under stress from nitrogen deficiency. Although symptoms of tomato early blight may appear in the early stages of the cropping season, susceptibility to the

disease increases with the age of plant tissues, especially after fruit and tuber initiation (Schultz and Ronald, 2009). Symptoms of tomato early blight appear within a week of infection.

2.7.6. Global distribution of tomato early blight

Tomato early blight certainly figures among the most prevalent challenges in tomato production worldwide (Rottem, 1994). It occurs in every continent, wherever tomatoes are grown and thrives in many climatic zones. Occurrence of dew in semi-arid zone favors tomato early blight development. Although the pathogen is more prevalent in field crops, it sometimes damages crops in greenhouses (Rottem, 1994).

2.7.7. Management of tomato early blight

Although tomato early blight is a localized infection, it has been reported to be difficult to control. This is often associated to the fact that *A. solani* has a wide host range, many infective strains and prolonged active phase. Losses in tomato yield reaching 78% have been reported for disease severity oscillating 72%. An increase of 1% in disease severity reduces tomato yield up to 1.4% (Yadav and Dabbas, 2012).

Tomato early blight can be managed by one or a combination of three approaches: cultural approaches, chemical application and use of tolerant cultivars (Adhikari *et al.*, 2017). A few varieties of tomato have been reported to be tolerant to early blight. However, tomato early blight tolerant cultivars have not performed well in terms of yield (Yadav and Dabbas, 2012). Moreover, it is not easy to transfer resistance to most tomato cultivars (Pandey *et al.*, 2003). Thus, cultural management strategies in addition to regular applications of synthetic fungicides are the most common measures for tomato early blight management (Foolad *et al.*, 2008). Most common chemical compounds applied globally under field conditions for managing tomato early blight include: Mancozeb, Zineb, Ridomil MZ-72, Saaf and copper oxychloride, Propiconazole,

Thiophanate Methyl, Propamocarb, Azoxystrobin, Cymoxanil, Propineb and Chlorothalonil among others (Singh and Singh, 2002; Mishra, 2012). These chemicals are required to be applied on a basis of seven days to effectively manage the pathogen (Li, 2012).

Most common cultural practices involved in managing tomato early blight include; sanitation, rotating tomatoes with non-host crops for period of two to three years, use of pathogen-free seeds and transplants, elimination of infected plant material from the garden and management of volunteer crops and weeds (like potato and horsenettle) to reduce the inoculum source. Proper irrigation strategies such as irrigating early in the morning to lower the duration of leaf wetness, the use of furrow and drip irrigation as opposed to overhead irrigation are useful in tomato early blight management. Furthermore, maintenance of plant vigor through adequate addition of nitrogen and phosphorus specifically during the fruiting stage, has been reported to have significant contribution to tomato early blight management (Chaerani and Voorrips, 2006; Li, 2012). Water treatment of infected seeds at 50°C for 25 minutes prior to sowing is beneficial for preventing seedborne infection (Neils *et al.*, 2015).

Management of early blight can be achieved through use of plant extracts (Neils *et al.*, 2015) as well as antagonistic cells and/or their secondary metabolites (Sultan, 2012; Neils *et al.*, 2015). Most reported bio-agents involved in managing early blight include; *Pseudomonas aeruginosa*, *P. putida*, *P. cepacia*, *P. gladioli*, *P. fluorescens*, *Trichoderma viride*, *T. harzianum* and *Bacillus subtilis*. Modes of action of most antagonistic microorganisms are not well known. However, these have been associated with one or the combination of the following strategies: direct parasitism which often leads to the death of plant pathogens, nutrient and space competition constraining pathogen growth and direct production of antibiotic compounds hindering the development of plant pathogens (Heydari and Pessarakli, 2010). *Pseudomonas gladioli* triggers

systemic resistance of host plants through accumulation of greater amounts of phenol and increased peroxidase activity (Jagadeesh and Jagadeesh, 2009). *Bacillus subtilis* is a plant growth-promoting rhizobacterium which induces production of growth related compounds and enzymes associated with disease resistance. These include; peroxidase, polyphenol oxidase and superoxide dismutase among others (Neils *et al.*, 2015). Extracts of various plants including *Cinnamomum zeylanicum*, *Ferula foetida*, *Glycyrrhiza glabra*, *Hemidesmus indicus*, *Syzygium aromaticum* among others, have been reported to induce antifungal activity against tomato early blight pathogen (Yeole *et al.*, 2014).

2.8. Overview on bio-control agents

2.8.1. Description and modes of action for *Bacillus subtilis*

Bacteria under *Bacillus subtilis* group are gram positive, form flagella which grow at peritrichous positions and serve as propagative spores (George, 2001; Slepecky and Hemphill, 2006). They are mainly found in soil and in water (Slepecky and Hemphill, 2006) but can survive in diverse environments, often with severe variations in temperature and nutrients (Driks, 2004). They have several features beneficial for the study of chromosomal replication. *Bacillus* belongs to the family *Bacillaceae*, order Bacillales, class Bacilli, phylum Firmicutes (George, 2001). Distinguishing feature of *Bacillaceae* family is production of circular, ovate or tubular endospores. Species of *Bacillus* genus are differentiated from other species of *Bacillaceae* family by their aerobic nature (strict or facultative), their ability to produce catalase and their rod shape nature (George, 2001; Slepecky and Hemphill, 2006).

Several modes of action are employed (individually or synergistically) by *B. subtilis* strains to hinder development of phytopathogens. These comprise; competition for available space and nutrients, antibiosis and induction of host plant defense mechanisms (Wang *et al.*, 2018).

Antibiosis results from emission of secondary metabolites along with lipopeptides, enzymes and various low molecular weight volatile compounds. Lipopeptide compounds include: surfactin, fengycin, iturin (Torres *et al.*, 2016), bacitracin (Rukmini *et al.*, 2015). Enzymes include chitinase (Liu *et al.*, 2011) and chitosanase (Wang and Yeh, 2008). Volatile compounds produced by *B. subtilis* strains include; 2-nonanone, 2-methylpyrazine and β -benzeneethanamine which hinder development of various fungal pathogens by preventing their mycelium from growing and by preventing their spores from germinating (Zheng *et al.*, 2013). Some volatile compounds secreted by *B. subtilis* are also known to induce activities that enhance plant growth (Compant *et al.*, 2005).

Abdelmoteleb *et al.* (2017) evaluated the *B. subtilis* strain “ALICA” against various plant pathogenic fungi including *A. alternata* and reported production of antifungal lipopeptides such as subtilosin, subtilisin and 2 hydrolytic enzymes; β -1,3-glucanase and protease which were suspected to degrade components of fungal cell walls including β -1,4-glucan and the glucosidic bonds.

Ramyabharathi and Raguchander (2014) tested the strain EPCO16 of *B. subtilis* against *Fusarium oxysporum* f.sp. *lycopersici* and reported production of antifungal metabolites including; bacillomycin, fengycin, iturin, and bacilysin in addition to volatile compounds with antifungal activities including; hexadecanoic acid methyl ester, dodecanoic acid, pentadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester 1,2-Benzenedicarboxylic acid and dibutyl ester. They also recorded higher reduction in the disease incidence and improved plant growth and fruit yield for plant treated with the antagonist compared to control plants.

2.8.2. Description and modes of action for *Pseudomonas fluorescens*

Pseudomonas fluorescens is a gram staining and starch hydrolysis negative, catalase, gelatin liquefaction, oxidase and fluorescent pigmentation positive bacterium (Meera and Balabaskar, 2012). The bacterium is rod-shaped, moves by means of flagella (Soesanto *et al.*, 2011). *Pseudomonas* genus belongs to the family *Pseudomonadaceae*, order Pseudomonadales, class Gammaproteobacteria (Spring *et al.*, 2015), phylum Proteobacteria (Tamez-Guerra *et al.*, 2017). *Pseudomonas fluorescens* is a saprophytic bacterium mostly found in soil, water (Nepali *et al.*, 2018), aquatic environments (Nagel *et al.*, 2012), in the rhizosphere of various crop plants (Maurya *et al.* 2014; Nepali *et al.*, 2018). *Pseudomonas* members are primarily aerobes (Franzetti and Scarpellini, 2007). Optimal growth temperatures for *P. fluorescens* range between 25-35⁰C (Soesanto *et al.*, 2011).

Fluorescent pseudomonads are known for activities which impede establishment and development of plant pathogens (Jain and Das, 2016). In addition to competing for available nutrients and space, *P. fluorescens* is known for the production of secondary metabolites including antibiotics along with; 2,4-diacetyl phloroglucinol, phenazine, pyoluteorin, and biosurfactant antibiotics (Angayarkanni *et al.*, 2005), iron chelating siderophores such as salicylic acid, pyochelin and pyoverdine (Ramanujam *et al.*, 2015), hydrogen cyanide, lytic enzymes including chitinase and β -1,3-glucanase among others (Nandakumar *et al.*, 2002). Production of siderophores as an antagonistic strategy is used by *P. fluorescens* mainly in environments where competition for available iron is required (Deveau *et al.*, 2016). Lytic enzymes are responsible for the digestion of chitin, β -1,3-glucan and proteins composing cell walls of phytopathogenic fungi. *Pseudomonas* species have also been associated with activities favoring plant growth along with secretion of plant hormones such as auxins, cytokinins and

gibberellins; and solubilization of essential minerals such as nitrogen, phosphorous, iron (Lugtenberg and Kamilova, 2009).

Dawoud *et al.* (2012) evaluated *P. fluorescens* and *P. putida* against tomato pathogens including *A. solani* and *P. syringae* and reported plant growth promoting activities and inhibition of the percent disease index for both infections resulting from the production of siderophores, hydrogen cyanide, ammonia and Indole-3-acetic acid by both antagonistic bacteria.

Ramyasmruthi *et al.* (2012) evaluated *P. fluorescens* against *Alternaria alternata*, *A. brassicola*, *A. brassicae*, *Colletotrichum gloeosporioides in vitro* and on chilli seeds and reported concurrent production of siderophore, indole-3-acetic acid, hydrogen cyanide, phosphate solubilisation among others as mechanisms used by the antagonist to hinder growth of plant pathogens and promote plant growth.

2.8.3. Description and modes of action of *Trichoderma* species

Trichoderma genus belongs to the family - *Hypocreaceae*, order - Hypocreales, class - Sordariomycetes, sub-division - Pezizomycotina, division - Ascomycota. Recent studies have phylogenetically allocated more than 200 species to *Trichoderma* genus based on rpb2 sequence (Druzhinina *et al.*, 2006). *Trichoderma* species are most frequently found in nearly all types of ecosystem including agricultural, forest, salt and desert soils of all climates. They often form opportunistic avirulent symbiotic associations with varied plant species ranging from woody to herbaceous species (Harman *et al.*, 2004; Błaszczuk *et al.*, 2014).

Trichoderma spp. form septate hyaline hyphae, branched hyaline conidiophores which are occasionally arranged pyramidally, flask-shaped hyalide phialides attaching to the conidiophores at right angles and arranged in a solitary or clustery manner. Single-celled, round or ellipsoidal conidia that are generally green in colour and displayed in sticky heads at phialide tips have been

reported (De Hoog *et al.*, 2000). Complete growth of *Trichoderma* colonies can be achieved at 25⁰C on PDA in 5 days since *Trichoderma* spp. are fast growing. Colonies are white in obverse view of PDA plates. Scattered blue-green to yellow-green patches resulting from the formation of conidia and often forming concentric rings are sometimes observed on the plates (De Hoog *et al.*, 2000). *Trichoderma* species have been reported to have a range of activities which hinder establishment and development of plant pathogens. These include; mycoparasitism, emission of antibiotics, competition and induction of host-plant systemic resistance. Furthermore, *Trichoderma* species have been reported for activities that promote growth of host plants and improve productivity. Interestingly, these modes of action are often synergistically employed by *Trichoderma* spp. rendering *Trichoderma* spp. more efficient against plant pathogens (Vinale *et al.*, 2008; Saba *et al.*, 2012; Vinale *et al.*, 2014).

Mycoparasitism process starts with pathogen recognition followed by coiling of *Trichoderma* hyphae around plant pathogen hyphae. Thereafter, *Trichoderma* spp. penetrate plant pathogen cell wall through secretion and release of cell wall-degrading enzymes of which the mutanase α -1,3-glucanase is well known (Viterbo *et al.*, 2002, Nusret and Steven, 2004).

Antibiosis results from the production of microbial compounds that are harmful to phytopathogens. *Trichoderma* have been associated with the emission of a range of antifungal secondary metabolites. These include; pyrones (e.g. 6-pentyl- α -pyrone), koninginins, viridins, azaphilones, isocyano metabolites and peptaibols among others (Vinale *et al.*, 2014). These secondary metabolites are friendly to the environment and can be applied to hinder the development of phytopathogens (Vinale *et al.*, 2009).

The competitive suppressive activity of *Trichoderma* spp. results from their ability to more efficiently use the available nutrients since they grow faster than most plant pathogens and colonize free spaces in their environment (Harman *et al.*, 2004; Waghunde *et al.*, 2016).

Kumar *et al.* (2015) evaluated *T. harzianum* against Fusarium wilt of tomatoes and noted that the mycelium of the pathogen was mycoparasitized by *T. harzianum* mycelium. They also recorded lower disease incidence and increased plant height, dry weight and quantity of harvested fruits for plants treated with the antagonist compared to control plants.

Rani *et al.* (2017) tested *T. harzianum* against *A. solani* *in vitro* and recorded inhibition of the pathogen radial growth by the antagonist. They also evaluated the antagonist against *A. solani* under field conditions and reported reduction of the disease intensity for tomato early blight and increased tomato yields for plants treated with the antagonist compared to untreated plants.

CHAPTER THREE: EFFECTS OF BIO-CONTROL AGENTS ON RADIAL GROWTH OF *Alternaria solani*

3.1. Abstract

Tomato fruits are important for a healthy diet and are important sources of income for many households worldwide. The fungus *Alternaria solani* causes early blight which is a common threat to tomato production worldwide. Integration of bio-control agents (BCAs) in tomato early blight management is supportive of sustainable agricultural production as it lowers the dependence on synthetic chemicals known for their potential hazard to humans and the environment. This study was carried out to evaluate *in vitro* activities of 10 *Trichoderma* spp. isolated from the soil, 19 *Bacillus* isolates and one commercial *Pseudomonas fluorescens* on *A. solani* isolated from infected tomato leaves. This study was carried out in Plant Pathology Laboratory at the Department of Plant Science and Crop Protection, University of Nairobi. Experiments were carried out in five replicates in a Completely Randomized Design following the dual culture technique. Diameter of *A. solani* colony was measured and percent growth inhibition was calculated. Comparison of means was done using Fischer's protected LSD test at 5%. All the BCAs significantly inhibited the radial growth of *A. solani*. *Trichoderma* isolates had higher inhibitory effects over radial growth of *A. solani* with percent growth inhibition of 80.9 and 82.2% for Tricho 7 and Tricho 10 respectively. *Trichoderma* isolates exhibited growth inhibition zones and grew over *A. solani* colonies. *Bacillus* isolates with 56.6 and 54.1% of percent growth inhibition for CA51 and CB12 respectively, were more effective in inhibiting the radial growth of *A. solani* compared to commercial *Pseudomonas fluorescens* with a percent growth inhibition of 47.6%. *Bacillus* isolates, unlike commercial *Pseudomonas fluorescens* exhibited growth inhibition zones. BCAs are effective against *A. solani in vitro* and further evaluations under field and greenhouse conditions need to be done.

3.2. Introduction

Tomato plants are cultivated all over the world for their fruits (Monte *et al.*, 2013). Tomato fruits are important sources of nutrients along with lycopene reported with activities lowering incidences of prostate, lung and digestive tract cancer (Giovannucci, 2002). In addition, tomato fruits contain vitamins A, B and C and other health beneficial nutrients (Masinde *et al.*, 2011). Tomato production generates income for numerous families in Kenya (Sigei *et al.*, 2014) and all over the world (Monte *et al.*, 2013). The fungus *Alternaria solani* causes early blight which is a major challenge to tomato production in Kenya (Mwangi *et al.*, 2015) and all over the world (Hou and Huang, 2006). *Alternaria solani* leads to massive losses when it is not managed both at harvest and after harvest. Synthetic chemicals are most commonly used for early blight management as they quickly knock out the disease and are easily accessible (Foolad *et al.*, 2008; Mishra, 2012). However, regular application of synthetic chemicals leads to their accumulation in soil, water and air in addition to inducing chemical resistance in plant pathogen populations (Rojo *et al.*, 2007; Nderitu *et al.*, 2007). Accumulation of synthetic chemicals is associated with side effects on wild animals (Nderitu *et al.*, 2007). Moreover, misuse of synthetic chemicals is often associated with excessive chemical residues on crop produce resulting in harmful effects to consumers (Fabro and Varca, 2011). Development of disease management approaches with harmless effects on the environment and humans is necessary for sustainable agricultural production (Mamgain *et al.*, 2013). Several BCAs have been reported to have inhibitory activity over growth of plant pathogens (Zhao *et al.*, 2008). BCAs are biodegradable and have few effects on the environment and human health (Gupta *et al.*, 2014). Various modes of action against plant pathogens namely; competition for available resources, secretion of metabolites hindering pathogen growth are associated with BCAs (Alabouvette *et al.*, 2006). Understanding

interactions between plant pathogens and BCAs is necessary in integrating BCAs in the management of plant diseases (Ngoc, 2013). *In vitro* studies are useful in evaluating inhibitory activities of BCAs on pathogen growth. This study was carried out to evaluate the inhibitory effects of *Trichoderma* isolates, *Bacillus* isolates and commercial *Pseudomonas fluorescens* on growth of *A. solani* under *in vitro* conditions.

3.3. Materials and methods

All laboratory experiments pertaining to this study were carried out in Plant Pathology Laboratory at the Department of Plant Science and Crop Protection, University of Nairobi.

3.3.1. Isolation and identification of *Alternaria solani*

Leaves of tomato plants exhibiting early blight symptoms were obtained from tomato plants at Kabete Field Station and carried in paper bags. These were used for isolation of *A. solani* following the standard tissue isolation technique. Using sterile blades, diseased leaves were chopped into small pieces, which were surface sterilized for 60 seconds in sterile petri dishes containing a solution of 1% sodium hypochlorite. Surface sterilized tissues were rinsed in four changes of sterile distilled water using forceps (Narayanasamy, 2011). Rinsed tissues were dried on sterile paper tissues and transferred to Petri plates containing sterilized PDA medium. The plates were then incubated at 28⁰C for seven days for growth and sporulation of the pathogen (Vaghabhai, 2016). Seven days later, single spore colonies were transferred using a sterilized inoculating needle, to petri dishes containing sterilized PDA to obtain pure culture of *A. solani* (Yadav and Dabbas, 2012). *A. solani* identification was based on colony features, hyphal and conidial characteristics along with shape, colour and septation. Hyphal and conidial characteristics were determined using a light microscope.

3.3.2. Inoculum preparation

Ten ml of sterile distilled water were poured on petri dishes each holding 14 days old single spore colonies. Colonies were scraped using a sterile glass slide. To remove debris, the resulting conidial suspension was sieved through a sterile muslin cloth. Using a haemocytometer the concentration of the suspension in conidia was calculated and adjusted to 3×10^6 spores/ml through addition of water.

3.3.3. Raising of tomato seedlings

Tomato seedlings were raised under greenhouse conditions by sowing five seeds from Rio Grande variety in pots of 22 cm diameter. Thinning was done to three plants per plot when tomato seedlings reached a height of 10 cm (Muiru, 2000). Pots were each filled with five kg of the potting medium which comprised of a mixture of sand and sandy loam soil in a ratio of 2:1. The medium was autoclaved at 121°C for one hour (Selim, 2015) and allowed to cool for seven days before use (Muiru, 2000). Watering and fertilization was done as per requirements.

3.3.4. Pathogenicity test of *Alternaria solani*

To confirm virulence of *A. solani*, pathogenicity test was carried out under greenhouse conditions following Koch's postulates. Using a hand sprayer, 40 day old seedlings raised as described in section 3.3.3 were sprayed with 20 ml of *A. solani* conidial suspension at 3×10^6 spores/ml prepared as described in section 3.3.2 (Hassanein *et al.*, 2010). Sterile water was used for the control plants. To maintain a high relative humidity required for *A. solani* infection, plants were each covered with plastic bags for 48 hours. Early blight symptoms were observed on the inoculated plants and the pathogen was re-isolated from the leaves and cultured. Morphological and cultural characteristics of the re-isolated pathogen were compared to those of the original pathogen (Kumar, 2017).

3.3.5. Isolation and identification of *Trichoderma* isolates

To isolate *Trichoderma* spp. from the soil, the serial dilution technique was followed. Soil samples collected from cabbage and coffee plantations were air dried. One gram of air dried soil samples was weighed and suspended in nine ml of sterilized distilled water and shaken properly. One ml of the obtained solution was transferred to nine ml of sterilized distilled water to form a soil suspension at 10^{-1} and the process was continued until dilution at 10^{-5} , 10^{-6} and 10^{-7} were attained. Two hundreds μl aliquots of soil suspensions at 10^{-5} , 10^{-6} and 10^{-7} were spread on Petri plates containing PDA plates using a sterile glass rod and incubated at 28°C for seven days (Kannangara *et al.*, 2016). To obtain pure cultures, single spore colonies were sub-cultured on PDA plates. Colony characteristics and morphological features of hyphae, conidiophores and conidia observed under light microscope were used for *Trichoderma* isolates identification. The morphological keys of *Trichoderma* genus developed by Watanabe (2010) were used for identification.

3.3.6. Isolation of *Pseudomonas fluorescens* from commercial formulation

Bio-cure; a commercial formulation containing the rhizobacterium *Pseudomonas fluorescens* was purchased and used as a source of the bacterium. Using sterile micropipette, one ml of the formulation was measured and diluted as recommended for the *in vitro* evaluation of the bacterium against *A. solani*.

3.3.7. *In vitro* activity of bio-control agents against *Alternaria solani*

BCAs were evaluated for their inhibitory activities against *A. solani* *in vitro* growth following the dual culture technique. Using a sterile cork borer, discs of five mm diameter were cut from the edge of seven day old cultures of *A. solani* and placed at the center of PDA plates. Four discs of the same diameter were cut from the edge of seven day old cultures of respective *Trichoderma*

isolates and placed two cm from the edge of plates containing pathogen discs at four equidistant points (Sundaramoorthy and Balabaskar, 2013). On the other hand, colonies of five mm diameter from respective bacterial BCAs were spot inoculated using sterile glass rods at four equidistant points in PDA plates containing five mm discs of *A. solani* colony at the center (Shahzaman *et al.*, 2016). Due to its slow growth, *A. solani* discs were plated three days before bacterial BCAs. In control plates, only pathogen discs of the same diameter were plated at the center. All treatments were replicated five times and the plates were incubated at 28°C. Plates were arranged in a CRD. Diameter of the pathogen colony was measured daily in each treatment until no increase in pathogen diameter was noticed in the control plates. Growth inhibition zones were measured in mm and characterized as distinct or faint. Diameter of *A. solani* colony in the presence of respective BCAs was compared to diameter of *A. solani* in the control. Percent *A. solani* radial growth inhibition was calculated as demonstrated by Arora and Dwivedi (1979):

$$\text{PGI} = \frac{C-T}{C} \times 100$$

Where, PGI= Percent growth inhibition, C=diameter of *A. solani* colony in control plates (mm), T=diameter of *A. solani* colony in respective treatment (mm).

The percent growth inhibition was qualified as “low” when it ranged between 0-50%, “medium” when it ranged between 51-70% and “high” when it ranged between 71-100%.

Since the number of BCAs was high (19 *Bacillus* and 10 *Trichoderma* isolates), preliminary experiments were carried out to select the most effective isolates. These experiments involved plating several *Trichoderma* isolates in the same plate against *A. solani*. A disc of five mm diameter from the edge of a seven day old culture of *A. solani* was plated at the center of a Petri dish containing sterile PDA. Then four discs of five mm diameter from four different *Trichoderma* isolates were plated two cm from the edge of the Petri plate at four equidistant

points using a sterile cork borer. In the case of *Bacillus* isolates, the same procedure was followed except that *Bacillus* colonies were spot inoculated using sterile glass rods. In the control plates, only pathogen discs were plated at the center. Colonies from each antagonist were plated in four different Petri plates. Seven day after plating, the diameter of pathogen colony was measured (in mm) from the center of Petri plates in direction to the antagonist colony.

3.3.8. Data analysis

All the data were investigated by ANOVA using Genstat® 14th edition. Comparison of means was done using Fisher's protected LSD test at $p \leq 0.05$.

3.4. Results

3.4.1. Isolation and identification of *Alternaria solani*

Pure *A. solani* colonies were obtained on PDA and were used for *A. solani* identification. Ten day old single spore colonies were dark on the back side of the plates, greyish on the front side. The hyphae were septate, branched and brownish. Conidiophores were short, septate and brownish. Conidia were large, brownish, arising singly or in pair, displaying 0-2 longitudinal and 3-4 transversal septa (Plate 3.1).

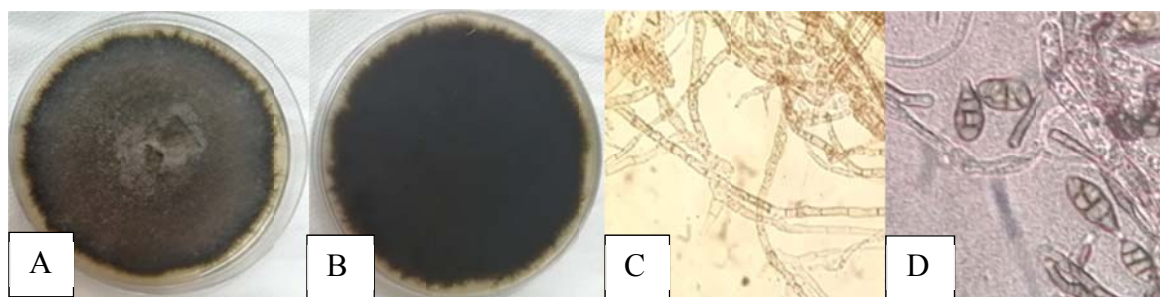


Plate 3.1: Morphological and cultural characteristics of isolated *Alternaria solani*

A: *Alternaria solani* ten day old colony (obverse), B: *Alternaria solani* ten day old colony (reverse), C: *Alternaria solani* hyphae (x40), D: *Alternaria solani* conidia (x40).

3.4.2. Pathogenicity test for *Alternaria solani*

Thirty day old tomato seedlings were inoculated with *A. solani* inoculum prepared as described in section 3.3.2. Tomato plants were observed with early blight symptoms on the twentieth day after plant inoculation with *A. solani*. Inoculated leaves exhibited dark brown oval to angular spots measuring 2-5 mm with concentric rings and becoming larger with time. Spots were surrounded by distinct to faint chlorotic lesions (Plate 3.2). *A. solani* was re-isolated from inoculated leaves following the standard tissue isolation technique as described in section 3.3.1. Single spore colonies of re-isolated pathogen exhibited morphological features which were

comparable to those described in section 3.4.1. Observed conidial, hyphal features were comparable to those illustrated in section 3.4.1.



Plate 3.2: Early blight symptoms on infected tomato leaf and healthy tomato leaf

A: Tomato leaf infected with *Alternaria solani*, B: Healthy tomato leaf.

3.4.3. Isolation and identification of *Trichoderma* isolates

Trichoderma spp. were isolated from cabbage and coffee soils at Kabete Field soils and pure cultures were obtained as described in section 3.3.5. These were coded as Tricho 1, Tricho 2, Tricho 3, Tricho 4, Tricho 5, Tricho 6, Tricho 7, Tricho 8, Tricho 9 and Tricho 10. Seven day old colonies were used in identification. Colonies formed by Tricho 1, Tricho 4, Tricho 5, Tricho 6, Tricho 7, Tricho 8 and Tricho 10 were greyish on the back side of the plates and greenish on the front side and were forming scattered patches appearing in concentric rings. Colonies formed by Tricho 2, Tricho 3 and Tricho 9 were double coloured (white and green) on the front side of the plates and light brownish on the back side and displayed no scattered patches. Hyphae were septate, hyaline and green coloured. Conidiophores were septate, hyaline, branched and

forming verticillate phialides. Conidia were small in size, single celled, ovate, green coloured and released at the top of phialides in masses (Plate 3.3).

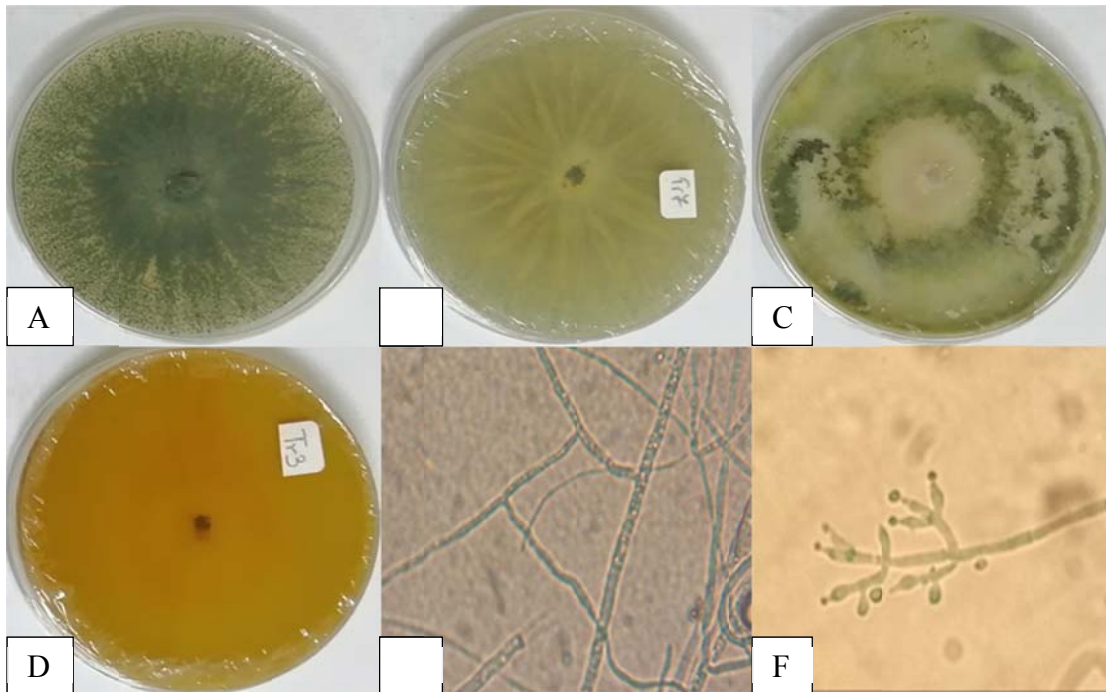


Plate 3.3: Morphological and cultural characteristics of *Trichoderma* isolates

A: Tricho 7 colony (obverse), B: Tricho 7 colony (reverse), C: Tricho 3 colony (obverse), D: Tricho 3 colony (reverse), E: *Trichoderma* hyphae (x40), F: *Trichoderma* conidiophores and conidia (x40).

3.4.4. Activity of bio-control agents against *Alternaria solani*

3.4.4.1. Preliminary screening of *Bacillus* isolates against *Alternaria solani*

Preliminary screening was carried out to select the most active *Bacillus* isolates for further evaluation. All the isolates significantly inhibited *A. solani* radial growth compared to the control (Figure 3.1). All the isolates except for CB3 and CB22 showed growth inhibition zones between *A. solani* colony and their respective colonies. CA51 and CB12 isolates (with 48.5 and 49.7% of percent growth inhibition respectively) were the most effective against *A. solani* radial growth. These were selected for further evaluation.

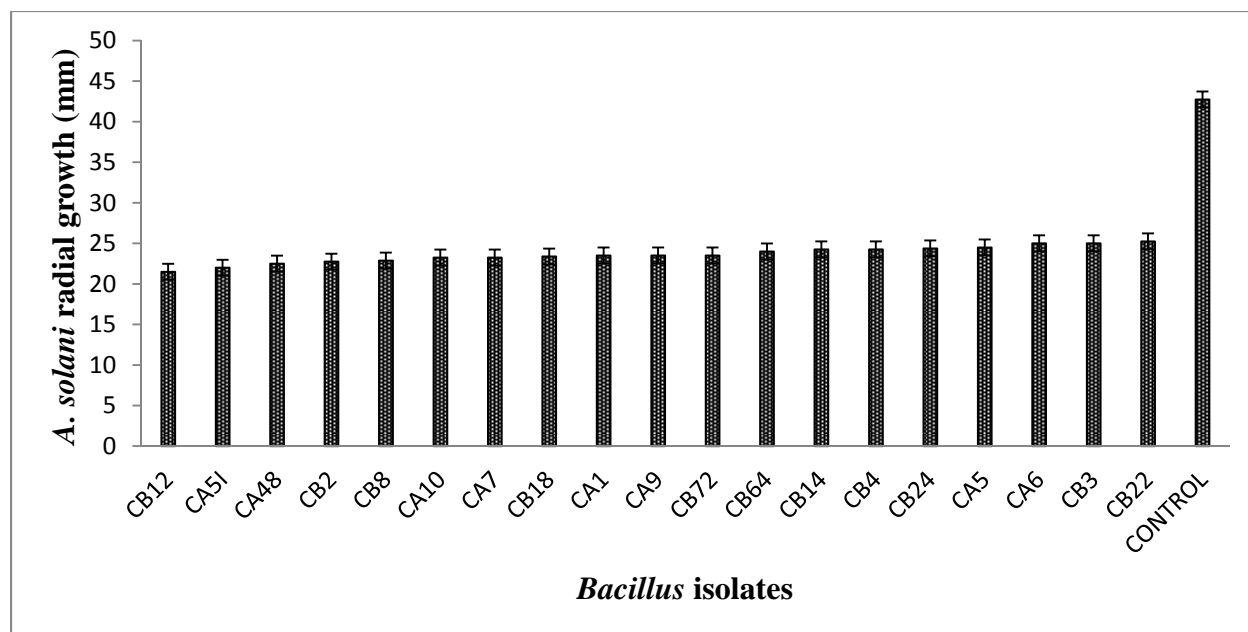


Figure 3.1: Mean diameter of *Alternaria solani* in the presence of *Bacillus* isolates

3.4.4.2. *In vitro* activity of *Bacillus subtilis* isolates selected from preliminary screening and *Pseudomonas fluorescens* against *Alternaria solani*

Selected *Bacillus* isolates from preliminary experiments and *Pseudomonas fluorescens* isolated from Bio-cure were evaluated for their *in vitro* activity against *A. solani*. Pathogen colony diameter was measured (in mm) on a daily basis commencing on the third day after plating BCAs until the thirteenth day when no pathogen colony increase was noticed in the control plates. All *Bacillus* isolates and *P. fluorescens* significantly inhibited *A. solani* radial growth from the third day after they were plated until no *A. solani* colony increase was noticed in the control plates. Isolates CA51 and CB12 had medium percent growth inhibition (56.6 and 54.1% respectively) over the radial growth of *A. solani*. *Pseudomonas fluorescens* recorded lower percent growth inhibition (47.6%) (Table 3.1). Plates treated with either *Bacillus* isolate exhibited growth inhibition zones between the bacterial colonies and *A. solani* (Plate 3.4). Inhibition zones were distinct measuring 4-6 mm on the third day after plating bacterial BCAs.

Those zones later (4-5 days) reduced in size measuring 2-3 mm. On the other hand, plates treated with *P. fluorescens* did not show any growth inhibition zones.

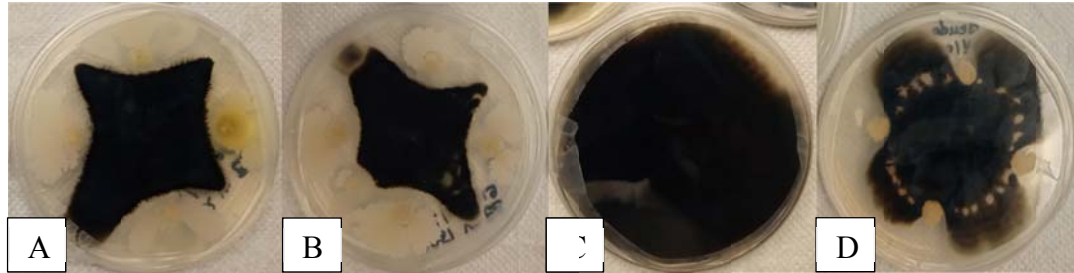


Plate 3.4: Effects of bacterial antagonists on the radial growth of *Alternaria solani*

A: CA51 against *Alternaria solani*, B: CB12 against *Alternaria solani*, C: *P. fluorescens* against *Alternaria solani*, D: Control. CA51 and CB12: respective *B. subtilis* isolates.

Table 3.1: *In vitro* activity of *Bacillus subtilis* isolates selected from preliminary screening and *Pseudomonas fluorescens* against *Alternaria solani*

Treatment	Time after plating bacterial antagonists (Days)					
	3	5	7	9	11	13
CA51	36.6a	35.9a	34.7a	35.2a	35.8a	35.7a
CB12	38.0a	37.9a	39.0b	38.8ab	38.7ab	37.7a
<i>Pseudomonas</i>	40.6a	40.9a	41.4b	41.4b	42.1b	43.1b
Control	53.5b	67.5b	74.9c	81.8c	82.2c	82.2c
LSD ($p \leq 0.05$)	4.8	5.9	4.0	3.9	4.7	4.0
% CV	8.3	9.5	6.1	5.7	6.9	5.9

Means accompanied by the same letter (s) in the same column are comparable ($p \leq 0.05$). Means represent pathogen colony diameter in mm. CA51 and CB12: *Bacillus subtilis* isolates; *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure); LSD: Least significant difference; % CV: Percent of coefficient of variation.

3.4.4.3. Preliminary screening of *Trichoderma* isolates against *Alternaria solani*

Ten *Trichoderma* isolates namely; Tricho 1, Tricho 2, Tricho 3, Tricho 4, Tricho 5, Tricho 6, Tricho 7, Tricho 8, Tricho 9 and Tricho 10 were isolated from the soil. Preliminary screening was carried out to select the most active isolates for further evaluation. All the isolates significantly inhibited *A. solani* radial growth (Figure 3.2). All the isolates except for Tricho 9, Tricho 4 and Tricho 2 exhibited distinct growth inhibition zones measuring 3-8 mm on the third

day after they were plated. All the isolates grew over *A. solani* colony. Isolates Tricho 7 and Tricho 10 (with 65.3 and 64.4% of percent growth inhibition respectively) were most effective against *A. solani*. These were selected for further evaluation.

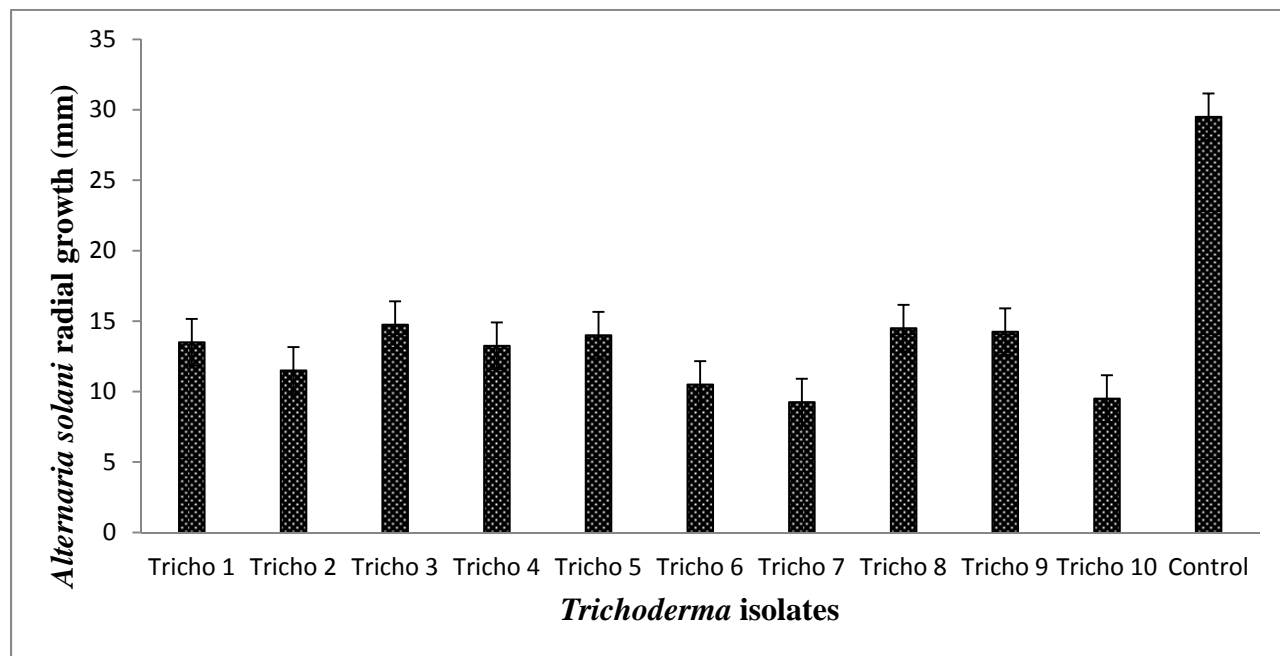


Figure 3.2: Mean *Alternaria solani* diameter in the presence of *Trichoderma* isolates

3.4.4.4. *In vitro* activity of *Trichoderma* isolates selected from preliminary screening against *Alternaria solani*

Selected *Trichoderma* isolates from preliminary screening experiments namely Tricho 7 and Tricho 10 were evaluated for their activity over *A. solani* radial growth. *Alternaria solani* colony diameter was measured (in mm) on a daily basis commencing on the third day after plating *Trichoderma* isolates until the thirteenth day when no *A. solani* colony growth increase was noticed in control plates. Both *Trichoderma* isolates significantly inhibited *A. solani* radial growth from the third day after they were plated until the thirteenth day (Table 3.2) with percent growth inhibition of 80.9 and 82.2% for Tricho 7 and Tricho 10 respectively. Both isolates exhibited distinct growth inhibition zones between their respective colonies and *A. solani*

colonies (Plate 3.5). Growth inhibition zones ranged between 5-9 mm on the third day after plating *Trichoderma* isolates. Sizes of growth inhibition zones later started reducing (up to 2 to 5 mm on the thirteenth day).

Table 3.2: *In vitro* activity of *Trichoderma* isolates selected from preliminary screening against *Alternaria solani*

Treatment	Time after plating fungal antagonists (Days)					
	3	5	7	9	11	13
Tricho 7	15.7a	16.5a	16.2a	15.4a	15.4a	15.7a
Tricho 10	14.9a	15.1a	14.9a	15.0a	15.3a	14.6a
Control	53.5b	67.5b	72.9b	81.8b	82.2b	82.2b
LSD ($p \leq 0.05$)	5.4	5.0	4.6	4.0	3.5	4.0
% CV	13.1	10.5	8.9	7.4	6.5	7.3

Means accompanied by the same letter (s) in the same column are comparable ($p \leq 0.05$). Means represent pathogen colony diameter in mm. LSD: Least significant difference; % CV: Percent of coefficient of variation. Tricho 7 and Tricho 10: *Trichoderma* isolates.

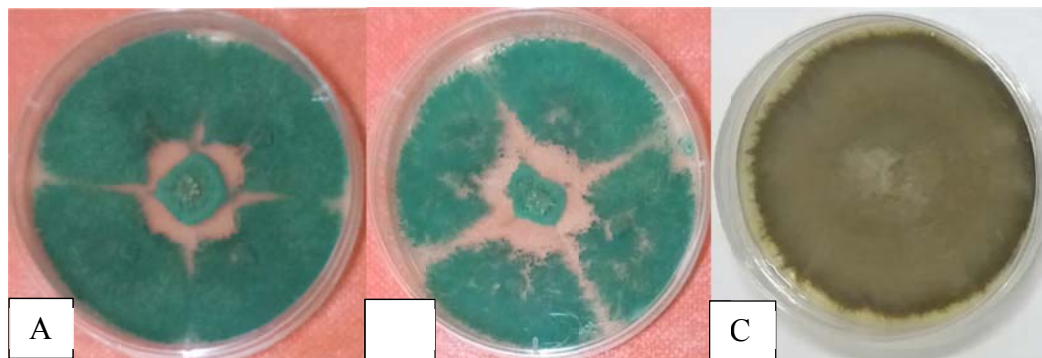


Plate 3.5: Effects of *Trichoderma* isolates on the radial growth of *Alternaria solani*

A: Tricho 10 against *Alternaria solani*, B: Tricho 7 against *Alternaria solani*, C: Control.

3.5. Discussion

Alternaria solani was isolated from infected tomato leaves. Ten day old colonies were dark on the back side and greyish on the front side of PDA plates. Hyphae were branched, septate, dark brown and turning dark with time. Conidiophores were short, brownish and septate. Conidia were brownish and septate with 3-4 transversal septa and 0-2 longitudinal septa. These characteristics corroborate findings of Kumar (2017).

Pathogenicity test was carried out in the greenhouse to confirm pathogenicity of *A. solani* isolates. Twenty days after inoculation of seedlings with *A. solani*, oval to angular dark brown spots measuring 2-5 mm, displaying concentric rings and surrounded by distinct to faint chlorotic lesions were observed. Spots were becoming larger with time until entire tomato leaves were blighted. These symptoms correspond to those associated with *A. solani* on tomato leaves from other studies (Ngoc, 2013; Neils *et al.*, 2015; Kumar, 2017).

Trichoderma isolates were isolated from forest and agricultural soil samples and pure colonies were obtained on PDA plates. Seven day old colonies were greyish or light brownish at the back of the plates and greenish or green-whitish at the front. Hyphae were septate and hyaline. Conidiophores were septate, hyaline, branched and forming verticillate phialides. Single celled, ovate and greenish phialosporous conidia were observed. These characteristics corroborate with those reported for *Trichoderma* species by De Hoog *et al.* (2000) and Watanabe (2010).

Results of this study reveal that *Trichoderma* isolates and bacterial BCAs are effective in inhibiting *A. solani* radial growth. *Trichoderma* isolates were most effective and recorded higher

percent growth inhibition. On the other hand, among bacterial BCAs, *B. subtilis* isolates with medium percent growth inhibition were more effective compared to *P. fluorescens* with low percent growth inhibition. Thus, the BCAs recorded differences in their effectiveness against *A. solani*. It has been reported that the susceptibility of a plant pathogen to BCAs can vary with the BCAs (Tapwal *et al.*, 2015).

In this study, *Trichoderma* isolates were more effective in inhibiting *A. solani* radial growth compared to all bacterial antagonists. This could be associated with the fact that *Trichoderma* species employ varied mechanisms of action to impede development of phytopathogens (Vinale *et al.*, 2009; Nusret and Steven, 2004) in addition to their fast growing ability (Harman *et al.*, 2004; Waghunde *et al.*, 2016). This corroborates the results of Dalpati *et al.* (2010) who stated that *Trichoderma harzianum* had a higher inhibitory activity against *Alternaria macrospora* inciting leaf spot in cotton compared to *Bacillus subtilis* and *Pseudomonas fluorescens*. However, compared to the findings of this study, Sundaramoorthy and Balabaskar (2013) reported a lower activity for *Trichoderma harzianum* over *Fusarium oxysporum* f. sp. *lycopersici* radial growth. This might be associated with the fact that different plant pathogens have varied susceptibility to most microbial antagonists along with *Trichoderma* species. Variability in susceptibility of plant pathogens to *Trichoderma* species was confirmed by Tapwal *et al.* (2015) who reported that *T. viride* and *T. harzianum* had varied *in vitro* inhibitory effects on *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Fusarium oxysporum* and *Rhizoctonia solani*. Similar findings were reported by Srivastava (2008) who tested five *Pseudomonas* strains and noticed variability in their antifungal activity against plant pathogens

including *Alternaria cajani*, *Curvularia lunata*, *Fusarium* spp., *Bipolaris* spp. and *Helminthosporium* spp.

Findings from this study recorded presence of growth inhibition zones between *Trichoderma* spp. colonies and those of *A. solani*. Presence of growth inhibition zones suggested the formation of secondary metabolites which inhibit pathogen growth. Several secondary metabolites that inhibit the growth of plant pathogens have been isolated from *Trichoderma* spp. These include; pyrones, koniginins, viridins, azaphilones, butenolides, diketopiperazines and peptaibols among others (Vinale *et al.*, 2009). In addition to formation of growth inhibition zones, *Trichoderma* isolates used in this study grew over *A. solani* colonies. This may be related to the capacity of *Trichoderma* species to mycoparasitize plant pathogen hyphae by secreting cell wall degrading enzymes such as the mutanase α -1,3-glucanase (Viterbo *et al.*, 2002; Nusret and Steven, 2004). These results are in agreement with those recorded by Tapwal *et al.* (2015) who evaluated *T. viride* and *T. harzianum* against *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Fusarium oxysporum* and *Rhizoctonia solani* and reported that in addition to forming growth inhibition zones, *Trichoderma* antagonists grew over pathogen colonies.

In this study no growth inhibition zones were noticed between *Pseudomonas fluorescens* colonies and those of *A. solani*. These results corroborate those of Pandey *et al.*, (2006) who evaluated *Pseudomonas corrugata* against *Alternaria alternata* and *Fusarium oxysporum* and reported significant radial growth reductions of both pathogens by the bacterium without forming any growth inhibition zones.

Findings from this study reported formation of growth inhibition zones between colonies of either *Bacillus* isolates and *A. solani* colonies. This suggested the formation of secondary metabolites that inhibit growth of plant pathogens. *Bacillus* isolates have been reported to produce several secondary metabolites that hinder growth of plant pathogens. These include: bacillomycin, fengycin, iturin, and bacilysin, hexadecanoic acid methyl ester, dodecanoic acid, pentadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester and 1,2-Benzenedicarboxylic acid among others (Ramyabharathi and Raguchander, 2014). These findings corroborate those of Abdalla *et al.* (2014) who tested *Bacillus* stains against *Alternaria alternata* isolated from tomato and reported formation of growth inhibition zones between *A. alternata* colonies and those of *Bacillus*.

BCAs namely; *Trichoderma* isolates, *Bacillus* isolates and *P. fluorescens* were effective against *A. solani* *in vitro* growth. Various modes of action along with mycoparasitism, competition and antibiosis are used by BCAs to hinder growth of *A. solani*.

CHAPTER FOUR: EFFECTIVENESS OF BIO-CONTROL AGENTS IN THE MANAGEMENT OF TOMATO EARLY BLIGHT IN THE FIELD AND THE GREENHOUSE

4.1. Abstract

Integrating BCAs in managing tomato early blight can minimize the dependence on synthetic chemicals which are potentially hazardous to humans and the environment. In this study, five promising BCAs with significant inhibitory effects over *A. solani in vitro* growth, were selected and evaluated for their effectiveness in managing early blight in tomatoes in the fields at Kabete Field Station and at KALRO Mwea and in the greenhouse. BCAs included two *Trichoderma* isolates (Tricho 7, Tricho 10), two *Bacillus subtilis* isolates (CA51 and CB12) and one *Pseudomonas fluorescens* strain. Water and Tower (Metalaxyl 8% + Mancozeb 64%) were used as control and standard check respectively. Each treatment was replicated thrice. The experimental design was a Randomized Complete Block Design in triplicate. Data were taken on disease and plant parameters. Percent disease index and the area under disease progress curve (AUDPC) were calculated. Marketable fruits were selected from harvested fruits and weighed. Comparison of means was done using Fischers' protected LSD test (at $p \leq 0.05$) using GenStat[®] 14th edition. Percent disease incidence was significantly lower in all treatments compared to control treatment at both experimental sites. The percent disease incidence recorded for Tower was comparable to percent disease incidence recorded for most BCAs. Percent disease severity and the percent disease index were significantly lower in all treatments compared to control treatment at both experimental sites and in the greenhouse. Percent disease index ranging between 64.7 and 86.1% were recorded on the 90th day after transplanting for the control while the other treatments recorded percent disease index ranging between 28.4 to 53.1%. All

treatments significantly recorded higher quantities of marketable fruits compared to control treatment at both experimental sites and in the greenhouse. However, Tower recorded higher quantities compared to all the BCAs at both experimental sites. In the greenhouse, Tower and both *Trichoderma* isolates recorded higher quantities.

BCAs are effective in managing early blight in tomatoes under greenhouse and field conditions and minimize yield losses caused by the infection.

4.2. Introduction

Tomato is a solanaceous plant cultivated in nearly all countries around the world for its fruits which are important source of income (Abd-El-Kareem *et al.*, 2006; Monte *et al.*, 2013). Tomato fruits are consumed as salad or in various forms of processed food and are great sources of vitamins and other nutrients (Masinde *et al.*, 2011). The fungus *A. solani* interferes with tomato production worldwide by causing early blight which can lead to enormous crop losses (Adhikari *et al.*, 2017). Most tomato cultivars are susceptible to the disease. A few cultivars with moderate tolerance to tomato early blight have been developed from wild tomato species through conventional breeding programs. However, tomato early blight tolerant cultivars are of low agronomic or commercial quality. Most common early blight management approaches include cultural practices and application of agrochemicals (Adhikari *et al.*, 2017). However, continuous applications of synthetic chemicals lead to the formation of pathogen strains that are resistant to chemicals (Rojo *et al.*, 2007). Formation of pathogen biotypes which are resistant to chemicals has been associated with intensification of chemical application resulting in high pesticide residue levels on tomato produce with the associated harmful effects on humans. Excessive applications of synthetic chemicals have been associated with pollution of air and water which is hazardous to wild animals (Fabro and Varca, 2011). Integration of disease management

approaches with fewer effects on the environment and humans is important for sustainable agricultural production (Alabouvette *et al.*, 2006). BCAs are known to be effective in managing plant diseases and due to their bio-degradability they are friendly to the environment (Zhao *et al.*, 2008). Various mechanisms along with mycoparasitism, antibiosis, competition and improvement of plant defense mechanisms are employed by BCAs to hinder the growth and development of plant pathogens (Benítez *et al.*, 2004). This study was conducted to evaluate five promising isolates of BCAs selected from *in vitro* for their effectiveness in managing tomato early blight in the field and in the greenhouse. These included; two *Trichoderma* isolates coded Tricho 7 and Tricho 10, two *Bacillus* isolates coded CA51 and CB12 and one commercial *Pseudomonas fluorescens*.

4.3. Material and methods

4.3.1. Description of the study sites

Greenhouse experiments pertaining to this study were conducted at Kabete Field Station, University of Nairobi. Field evaluations were carried out at Kabete Field Station and at KALRO Mwea. Kabete Field Station is located in Nairobi County which is in the Agro-ecological zone (AEZ) III and has a bimodal distribution of rainfall. The area is located at an altitude of 1,829 m beyond sea level and receives approximately 1,000 mm of rainfall annually with mean annual maximum temperature being 23⁰C and minimum going up to 13⁰C. The soils are humic nitosols with kaolinite clay minerals. The soils are deep with reliable drainage and usually dark brown to brown. All these conditions are conducive for tomato production.

KALRO Mwea is located in Kirinyaga County which belongs to the AEZ II. Kirinyaga County has a bimodal distribution of rainfall categorized by a long rain season (March-June) and a short rain season (October-December). The area receives annual rainfall ranging from 500-1,250 mm

and averaging 850 mm. The temperatures in the area range between 15.6⁰C to 28.6⁰C and average 22⁰C. Mwea is in LM4 and falls under an altitude of 1,159 m beyond sea level. The region has well drained nitosols and reliable source of water for irrigation (Jaetzold *et al.*, 2006; Kamanu *et al.*, 2012). All these conditions are ideal for tomato cultivation.

4.3.2. Sampling protocol for field evaluations

Field experiments were conducted between March 2019 and June 2019. Tomato seeds from Rio Grande which is an early blight susceptible variety were purchased from local market. These were sown in nursery beds. The resultant seedlings were transplanted in plots of 2 meter x 1.5 meter. Each plot comprised four rows consisting of five plants each. Distance between rows was 0.5 meter and distance between plants was 0.5 meter. On the other hand, plot to plot distance was one meter. A total of seven treatments including; two *Trichoderma* isolates coded Tricho 7 and Tricho 10, two *Bacillus subtilis* isolates coded CA51 and CB12, Bio-cure (a commercial formulation of the rhizobacterium *Pseudomonas fluorescens*), one standard check (TOWER 72 WP; 64% Mancozeb and 8% Metalaxyl) and one negative control (spray with water only) were applied. The experimental design was a Randomized Complete Block Design (RCBD) in triplicates.

4.3.3. Production of culture filtrates from bio-control agents

4.3.3.1. Production of cultures filtrates from *Bacillus subtilis* isolates

Culture filtrates from *B. subtilis* isolates were produced in Tschen's shaken liquid media as described by Loeffler *et al.*, (1986). Composition of the medium was as follows: 15 gram of glucose, 15 ml of glycerol, five gram of (NH₄)₂SO₄, 15 gram of soybean meal, one gram yeast extract, five gram of NaCl and five gram of CaCO₃ in 1,000 ml of distilled water (pH, 7.5). One hundred ml of each medium were prepared in 250 ml of Erlenmeyer flasks. Sterilization of the

medium was done at 121⁰C at 1 bar pressure for 15 minutes. The medium was cooled after sterilization.

Ten day old colonies of *Bacillus subtilis* previously sub-cultured on nutrient agar in plastic Petri dishes were submerged with 10 ml of sterile distilled water. Dislodging the colonies was done by scrapping with a sterile glass slide. Resultant suspensions were aseptically transferred into Erlenmeyer flasks each containing sterilized Tschén's liquid medium. Flasks were then properly sealed with cotton wool and Aluminum foil to avoid contamination and incubated on circulatory shaker at 125 rpm at (20±2)⁰C in the dark as recommended by Baker *at al.*, (1985) for seven days. To obtain culture filtrate of the bacterium, centrifugation of resultant fermentation broths was done at 5000 rpm for 15 minutes. Resultant culture filtrate was decanted into 500 ml sterile Erlenmeyer flasks. Flasks were then sealed properly with cotton wool and Aluminum foil and stored in a refrigerator at 4⁰C.

4.3.3.2. Production of culture filtrates from *Trichoderma* isolates

To produce culture filtrates from *Trichoderma* isolates, Czapek Dox Broth medium was used. Composition of the medium was as follows: 30.00 grams of Sucrose, 3.00 grams of sodium nitrate, 1.00 gram of dipotassium hydrogen phosphate, 0.50 gram of magnesium sulphate, 0.50 gram of potassium chloride, 0.01 gram of Ferrous sulphate in 1,000 ml of distilled water (final pH at 25⁰C: 7.3±0.2). Approximately 14.004 grams of the medium were weighed and transferred into 1,000 ml conical flasks. Four hundred ml of distilled water were poured and the blend was shaken to dissolve. The blend was sterilized by autoclaving at 121⁰C at one bar pressure for 15 minutes. The medium was then cooled before use. Seven day old colonies from *Trichoderma* isolates previously sub-cultured in plastic petri dishes containing PDA were dislodged by flooding the cultures with 20 ml of sterile distilled water and by scrapping the

culture surface with sterile glass slides. The resultant suspension was aseptically transferred into conical flasks each containing 400 ml of the medium. To avoid contamination, flasks were then sealed with cotton wool and aluminum foil. Flasks were incubated at room temperature (20 ± 2)⁰C on laboratory benches for 9 days (Mobisa, 2002). Resultant fermentation broth was filtered using a sterile muslin cloth. Resultant culture filtrate was kept in 1,000 ml sterile conical flasks. Flasks were then properly sealed with cotton wool and Aluminum foil and stored in a refrigerator at 4⁰C.

4.3.4. Application of the treatments for greenhouse and field experiments

Treatments were applied on a 10 day interval commencing 20 days after transplanting. BCAs included two isolates of *Trichoderma* spp. coded; Tricho 7 and Tricho 10, two *Bacillus subtilis* isolates coded CA51 and CB12 and Bio-cure (a commercial formulation of *Pseudomonas fluorescens*). One thousand ml of culture filtrates from isolates from respective BCAs prepared as described in sections 4.3.3.1 and 4.3.3.2 were thoroughly mixed with 1 ml acquawet (allowing them to stick on the leaf surface) and sprayed on leaves of tomato plants using hand sprayers. Since cases of phytotoxicity were recorded on tomato leaves for the normal strength, the culture filtrates were diluted to half strength by adding equal volume of sterile water. Tower (synthetic chemical) and Bio-cure were applied according to the manufactures' guidelines. A total of 6 sprays were done for the whole cropping season.

4.3.5. Field evaluation of bio-control agents

BCAs selected from the *in vitro* tests were evaluated for their effectiveness in managing tomato early blight in the field. Thirty days old tomato seedlings raised in nursery beds were transplanted in field plots as described in section 4.3.2 (Baka and Rashad, 2016). Application of

the treatments was done as described in sections 4.3.4. Fertilization, insecticide application, irrigating and weeding were carried out as per standard agronomic practices.

Tomato early blight was assessed on a 10 day interval commencing 20 days after transplanting until the end of harvesting (at 90 days after transplanting). Data collection was done on disease distribution, disease incidence and disease severity. Disease distribution was evaluated on a 0-2 scale, where 0 = no disease in the entire plot, 1 = disease existing in half of the plants in the plot, and 2 = disease existing over the whole plot. Evaluation of disease incidence was done on five plants randomly chosen from middle rows from each plot. The number of blighted compound leaves out of the total number of compound leaves per plant was considered. The proportion was converted to percent, where 0%= no disease and 100% = all compound leaves infected. Assessment of disease severity was done on five plants randomly chosen from middle rows. Five leaves randomly selected from each plant were considered. Evaluation of disease severity was done on a 0-5 scale modified from Pandey *et al.* (2003) by Kumar (2017), as follows: 0 = entire leaf free from disease, 1 = necrotic spots covering nearly 1-10% of leaf area, 2 = nearly 11-25% of leaf area covered by necrotic spots, 3 = necrotic spots nearly covering 26-50% of leaf area, 4 = 51-75% leaf area blighted, 5 = more than 75% leaf area blighted. Percent disease severity was calculated from data on disease severity.

Percent disease index (PDI) was calculated for each plot using the scores on disease distribution, disease incidence and disease severity as follows:

$$\text{PDI} = \frac{\text{Distribution score} + \text{Incidence score} + \text{Severity score}}{\text{Cumulative maximum disease score (8)}} \times 100$$

The AUDPC was calculated as follows:

$AUDPC = \Sigma[(X_{i+1} + X_i)/2] [t_{i+1} - t_i]$; where Σ =Sum total of the disease, X_i = Disease measure (percent disease severity in the case of this study) on the first assessment, X_{i+1} =Disease measure of the subsequent assessment, t_i = time on the first assessment (in days), t_{i+1} =time of the subsequent assessment (in days).

4.3.6. Greenhouse evaluation of bio-control agents

Selected antagonists from *in vitro* experiment were evaluated for their efficacy in managing early blight in tomatoes in the greenhouse. Pots of 22 cm diameter were packed with 5 Kg of the potting medium prepared as illustrated in section 3.3.3. Tomato seedlings from Rio Grande (a tomato early blight susceptible variety) were raised as described in section 3.3.3 and used for the experiment. Thinning was done as described in section 3.3.3. Inoculation of plants with the pathogen was done as described in section 3.3.4 and application of BCAs was done as described in section 4.3.4. Each treatment was replicated four times. BCAs were applied on a 10 day interval commencing 50 days after sowing tomato seeds. A total of six sprays were done. One week after the first application, tomato plants were inoculated with the *A. solani* conidia. To maintain high relative humidity conditions, plants were covered for 48 hours. Polythene bags were used (Yadav and Dabbas, 2012; Nashwa and Abo-Elyousr, 2012). Tomato early blight was assessed from each plant as described in section 4.3.5. The AUDPC and percent disease index for tomato early blight were calculated as described in section 4.3.5 except that for the percent disease index the data on disease distribution was not included (the cumulative disease score was thus 6).

4.3.7. Assessment of plant growth parameters

To evaluate the effects of BCAs on growth of tomato plants, data were collected on parameters including; plant height, the number of branches per plant and the number of compound leaves per plant, at an interval of 10 days commencing 20 days after transplanting until 90 days. Tomato fruits at pink or ripe stage were harvested and weighed per treatment on a weekly basis. Marketable fruits were selected from harvested fruits and weighed. For field data, the quantity of marketable fruits from each plot was extrapolated to tons per hectare (Tons/ha). On the other hand, for greenhouse experiment, the yield of marketable fruits was converted to Kg per plant (Kg/plant).

4.3.8. Data analysis

Data were analyzed as described in section 3.3.8. Correlations between parameters; tomato early blight measurement parameters and tomato plant growth parameters were tested by the two-tailed correlation coefficient of Pearson using IBM[®] SPSS[®] statistics 20.

4.4. Results

4.4.1. Effects of bio-control agents on tomato early blight percent disease incidence

At Kabete field Station the percent disease incidence did not significant ($p \leq 0.05$) differ among treatments on the 20th day after transplanting. Significant ($p \leq 0.05$) increases were recorded for the percent disease incidence in all the treatments with time. However, all the treatments significantly ($p \leq 0.05$) reduced the percent disease incidence compared to control treatment over time (Appendix 1). On the 90th day after transplanting, percent disease incidence ranged between 13.3 and 27.1% for all treatments while for control treatment, percent disease incidence was 69.9%. No significant ($p \leq 0.05$) differences were recorded over time in reducing the percent disease incidence between respective BCAs and the standard chemical (Tower) (Table 4.1).

Table 4.1: Effects of bio-control agents on tomato early blight percent disease incidence at Kabete Field Station

Treatment	Time after transplanting (Days)							
	20	30	40	50	60	70	80	90
CA51	10.1a	8.4ab	24.2a	37.2a	40.8a	44.7a	24.7a	23.9ab
CB12	9.4a	7.6ab	26.6a	36.1a	41.8a	31.6a	29.4a	27.1b
Tricho 7	9.2a	8.7ab	23.0a	30.9a	41.8a	36.6a	32.3a	25.8b
Tricho 10	10.4a	7.7ab	23.5a	37.1a	40.0a	33.3a	25.5a	24.1ab
<i>Pseudomonas</i>	10.4a	9.4b	21.0a	33.0a	40.6a	36.5a	30.4a	20.5ab
Tower	10.7a	5.3a	26.6a	35.1a	40.8a	33.1a	26.4a	13.3a
Water	10.7a	14.2c	51.2b	76.6b	78.4b	67.9b	71.5b	69.9c
LSD ($p \leq 0.05$)	2.0	3.9	14.8	19.0	10.6	15.9	17.6	12.4
% CV	11.1	24.9	29.7	26.2	12.9	22.1	28.8	23.9

Means accompanied by the same letter (s) in the same column are comparable ($p \leq 0.05$). LSD: Least significant difference, % CV: Percent of coefficient of variation, CA51 and CB12: *Bacillus subtilis* isolates, Tricho 7 and Tricho 10: *Trichoderma* isolates, *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure). Tower: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

At KALRO Mwea, the percent disease incidence did not significantly ($p \leq 0.05$) differ among the treatments on the 20th day after transplanting. In all the treatments, significant ($p \leq 0.05$) increases were recorded for the percent disease incidence over time. However, all treatments significant ($p \leq 0.05$) reduced the percent disease incidence compared to control treatment over time (Appendix 2). On the 90th day after transplanting, percent disease incidence ranging between 12.2 and 15.5% were recorded for all treatments while for control treatment, a percent disease incidence of 35.4% was recorded. Over time, effects recorded for Tower in reducing the percent disease incidence were comparable ($p \leq 0.05$) to all the BCAs except for the isolate CB12 with lower effects compared to Tower (Table 4.2).

Table 4.2: Effects of bio-control agents on tomato early blight percent disease incidence at KALRO Mwea

Treatment	Time after transplanting (Days)							
	20	30	40	50	60	70	80	90
CA51	9.0a	8.6a	19.1ab	20.9b	17.6b	11.6a	12.7a	13.2a
CB12	8.9a	8.0a	19.7ab	19.5ab	18.9b	11.3a	15.3a	14.5a
Tricho 7	8.7a	9.3a	21.3ab	20.9b	15.1b	11.4a	12.1a	13.8a
Tricho 10	8.2a	8.4a	21.5b	21.0b	13.0ab	11.5a	10.0a	15.5a
<i>Pseudomonas</i>	8.4a	8.8a	18.8ab	20.4b	13.6ab	11.2a	12.6a	13.8a
Tower	8.4a	6.0a	15.3a	16.1a	8.0a	10.8a	9.4a	12.2a
Water	8.1a	18.9b	42.0c	45.7c	37.0c	28.0b	27.8b	35.4b
LSD ($p \leq 0.05$)	2.4	5.3	6.1	4.2	6.2	4.5	6.1	9.2
% CV	15.9	30.4	15.2	10.0	19.7	18.5	24.2	30.5

Means accompanied by the same letter (s) in the same column are comparable ($p \leq 0.05$). LSD: Least significant difference, % CV: Percent of coefficient of variation, CA51 and CB12: *Bacillus subtilis* isolates, Tricho 7 and Tricho 10: *Trichoderma* isolates, *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure). Tower: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

In the greenhouse, the percent disease incidence for tomato early blight was comparable ($p \leq 0.05$) among all the treatments on the 20th day after transplanting. Significant ($p \leq 0.05$) increases in the percent disease incidence were recorded for all the treatments over time. Over time, all treatments did not significantly ($p \leq 0.05$) reduce the percent disease incidence compared to control treatment (Appendix 3). However, on the 60th, 70th, 80th and 90th days after transplanting; Tower, isolates Tricho 10 and CB12 significantly ($p \leq 0.05$) reduced the percent disease incidence compared to control treatment. On the 90th day after transplanting, percent disease incidence ranged between 6.9 and 16.2% for all treatments while for control treatment, percent disease incidence was 17.9% (Table 4.3).

Table 4.3: Effects of bio-control agents on tomato early blight percent disease incidence in the greenhouse

Treatment	Time after transplanting (Days)							
	20	30	40	50	60	70	80	90
CA51	4.6a	11.2b	16.0ab	8.5ab	7.0a	8.4ab	10.1abc	10.5ab
CB12	4.8a	6.5ab	9.6ab	8.1ab	5.7a	5.7a	9.0ab	10.3ab
Tricho 7	4.9a	8.5ab	11.2ab	8.0ab	7.7ab	7.7a	8.8ab	11.0abc
Tricho 10	5.6a	3.8a	6.0a	4.8a	6.2a	5.9a	6.2a	6.9a
<i>Pseudomonas</i>	4.8a	6.7ab	19.5b	14.4b	11.2c	13.1b	13.2bc	16.2bc
Tower	4.7a	4.1a	7.4a	5.2a	5.1a	4.5a	6.7a	8.1a
Water	5.1a	7.0ab	14.8ab	13.8b	11.0bc	12.6b	14.7c	17.9c
LSD ($p \leq 0.05$)	7.7	5.5	10.1	8.1	3.4	4.8	4.8	7.3
% CV	64.3	54.1	56.2	60.9	29.7	38.9	32.9	42.3

Means accompanied by the same letter (s) in the same column are comparable ($p \leq 0.05$). LSD: Least significant difference, % CV: Percent of coefficient of variation, CA51 and CB12: *Bacillus subtilis* isolates, Tricho 7 and Tricho 10: *Trichoderma* isolates, *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure). Tower: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

4.4.2. Effects of bio-control agents on tomato early blight percent disease severity

Percent disease severity for tomato early blight was evaluated as described in section 4.3.5 and used to generate disease progress curves for all the treatments. At Kabete Field Station, the percent disease severity was comparable ($p \leq 0.05$) among all the treatments on the 20th day after transplanting. Over time, significant ($p \leq 0.05$) increases were recorded for the percent disease severity in all the treatments. However, over time, all the treatments significantly ($p \leq 0.05$) reduced the percent disease severity compared to control treatment (Appendix 4). On the 90th day after transplanting, percent disease severity ranging between 40.5 and 51.5% were recorded for all treatments while for control treatment, a percent disease severity of 83.7 was recorded. Over time, effects recorded for Tower in reducing the percent disease severity were comparable ($p \leq 0.05$) to respective BCAs except for commercial *P. fluorescens* which recorded lower effect. No significant ($p \leq 0.05$) differences were recorded between bacterial and *Trichoderma* antagonists over time (Figure 4.1).

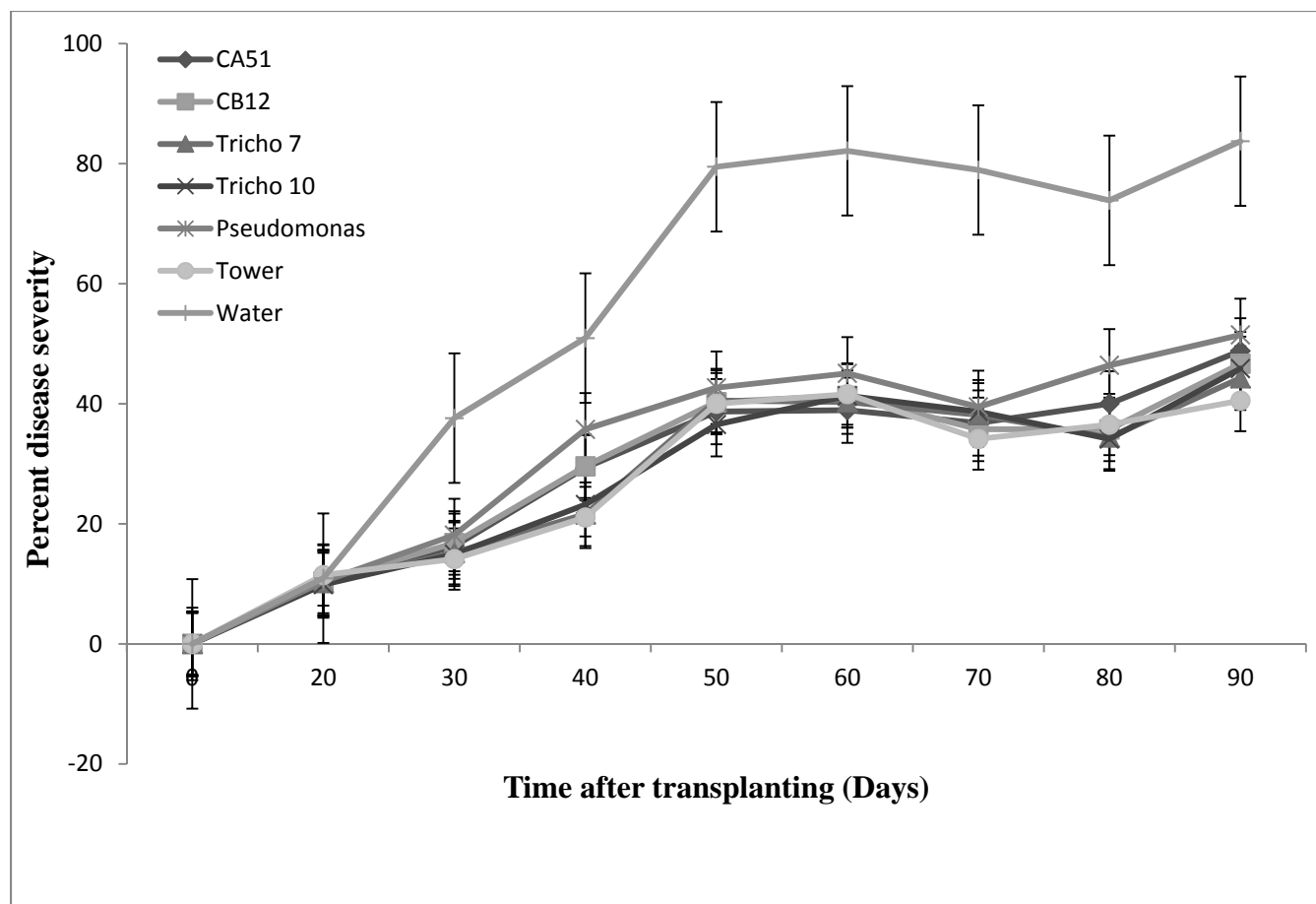


Figure 4.1: Effects of bio-control agents on tomato early blight disease progress curve at Kabete Field Station

CA51 and CB12: *Bacillus subtilis* isolates, Tricho 7 and Tricho 10: *Trichoderma* isolates, *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure). Tower: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

At KALRO Mwea, the percent disease severity for tomato early blight was comparable ($p \leq 0.05$) among all the treatments on the 20th day after transplanting. Over time, significant ($p \leq 0.05$) increases in the percent disease severity were recorded for all the treatments. However, over time, the percent disease severity was significantly ($p \leq 0.05$) lower in all treatments compared to control treatment (Appendix 5). On the 90th day after transplanting, percent disease severity ranged between 24.0 and 39.5% for all treatments while for control treatment, percent disease severity of 62.9% was recorded. The percent disease severity was significantly ($p \leq 0.05$) lower in

plots treated with Tower compared to those treated with respective BCAs except for *B. subtilis* isolates which recorded percent disease severity that was not significantly ($p \leq 0.05$) different from Tower. Over time, effects recorded for commercial *P. fluorescens* in reducing the percent disease severity were comparable ($p \leq 0.05$) to all BCAs except for CA51 which recorded a percent disease severity that was significantly ($p \leq 0.05$) lower (Figure 4.2).

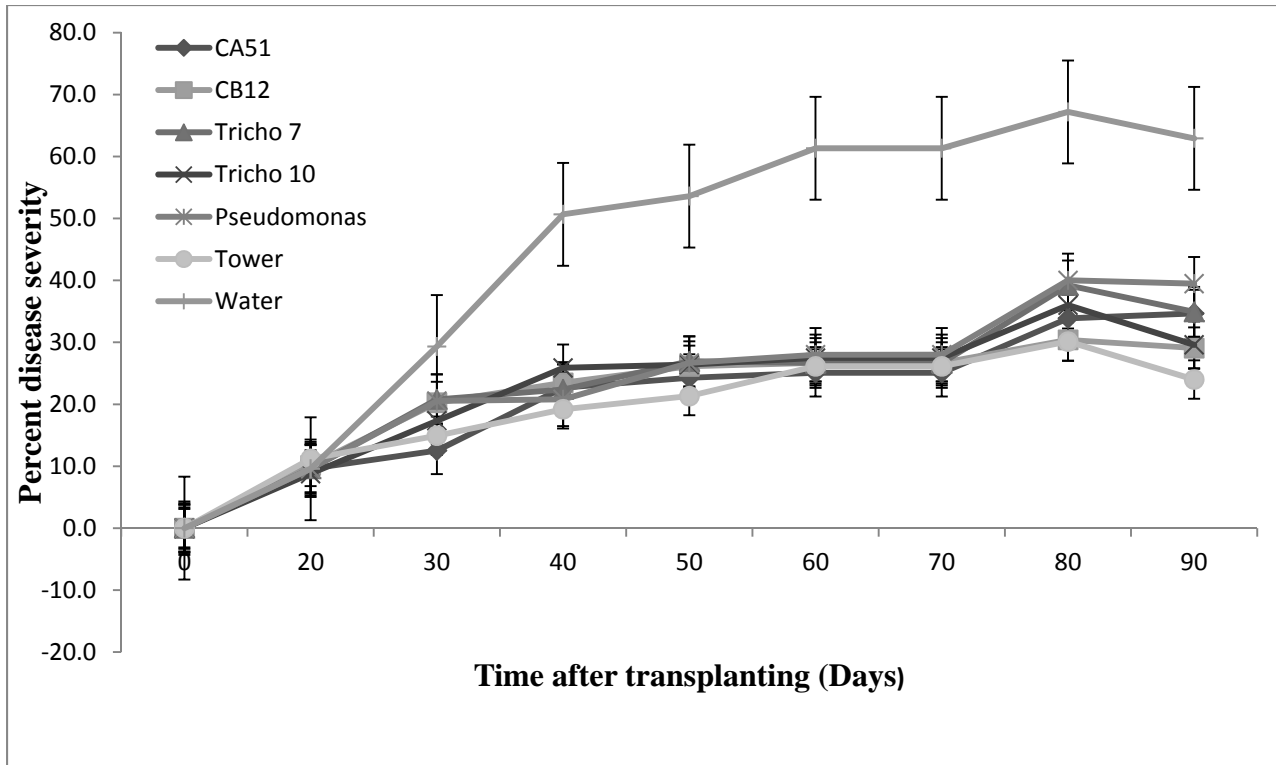


Figure 4.2: Effects of bio-control agents on tomato early blight disease progress curve at KALRO Mwea

CA51 and CB12: *Bacillus subtilis* isolates, Tricho 7 and Tricho 10: *Trichoderma* isolates, *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure). Tower: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

In the greenhouse, the percent disease severity for tomato early blight was comparable ($p \leq 0.05$) among treatments on the 20th day after transplanting. Over time, the percent disease severity significantly increased in all the treatments. However, over time, all treatments significantly ($p \leq 0.05$) reduced the percent disease severity compared to control treatment (Appendix 6). On

the 90th day after transplanting, percent disease index ranging between 32 and 48% were recorded for all treatments while for control treatment, percent disease severity was 74%. Over time, effects recorded for Tower in reducing the percent disease severity were comparable ($p \leq 0.05$) to respective BCAs except for commercial *P. fluorescens* which had a lower effect (Figure 4.3).

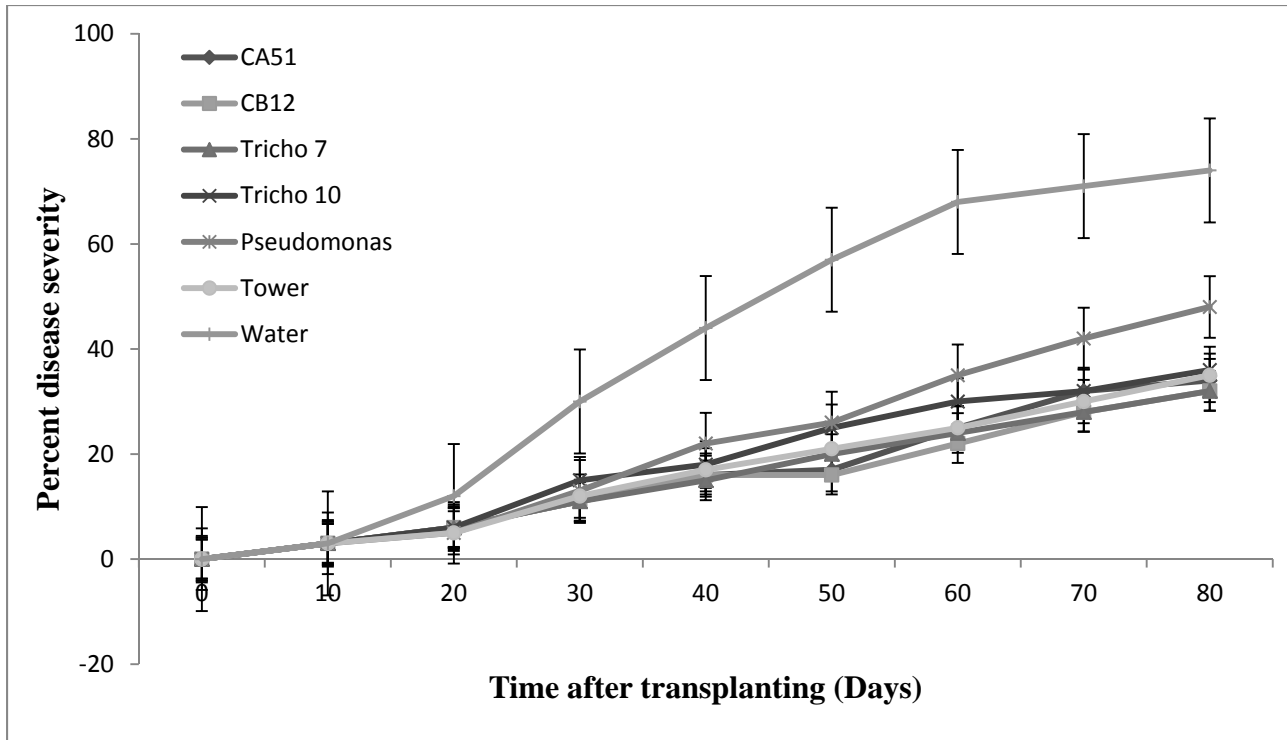


Figure 4.3: Effects of bio-control agents on tomato early blight disease progress curve in the greenhouse

CA51 and CB12: *Bacillus subtilis* isolates, Tricho 7 and Tricho 10: *Trichoderma* isolates, *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure). Tower: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

4.4.3. Effects of bio-control agents on tomato early blight percent disease index

At Kabete Field Station, the percent disease index did not significantly ($p \leq 0.05$) differ among treatments on the 20th day after transplanting. Over time, significant ($p \leq 0.05$) increases in the percent disease index were recorded in all the treatments. However, over time, all treatments

significantly ($p \leq 0.05$) reduced the percent disease index compared to control treatment (Appendix 7). On the 90th day after transplanting, percent disease index ranging between 42.8 and 53.1% were recorded for all treatments while for control treatment, a percent disease index of 86.1% was recorded. Over time, effects recorded for Tower in reducing the percent disease index did not significantly ($p \leq 0.05$) differ from BCAs except for commercial *P. fluorescens* with a lower effect compared to Tower. Compared to each other, bacterial and *Trichoderma* antagonists recorded comparable ($p \leq 0.05$) effects in reducing the percent disease index (Table 4.4).

Table 4.4: Effects of bio-control agents on tomato early blight percent disease index at Kabete Field Station

Treatment	Time after transplanting (Days)							
	20	30	40	50	60	70	80	90
CA51	14.3a	18.0a	33.0cd	43.4a	44.4a	44.8a	45.6bc	51.4bc
CB12	13.9a	15.8a	31.4bc	44.7a	46.9a	42.1a	42.3b	49.6bc
Tricho 7	14.6a	17.0a	26.8a	42.1a	43.7a	43.4a	40.9b	48.6bc
Tricho 10	14.5a	16.7a	28.7ab	40.4a	44.6a	42.9a	40.4b	47.9b
<i>Pseudomonas</i>	14.9a	16.8a	36.1d	44.6a	48.2a	45.5a	49.9c	53.1c
Tower	13.9a	16.6a	26.1a	42.3a	44.4a	40.1a	31.1a	42.8a
Water	15.2a	38.2b	61.2e	84.3b	86.1b	82.8b	80.1d	86.1d
LSD ($p \leq 0.05$)	1.5	3.3	3.2	9.4	7.51	5.8	7.0	5.0
% CV	5.7	8.6	5.2	10.9	8.2	6.7	8.1	5.2

Means accompanied by the same letter (s) in the same column are comparable ($p \leq 0.05$). LSD: Least significant difference, % CV: Percent of coefficient of variation, CA51 and CB12: *Bacillus subtilis* isolates, Tricho 7 and Tricho 10: *Trichoderma* isolates, *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure). Tower: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

At KALRO Mwea, the percent disease index for tomato early blight was comparable ($p \leq 0.05$) among the treatments on the 20th day after transplanting. Over time, significant ($p \leq 0.05$) increases were recorded for the percent disease index in all the treatments. However, over time,

significant ($p \leq 0.05$) reductions in the percent disease index were recorded in all treatments compared to control treatment (Appendix 8). On the 90th day after transplanting, percent disease index ranged between 30.3 and 41.0% for all treatments while for control treatment, a percent disease index of 68.8% was recorded. Tower recorded a significantly ($p \leq 0.05$) higher effect in reducing the percent disease index compared to respective BCAs. Respective BCAs recorded comparable ($p \leq 0.05$) effects in reducing the percent disease index (Table 4.5).

Table 4.5: Effects of bio-control agents on tomato early blight percent disease index at KALRO Mwea

Treatment	Time after transplanting (Days)							
	20	30	40	50	60	70	80	90
CA51	14.2a	20.6ab	28.2bc	31.1ab	34.0c	32.5a	38.2bc	38.7bc
CB12	15.0a	22.8ab	27.1ab	33.4b	32.4bc	33.5a	36.8b	35.8bc
Tricho 7	14.2a	25.0b	27.5bc	31.4ab	29.8ab	31.9a	39.8cd	37.3bc
Tricho 10	14.4a	24.0ab	31.3c	33.3b	32.9c	33.2a	38.8bc	35.0ab
<i>Pseudomonas</i>	14.1a	23.9ab	25.4ab	33.0b	34.4c	33.5a	41.2d	41.0c
Tower	14.7a	19.7a	23.5a	28.3a	28.6a	31.4a	33.8a	30.3a
Water	14.1a	34.5c	51.5d	58.4c	65.6d	66.4b	70.5e	68.8d
LSD ($p \leq 0.05$)	2.2	4.8	3.9	3.2	3.0	3.8	2.2	5.3
% CV	8.5	11.0	7.1	5.0	4.6	5.7	2.9	7.2

Means accompanied by the same letter (s) in the same column are comparable ($p \leq 0.05$). LSD: Least significant difference, % CV: Percent of coefficient of variation, CA51 and CB12: *Bacillus subtilis* isolates, Tricho 7 and Tricho 10: *Trichoderma* isolates, *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure). Tower: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

In the greenhouse, the percent disease index did not significantly ($p \leq 0.05$) differ among treatments on the 20th day after transplanting. The percent disease index recorded significant ($p \leq 0.05$) increases in all the treatments over time. However, significant reductions in the percent disease index were recorded for all treatments compared to control treatment over time (Appendix 9). On the 90th day after transplanting, percent disease index ranging between 28.4

and 42.7% were recorded for all treatments while for control treatment, the percent disease index was 64.7%. Effects recorded for Tower in reducing the percent disease index, were not significantly ($p \leq 0.05$) different from respective BCAs except for commercial *P. fluorescens* with a lower effect (Table 4.6).

Table 4.6: Effects of bio-control agents on tomato early blight percent disease index in the greenhouse

Treatment	Time after transplanting (Days)							
	20	30	40	50	60	70	80	90
CA51	3.3a	6.9ab	11.8a	13.6a	15.3a	22.2a	28.3a	30.1a
CB12	3.3a	6.1ab	10.8a	13.5a	14.3a	19.3a	24.8a	28.4a
Tricho 7	3.3a	6.4ab	11.0a	14.8a	18.0ab	21.3a	24.8a	28.5a
Tricho 10	3.4a	5.6a	13.5a	18.4ab	21.9bc	26.0ab	27.7a	31.1a
<i>Pseudomonas</i>	3.3a	5.3a	14.8a	20.9b	23.4c	31.3b	37.2b	42.7b
Tower	3.3a	4.8a	11.2a	16.0ab	18.3abc	21.6a	26.1a	30.5a
Water	3.4a	11.2b	27.5b	38.3c	49.3d	58.8c	61.6c	64.7c
LSD ($p \leq 0.05$)	5.3	5.3	10.1	5.2	5.2	6.9	6.3	5.8
% CV	68.1	54.3	47.2	18.0	15.2	16.3	12.9	10.7

Means accompanied by the same letter (s) in the same column are comparable ($p \leq 0.05$). LSD: Least significant difference, % CV: Percent of coefficient of variation, CA51 and CB12: *Bacillus subtilis* isolates, Tricho 7 and Tricho 10: *Trichoderma* isolates, *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure). Tower: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

4.4.4. Effects of bio-control agents on tomato early blight area under disease progress curve

At Kabete Field Station, significant reductions in the AUDPC for tomato early blight were recorded ($p \leq 0.05$) for all treatments compared to control treatment. AUDPCs ranging between 2,192 and 2,636 were recorded for all treatments while for control treatment the AUDPC was 4,557. No significant ($p \leq 0.05$) differences were recorded in reducing the AUDPC between Tower and respective BCAs except for commercial *P. fluorescens* which recorded a lower effect.

No significant ($p \leq 0.05$) differences were recorded between bacterial or *Trichoderma* antagonists (Figure 4.4).

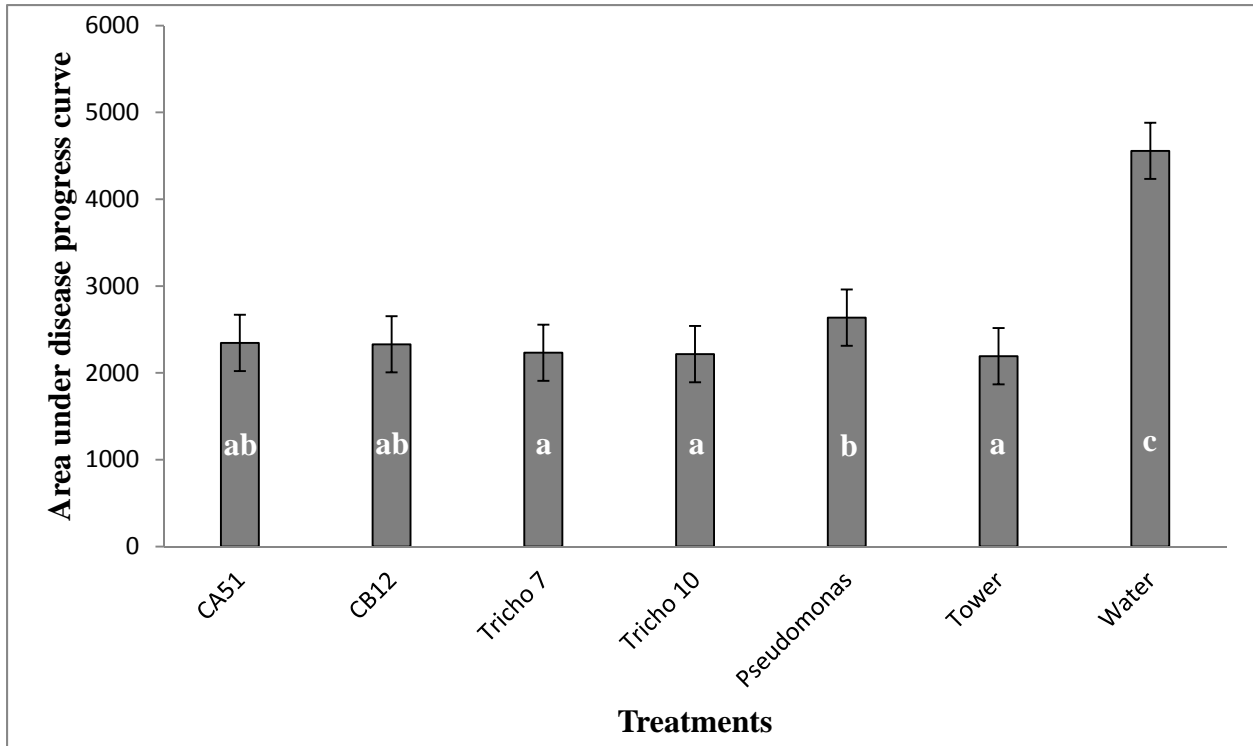


Figure 4.4: Effects of bio-control agents on tomato early blight area under disease progress curve at Kabete Field Station

Treatments with the same letter (s) are comparable ($p \leq 0.05$). CA51 and CB12: *Bacillus subtilis* isolates, Tricho 7 and Tricho 10: *Trichoderma* isolates, *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure). Tower: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

At KALRO Mwea, all treatments significantly ($p \leq 0.05$) reduced the AUDPC for tomato early blight compared to control treatment. AUDPCs ranging between 1,477 and 1,693 were recorded for all treatments while for control treatment an AUDPC of 3,291 was recorded. Tower recorded a significantly ($p \leq 0.05$) higher effect in reducing the AUDPC compared to respective BCAs except for *B. subtilis* isolates with comparable effects. Commercial *P. fluorescens* recorded a

significantly ($p \leq 0.05$) lower effect in reducing the AUDPC compared to respective BCAs except Tricho 7 with comparable effect (Figure 4.5).

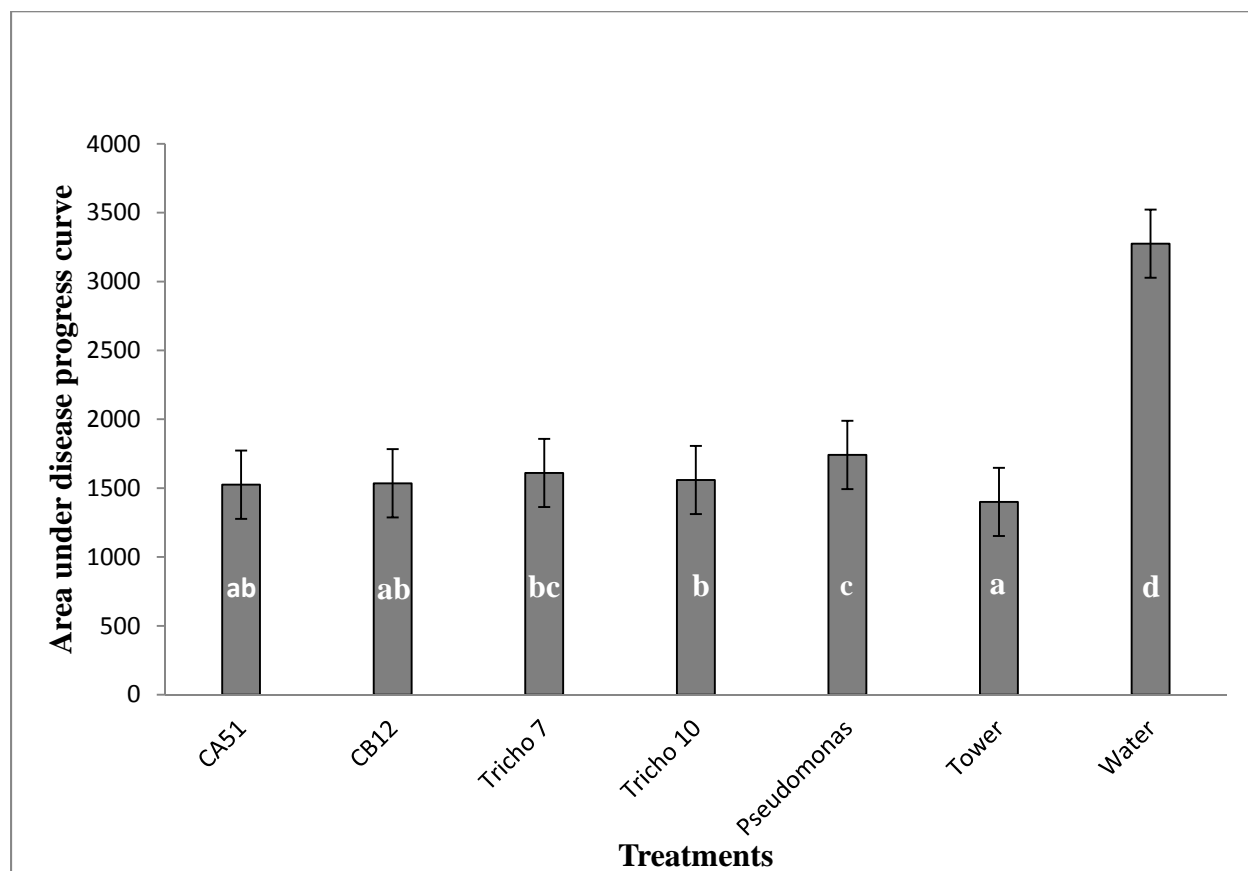


Figure 4.5: Effects of bio-control agents on tomato early blight area under disease progress curve at KALRO Mwea

Treatments with the same letter (s) are comparable ($p \leq 0.05$). CA51 and CB12: *Bacillus subtilis* isolates, Tricho 7 and Tricho 10: *Trichoderma* isolates, *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure). Tower: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

In the greenhouse, all treatments significantly ($p \leq 0.05$) reduced the AUDPC for tomato early blight compared to control treatment. AUDPCs ranging between 1,180 and 1,700 were recorded for all treatments while for control treatment, AUDPC of 3,290 was recorded. No significant ($p \leq 0.05$) differences were recorded in reducing the AUDPC between Tower and respective BCAs except for commercial *P. fluorescens* which recorded a lower effect. However, no

significant ($p \leq 0.05$) differences were recorded between commercial *P. fluorescens* and the isolate Tricho 10 (Figure 4.6).

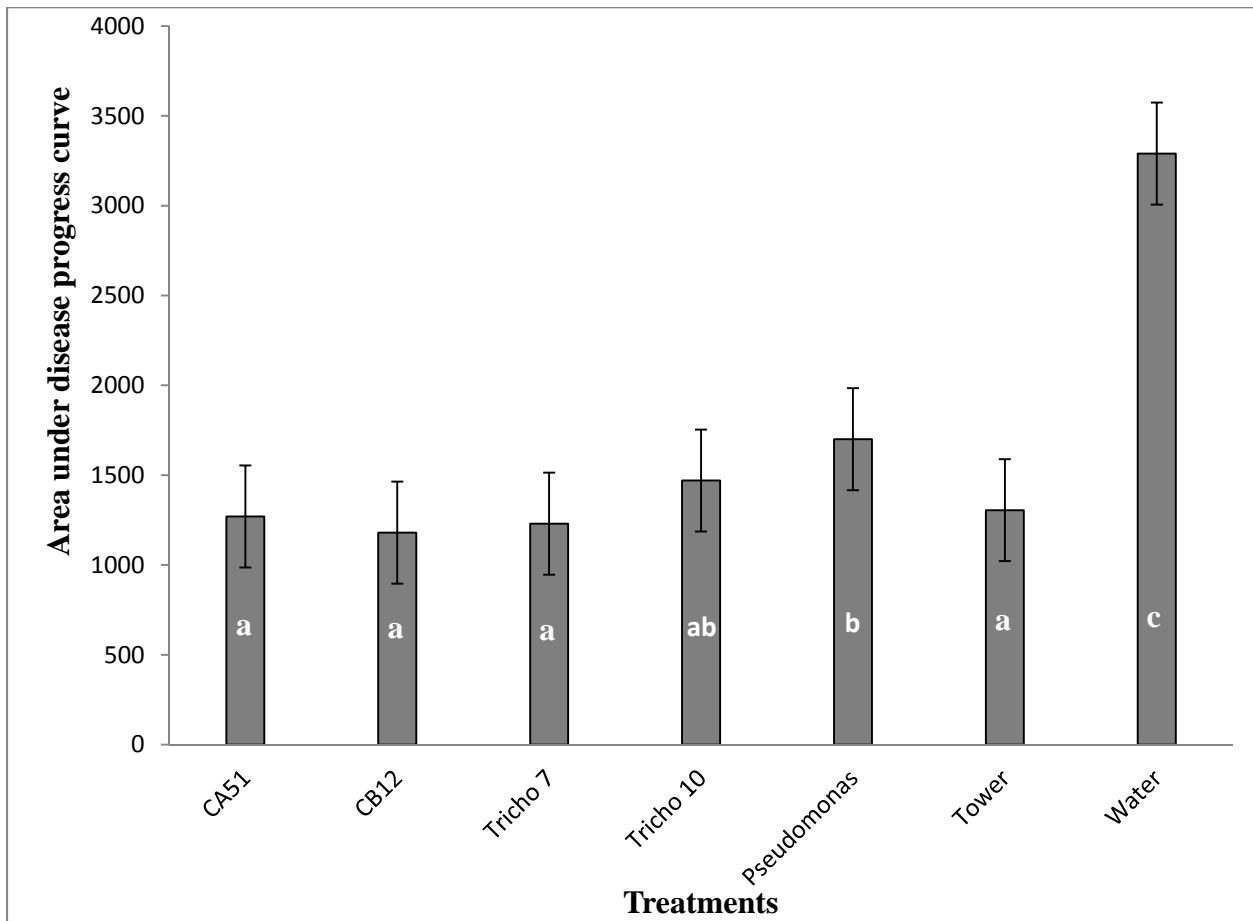


Figure 4.6: Effects of bio-control agents on tomato early blight area under disease progress curve in the greenhouse

Treatments with the same letter (s) are comparable ($p \leq 0.05$). CA51 and CB12: *Bacillus subtilis* isolates, Tricho 7 and Tricho 10: *Trichoderma* isolates, *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure). Tower: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

4.4.5. Effects of bio-control agents on tomato plant growth parameters

Over the growing season, no significant ($p \leq 0.05$) differences were recorded for plant height, the number of branches per plant and the number of compound leaves per plant between all the treatments at both experimental sites and in the greenhouse.

4.4.6. Effects of bio-control agents on the quantity of marketable tomato fruits

At Kabete Field Station, quantity of marketable tomato fruits was significantly ($p \leq 0.05$) higher in all treatments compared to control treatment. Mean quantities of marketable fruits ranging between 7.2 and 10.1 tons/hectare were recorded for all the treatments while for control treatment the mean quantity of marketable fruit was 3.8 tons/hectare. Tower recorded significantly ($p \leq 0.05$) higher quantity of marketable tomato fruits compared to all BCAs. No significant differences were recorded ($p \leq 0.05$) in the quantity of marketable fruits between bacterial or *Trichoderma* antagonists (Table 4.7).

Table 4.7: Effects of bio-control agents on quantity of marketable tomato fruits (Tons/hectare) at Kabete Field Station

Treatment	1 st Harvest	2 nd Harvest	3 rd Harvest	4 th Harvest	5 th harvest	Means
CA51	8.0b	9.1b	7.0ab	6.0b	5.9b	7.2bc
CB12	5.3abc	9.3bc	7.1ab	6.2b	5.8b	6.8b
Tricho 7	5.7abc	9.7bc	9.0b	9.2c	7.2b	8.1bc
Tricho 10	5.1abc	11.8c	7.7ab	9.5c	7.4b	8.3c
<i>Pseudomonas</i>	4.2abc	8.5b	8.9b	7.1b	6.8b	7.1bc
Tower	7.6ab	11.0bc	10.3b	11.8d	10.0c	10.1d
Water	3.9c	4.3a	4.3a	3.2a	3.0a	3.8a
LSD (≤ 0.05)	3.6	2.6	3.6	2.2	2.4	1.4
% CV	35.4	16.1	26.0	15.9	20.1	10.9

Means accompanied by the same letter (s) in the same column are comparable ($p \leq 0.05$). LSD: Least significant difference, % CV: Percent of coefficient of variation, CA51 and CB12: *Bacillus subtilis* isolates, Tricho 7 and Tricho 10: *Trichoderma* isolates, *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure). Tower: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

At KALRO Mwea, all treatments recorded significantly ($p \leq 0.05$) higher quantities of marketable tomato fruits compared to control treatment. Mean quantities of marketable fruits ranging between 8.1 and 11.7 tons/hectare were recorded for all the treatments while for control treatment mean quantity of 3.2 tons/hectare was recorded. The quantity of marketable fruits was

significantly ($p \leq 0.05$) higher with Tower treatment compared to all the BCAs. *Trichoderma* isolates recorded significantly ($p \leq 0.05$) higher quantities of marketable tomato fruits compared to respective BCAs agents (Table 4.8).

Table 4.8: Effects of bio-control agents on the quantity of marketable tomato fruits (Tons/hectare) at KALRO Mwea

Treatment	1 st Harvest	2 nd Harvest	3 rd Harvest	4 th Harvest	5 th Harvest	Means
CA51	9.4bc	7.8b	10.3bc	7.0b	5.8b	8.1b
CB12	8.7b	10.2bc	10.1bc	8.6bcd	8.8b	9.3c
Tricho 7	11.3bc	10.7bc	11.0bc	11.2cde	7.2b	10.3d
Tricho 10	11.1bc	11.7bc	10.8bc	11.7de	7.3b	10.5d
<i>Pseudomonas</i>	9.4bc	8.8bc	9.4b	7.9bc	5.7b	8.2b
Tower	12.8c	12.6c	12.2c	12.4e	8.6b	11.7e
Water	4.2a	3.3a	3.9a	2.7a	2.1a	3.2a
LSD ($p \leq 0.05$)	3.5	4.2	2.4	3.4	3.4	1.0
% CV	20.4	25.4	13.8	21.9	29.0	6.4

Means accompanied by the same letter (s) in the same column are comparable ($p \leq 0.05$). LSD: Least significant difference, % CV: Percent of coefficient of variation, CA51 and CB12: *Bacillus subtilis* isolates, Tricho 7 and Tricho 10: *Trichoderma* isolates, *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure). Tower: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

In the greenhouse, mean quantities of marketable tomato fruits were significantly ($p \leq 0.05$) higher in all treatments compared to control treatment. Mean quantities of marketable fruits ranging between 0.14 and 0.21 kg/plant were recorded for all treatments while for control treatment mean quantity of 0.06 kg/plant was recorded. Compared to respective BCA agents both *Trichoderma* isolates and Tower recorded significantly ($p \leq 0.05$) higher quantities of marketable tomato fruits (Table 4.9).

Table 4.9: Effects of bio-control agents on quantity of marketable tomato fruits (Kg/Plant) in the greenhouse

Treatment	1 st Harvest	2 nd Harvest	3 rd Harvest	4 th Harvest	5 th Harvest	Means
CA51	0.16b	0.16b	0.18b	0.12b	0.07ab	0.14b
CB12	0.15b	0.16b	0.20b	0.13bc	0.10bc	0.15b
Tricho 7	0.18b	0.28c	0.28d	0.17bcd	0.14c	0.21c
Tricho 10	0.18b	0.26c	0.26cd	0.16bcd	0.12bc	0.19c
<i>Pseudomonas</i>	0.15b	0.18b	0.21bc	0.14bc	0.11bc	0.16b
Tower	0.20b	0.27c	0.22bc	0.21d	0.10bc	0.20c
Water	0.07a	0.08a	0.07a	0.05a	0.03a	0.06a
LSD ($p \leq 0.05$)	0.06	0.07	0.06	0.06	0.06	0.03
% CV	25.4	24.1	18.8	28.3	43.8	13.1

Means accompanied by the same letter (s) in the same column are comparable ($p \leq 0.05$). LSD: Least significant difference, % CV: Percent of coefficient of variation, CA51 and CB12: *Bacillus subtilis* isolates, Tricho 7 and Tricho 10: *Trichoderma* isolates, *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure). Tower: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

4.4.7. Correlations between tomato early blight disease parameters and plant growth parameters

In the greenhouse, no significant ($p \leq 0.05$) correlations were recorded between different parameters. At Kabete Field Station, the percent disease incidence was significantly ($p \leq 0.05$) and negatively correlated with the number of branches per plant. No other significant ($p \leq 0.05$) correlation was recorded between different parameters at Kabete Field Station (Table 4.13).

Table 4.10: Correlations between tomato early blight parameters and plant growth parameters at Kabete Field Station

	% disease incidence	% disease severity	% disease index	AUDPC	Plant height	No of branches/Plant	No of compound leaves/Plant	Quantity of marketable fruits
% disease incidence	-							
% disease severity	0.857**	-						
% disease index	0.902**	0.992**	-					
AUDPC	0.856**	0.974**	0.979**	-				
Plant height	-0.219	0.058	-0.004	-0.017	-			
No of branches/Plant	-0.423*	-0.135	-0.192	-0.228	0.702**	-		
No of compound leaves/Plant	-0.554**	-0.169	-0.241	-0.179	0.570**	0.620**	-	
Quantity of marketable fruits	-0.820**	-0.837**	-0.858**	-0.802**	0.014	0.228	0.275	-

** : Significant at $p \leq 0.01$, * : Significant at $p \leq 0.05$, AUDPC: Area under disease progress curve, No: number, %: percent.

At KALRO Mwea, the percent disease incidence was significantly ($p \leq 0.05$) and negatively correlated with plant height and the number of branches per plant. Plant height recorded significant ($p \leq 0.05$) and positive correlations with the number of compound leaves and the quantity of marketable fruits at KALRO Mwea (Table 4.14). No other significant ($p \leq 0.05$) correlation was recorded among the parameters.

Table 4.11: Correlations between tomato early blight parameters and plant growth parameters at KALRO Mwea

	% disease incidence	% disease severity	% disease index	AUDPC	Plant height	No Branches/Plant	No of compound leaves/Plant	Quantity of marketable fruits
% disease incidence	-							
% disease severity	0.736**	-						
% disease index	0.824**	0.976**	-					
AUDPC	0.872**	0.924**	0.974**	-				
Plant height	-0.384*	-0.360	-0.363	-0.318	-			
No of branches/Plant	-0.481*	-0.296	-0.288	-0.302	0.327	-		
No of compound leaves/Plant	-0.606**	-0.168	-0.232	-0.286	0.379*	0.648**	-	
Quantity of marketable fruits	-0.766**	-0.919**	-0.942**	-0.901**	0.400*	0.220	0.137	-

** : Significant at $p \leq 0.01$, * : Significant at $p \leq 0.05$, AUDPC: Area under disease progress curve, No: number, %: percent.

4.4.8. Site interactions between Kabete Field Station and KALRO Mwea for tomato early blight disease parameters and tomato plant growth parameters

Site effects were evaluated for respective tomato early blight disease parameters and plant growth parameters. Significant ($p \leq 0.05$) interactions were recorded for the AUDPC (Appendix 10), the percent disease incidence (Appendix 11) and the weight of marketable fruits (Appendix 13). No significant ($p \leq 0.05$) interactions were recorded for the number of compound leaves per plant, the number of branches per plant, plant height, percent disease index and percent disease severity.

4.5. Discussion

Findings from this study reported significant reductions of the percent disease incidence for all treatments compared to control treatment at both experimental sites. Thus, applications of BCAs were effective in protecting tomato leaves from being infected by *A. solani*. These results are in agreement with the findings of Suleiman *et al.* (2017) who reported that *B. thuringiensis*, *P. fluorescens* and *T. viride* significantly reduced the percent disease incidence for early blight on potatoes under field conditions. Similarly, Udhav (2013) evaluated *B. subtilis*, *P. fluorescens* and *T. viride* against *A. solani* on potatoes under field conditions and reported significant reductions in the percent disease incidence. In this study, no significant differences in the percent disease incidence were reported (at $p \leq 0.05$) for all treatments in the greenhouse over time. These findings were in partial agreement with those of Deepthi (2006) who tested *T. harzianum*, *T. viride* and *P. fluorescens* against *A. vitis* and reported that the BCAs were not effective when they were applied 7 days after plant inoculation with the pathogen. However, the findings from this study are in contrast with those reported by Moges *et al.* (2012) who evaluated *P. fluorescens* and *B. subtilis* against *A. solani* on tomato in the greenhouse and reported significant reduction in the disease incidence by both antagonists. This may be explained by the fact that effectiveness of BCAs in controlling plant pathogens can be influenced by several factors along with the nature of the crop plant and the pathogen being controlled, environmental conditions such as nutrient availability, pH and temperature (Benítez *et al.*, 2004).

This study recorded significant reductions in the percent disease severity for tomato early blight for all treatments compared to control treatment at both experimental sites and in the greenhouse. Tower recorded comparable effects in reducing the percent disease severity for tomato early

blight with most BCAs. Thus, applications of BCAs on tomato leaves, prevented *A. solani* from spreading over the surface of tomato leaves. This minimizes the negative effects of the pathogen over the photosynthesis process. These findings corroborate those of Mishra *et al.* (2017) who evaluated *T. harzianum* and *P. fluorescens* against powdery mildew on garden pea and recorded significant reductions in the percent disease severity. Similar findings were reported by Verma *et al.* (2008) who evaluated *T. harzianum*, *T. viride* and *Bacillus subtilis* against *A. solani* on tomato in the greenhouse and reported significant reductions in the disease severity by all the BCAs. Similarly, Verma *et al.* (2018) evaluated *T. harzianum*, *T. viride* and *P. fluorescens* against *A. solani* on tomato in the greenhouse and reported significant reduction in disease severity for all the BCAs. However, Udhav (2013) evaluated *B. subtilis*, *P. fluorescens* and *T. viride* against *A. solani* on potato in open field and reported effectiveness for all the BCAs but noted a superior effect for Mancozeb compared to all the BCAs. Similarly, Sundaramoorthy and Balabaskar (2013) found that Carbendazim (0.1%) was most effective in reducing tomato wilt in the greenhouse. This is due to the fact that the effectiveness of BCAs can be influenced by the environmental factors, the nature of the plant pathogen and the plant itself (Benítez *et al.*, 2004).

This study recorded effectiveness for all BCAs in reducing the percent disease index for tomato early blight at both experimental sites and in the greenhouse. The application of BCAs reduces the intensity of tomato early blight by preventing *A. solani* from spreading from infected tomato leaves to healthy ones and from infected tomato plants to healthy ones. This corroborates the findings of Moges *et al.* (2012) who evaluated *B. subtilis* and *P. fluorescens* against *A. solani* in the greenhouse and reported significant reductions of the percent disease index by both antagonists. In this study, no significant differences were recorded between Tower and most

BCAs at Kabete Field Station. Similarly, Ngoc (2013) evaluated Ridomil (Metalaxyl 4% + Mancozeb 64%), *T. harzianum*, *T. viride*, *B. subtilis*, *Bacillus* spp. and *P. fluorescens* against *A. solani* on tomato in open field and reported that all the treatments significantly reduced the percent disease index compared to the untreated control but did not record any significant differences for Ridomil (a synthetic chemical) when compared to *P. fluorescens* or *Bacillus* spp. On the other hand, Tower recorded a significantly higher effect in reducing the percent disease index for tomato early blight at KALRO Mwea and in the greenhouse. This corroborates the findings of Ngoc (2013) who reported higher effect for Quintal (Carbendazim 25% +Iprodione) against *A. solani* on tomato plants in open fields compared to *T. harzianum*, *T. viride*, *Bacillus subtilis*, *Bacillus* spp., *P. fluorescens*. Similarly, Moges *et al.* (2012) reported that Mancozeb had a higher effect in reducing the percent disease index for tomato early blight compared to *B. subtilis* and *P. fluorescens* in the greenhouse.

Findings of this study recorded significant reductions of AUDPC for tomato early blight for all treatments compared to control treatment at both experimental sites and in the greenhouse. No significant differences were recorded in the reduction of AUDPC for most BCAs compared to Tower at both experimental sites and in the greenhouse. Application of BCAs slowed progression of tomato early blight. Similar findings were reported by Zegeye *et al.* (2011) who evaluated *P. fluorescens* and *T. viride* against *Phytophthora infestans* and recorded significant reductions of AUDPC by both BCAs. In their experiment, no significant differences were recorded in reducing the AUDPC between *T. viride* and Mancozeb (a synthetic chemical). Similarly Pute (2016) evaluated *B. subtilis* against *A. solani* on tomato and recorded significant reduction of AUDPC by the bacterium compared to the untreated control.

This study reported no significant effects of BCAs and Tower on tomato plant height, the number of branches per plant and the number of compound leaves per plant. These findings are in agreement with those reported by Ngoc (2013) who evaluated *T. viride*, *B. subtilis*, *P. fluorescens* and Ridomil against *A. solani* on tomatoes and recorded no significant effects for all the treatments on plant height and the number of branches per plant compared to untreated control. Similar findings were reported by Joseph *et al.* (2017) who evaluated *P. fluorescens* against *A. solani* and reported no effects of the antagonist on tomato plant height compared to the untreated control. Similarly, Ozbay and Newman (2004) evaluated the effects of three strains of *T. harzianum* on the growth of tomato plants and recorded no significant effects for the strain T95 on the number of leaves per plant compared to the control. However, Alemu and Alemu (2015) evaluated two strains of *P. fluorescens* on faba bean and reported increases in the number of branches per plant and plant height for the treated plants.

In this study, the quantities of marketable fruits were significantly higher in all treatments compared to water treatment at both experimental sites and in the greenhouse. Thus, application of BCAs on tomato plants minimizes the effects of *A. solani* over tomato plants and decreases associated yield losses. These findings are in agreement with those of Joseph *et al.* (2017), who evaluated *P. fluorescens* on tomato plant infected by *A. solani* and reported significant increases in fruit weight for the rhizobacterium over the untreated control. Similarly, Udhav (2013) evaluated *T. viride*, *B. subtilis* and *P. fluorescens* against *A. solani* on potato and reported significant increases in tuber yields for all the BCAs over the untreated control. The findings of this study also corroborate those of Suleiman *et al.* (2017) who evaluated *B. thuringiensis*, *P. fluorescens* and *T. viride* against *A. solani* on potato and reported significant increases in tuber

yields for all the treatments compared to the untreated control. Yadav (2014) evaluated *T. harzianum* and *P. fluorescens* against Fusarium wilt on tomatoes and reported increased tomato yield for both antagonists in open fields. Similarly, Sundaramoorthy and Balabaskar (2013) evaluated *T. harzianum* against Fusarium wilt on tomatoes in the greenhouse and reported increased fruit yield.

In this study, plant height recorded significant and positive correlations with the number of compound leaves per plant and the quantity of marketable fruits at KALRO Mwea. Similar findings were reported by Patel (2013) who recorded significant positive correlations for tomato plant height and number of primary branches, number secondary branches and fruit weight. The findings of this study recorded a significant and negative correlation between the percent disease incidence and the number of branches per plant at both experimental sites. A significant negative correlation between the percent disease incidence and plant height was also recorded at KALRO Mwea. This might be associated with the fact that the growth of tomato plant is weakened by a high infection with *A. solani* (Foolad *et al.*, 2008).

BCAs namely; *Trichoderma* isolates Tricho 7 and Tricho 10, *B. subtilis* isolates CA51 and CB12 and *P. fluorescens* were effective in managing tomato early blight under field and greenhouse conditions. Applications of commercial *P. fluorescens* and culture filtrates from *B. subtilis* and *Trichoderma* spp. reduced the intensity of tomato early blight under field and greenhouse conditions. Applications of BCAs agents were beneficial to tomato fruit production as they increased the quantity of marketable fruits. Integrating *Trichoderma* isolates Tricho 7 and Tricho 10, *B. subtilis* isolates CB12 and CA51 in the management of tomato early blight will be

beneficial for sustainable production of tomatoes as BCAs have fewer negative effects on the environment.

CHAPTER FIVE: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. General discussion

This study recorded effectiveness for *B. subtilis* isolates, *P. fluorescens* and *Trichoderma* isolates against the radial growth of *A. solani*. All the BCAs were effective in managing tomato early blight under field and greenhouse conditions. Thus, in this study, a strong correlation between the effectiveness of BCAs *in vitro* and their effectiveness *in vivo* was recorded against *A. solani*. This corroborates the findings from several other studies which demonstrated correlation between the effectiveness of BCAs against plant pathogens *in vitro* and their effectiveness *in vivo*. For instance, Babu *et al.* (2000a) tested *P. fluorescens* against *A. solani* and reported significant activities of the antagonist over the pathogen radial growth and effectiveness of the antagonist against the pathogen *in vivo*. Similar findings were reported by Selim, (2015) who evaluated five *Trichoderma* isolates and recorded effectiveness for all the antagonists against the radial growth of *A. solani* and in their effectiveness in managing tomato early blight *in vivo*. Similarly, Alemu and Alemu (2013) evaluated 12 isolates of *P. fluorescens* against *Botrytis fabae* causing chocolate spot on faba bean and reported that all the isolates effectively inhibited the mycelial growth of the pathogen *in vitro* and reduced the disease incidence and severity in pot culture through production of secondary metabolites. However, the effectiveness of BCAs *in vitro* is not always correlated with their effectiveness *in vivo*. For instance, Babu *et al.* (2000b) tested *T. harzianum* against *A. solani* and reported significant inhibition of the radial growth of the pathogen but recorded no effect of the antagonist over the pathogen in pot culture. Variations in the effectiveness of BCAs might result from the nature of the pathogen being controlled, the

plant variety and environmental conditions along with nutrient availability, pH and temperature (Benítez *et al.*, 2004).

In this study the radial growth of *A. solani* was inhibited by the presence of respective BCAs. Growth inhibition zones appearing between most BCAs colonies and *A. solani* colony were recorded. *Trichoderma* colonies grew over *A. solani* colony. It has been reported that, BCAs utilize various strategies such as mycoparasitism, antibiosis and competition for available nutrients and space to hinder growth of plant pathogens. The mechanisms can be used individually or synergistically (Benítez *et al.*, 2004). Presence of growth inhibition zones suggested production of growth inhibition compounds by the antagonists which hinder pathogen growth. Growing over the colonies of *A. solani* suggested the ability of *Trichoderma* isolates to mycoparasitize *A. solani* colonies (Viterbo *et al.*, 2002; Nusret and Steven, 2004).

In this study, culture filtrates from *B. subtilis* and *Trichoderma* isolates were used to manage tomato early blight under field and greenhouse conditions. The culture filtrates contained cells or spores and the metabolites from respective BCAs. Metabolites secreted by microbial antagonists have been reported to be better in controlling plant pathogens than the antagonist cells themselves (Sultan, 2012). Better disease inhibition has been reported when antagonist cells are applied together with their metabolites (Sultan, 2012).

In this study, increased tomato yields were recorded for plants treated with respective BCAs compared to water treated plants. BCAs minimized the effects of *A. solani* on tomato production through reduction of the disease intensity. BCAs have been reported to have activity that

promote plant growth and increase fruit production (Compant *et al.*, 2005; Vinale *et al.*, 2014; Jain and Das, 2016). The findings of this study corroborate those of Ramyabharathi and Raguchander (2014) who tested the strain EPCO16 of *B. subtilis* against *Fusarium oxysporum* f.sp. *lycopersici* and recorded increased plant growth and fruit yield in addition to the reduction of the disease incidence for plants treated with the antagonist compared to the untreated control plants. Similarly, Alemu and Alemu (2013) evaluated *P. fluorescens* against *Botrytis fabae* on Faba bean and reported induction of systemic resistance to the pathogen in faba bean plants by the antagonist in addition to promotion of plant growth. Rani *et al.* (2017) evaluated *T. harzianum* against *A. solani* in open fields and recorded reduction of the disease intensity for tomato early blight and increased tomato yields for plants treated with the antagonist compared to untreated plants.

5.2. Conclusion

BCAs namely; *Trichoderma* isolates Tricho 7 and Tricho 10, *B. subtilis* isolates CA51 and CB12 and *P. fluorescens* were effective in inhibiting the radial growth of *A. solani*. *Trichoderma* isolates Tricho 7 and Tricho 10 were the most effective followed by *B. subtilis* isolates CA51 and CB12.

All the BCAs were effective in reducing the percent disease index for tomato early blight under field and greenhouse conditions. All the BCAs significantly reduced the spread of tomato early blight infection from infected leaves to healthy ones. All the BCAs were also effective in reducing the spread of early blight infection over the leaf area. However, the standard chemical recorded higher effects in reducing the percent disease index compared to most BCAs.

Applications of all the BCAs minimized the effects of early blight on tomato production through reduction of disease severity. The quantities of marketable fruits were higher in plots treated with

respective BCAs compared to those treated with water. However, the synthetic chemical recorded the highest quantities of marketable fruit compared to respective BCAs under field condition while the *Trichoderma* isolates Tricho 7 and Tricho 10 recorded quantities of marketable fruits that did not significantly differ from the synthetic chemical in the greenhouse.

5.3. Recommendations

From the findings of this study it is recommendable that further studies be done:

- I. On integration of BCAs namely; *Trichoderma* isolates Tricho 7 and Tricho 10 and *B. subtilis* isolates CA51 and CB12 in the management of tomato early blight under greenhouse and field conditions.
- II. On making formulations from *Trichoderma* isolates Tricho 7 and Tricho 10 and *B. subtilis* isolates CA51 and CB12 for ease of storage and application and for prolonging shelf life.
- III. For eliciting the mechanisms of action for *Trichoderma* isolates Tricho 7 and Tricho 10 and *B. subtilis* isolates CA51 and CB12.
- IV. For more accurate identification of *Trichoderma* isolates Tricho 7 and Tricho 10.
- V. To elicit the effects of the combinations of respective *Trichoderma* and *B. subtilis* isolates on early blight.

CHAPTER SIX: REFERENCES

- Abdalla, S. A., Algam, S. A. A., Ibrahim, E. A. and El Naim, A. M. 2014. *In vitro* screening of *Bacillus* isolates for biological control of early blight disease of tomato in Shambat soil. *World Journal of Agricultural Research*, 2(2): 47-50.
- Abd-El-Kareem, F., El-Mougy, N. S., El-Gamal, N. G. and Fatouh, Y. O. 2006. Use of chitin and chitisan against tomato root rot disease under greenhouse conditions. *Research Journal of Agriculture and Biological Science*, 2(4): 147-152.
- Abdelmoteleb, A., Gonzalez-Mendoza, D., Troncoso-Rojas, R. and Gonzalez-Soto, T. 2017. Antifungal activity of autochthonous *Bacillus subtilis* isolated from *Prosopis juliflora* against phytopathogenic fungi. *The Korean Journal of Mycology*, 45(4): 385-391.
- Abdullahi, S. S., Vidya, H. C. and Joly, E. T. 2017. Bio-control of early blight of tomato using consortium of *Bacillus subtilis* and *Pseudomonas fluorescens*. *Life Sciences International Research Journal*, 4(1): 2347-2391.
- Acquaah, G. 2002. Horticulture: principles and practices. New Jersey: Prentice hall. ISBN 0-13-033125-2.
- Adhikari, P., Oh, Y. and Panthee, D. R. 2017. Current status of early blight resistance in tomato: An Update. *International Journal of Science*, 18: 2019; doi:10.3390/ijms18102019
- Alabouvette, C., Olivain, C. and Steinberg, C. 2006. Biological control of plant diseases: the European situation. *European Journal of Plant Pathology*, 114: 329-341.
- Alemu, F. and Alemu, T. 2013. Antifungal activity of secondary metabolites of *Pseudomonas fluorescens* isolates as a biocontrol agent of chocolate spot disease (*Botrytis fabae*) of faba bean in Ethiopia. *African Journal of Microbiology Research*, 7(47): 5364-5373.

- Alemu, F. and Alemu, T. 2015. *Pseudomonas fluorescens* isolates used as a plant growth promoter of faba bean (*Vicia faba*) *in vitro* as well as *in vivo* study in Ethiopia. American Journal of Life Sciences, 3(2): 100-108.
- Anderson, B., Dongo, A. and Pryor, B. M. 2008. Secondary metabolite profiling of *Alternaria dauci*, *A. porri*, *A. solani*, and *A. tomatophila*. Mycological Research, 112: 241-250.
- Angayarkanni, T., Kamalakannan, A., Santhini, E. and Predeepa, D. 2005. Identification of biochemical markers for the selection of *Pseudomonas fluorescens* against *Pythium* spp. In: Asian conference on Emerging Trends in Plant-Microbe Interactions. University of Madras, PP 295-303.
- Anonymous, 2016. Tomato production. PennState Extension <https://extension.psu.edu/tomato-production> (accessed on 15th June, 2018).
- Arora, D.K. and Dwivedi, R. S. 1979. Rhizosphere fungi of *Lens esculenta* Moench. antagonistic to *Sclerotium rolfsii* Sacc. Soil Biology and Biochemistry, 11(6): 563-566.
- Ashour, A. M. A. 2009. A protocol suggested for managing tomato early blight. Egyptian Journal of Phytopathology, 37(1): 9-20.
- Babu, J., Seetharaman, K., Nandakumar, R. and Johnson, I. 2000a. Bio-control efficacy of *Pseudomonas fluorescens* against *Alternaria solani* and tomato leaf blight disease. Annals of Plant Protection Sciences, 8: 252-254.
- Babu, S., Seetha, R. K., Nandakumar, R. and Johnson, I. 2000b. Efficacy of fungal antagonists against leaf blight of tomato caused by *Alternaria solani* (Ellis and Mart.) Jones and Grout. Journal of Biological control, 14: 79-81.

- Baka, Z. A. M. and Rashad, Y. M. 2016. Alternative control of early blight disease of tomato using the plant extracts of *Acacia nilotica*, *Achillea fragrantissima* and *Calotropis procera*. *Phytopathologia Mediterranea*, 55(1): 121-129.
- Baker, C. J., Stavely, J. R. and Mock, N. 1985. Bio-control of bean rust and *Bacillus subtilis* under field conditions. *Plant Disease*, 69: 770-772.
- Benítez, T., Rincón, A. M., Limón, M. C. and Codón, A. C. 2004. Bio-control mechanisms of *Trichoderma* strains. *International Microbiology*, 7(4): 249-260.
- Bhattacharjee, R. and Dey, U. 2014. An overview of fungal and bacterial biopesticides to control plant pathogens and diseases. *African Journal of Microbiology Research*, 8(7): 1749-1769.
- Bohs, L. 2005. Major clades in *Solanum* based on *ndhF* sequences. In “A Festschrift for William G. D’Arcy: the legacy of a taxonomist,” ed. Keating, R. C., Hollowell, V. C. and Croat, T. B. *Systematic Botany Missouri Botanical Garden*, 104: 27-49.
- Bose, T. K. and Som, M. G. 1986. *Vegetable Crops in India*. Nayaprakash Publishing, Calutta, PP 773.
- Bouis, H. 2003. Micronutrient fortification of plants through plant breeding; can it improve nutrition in Mana at low cost? *Proceedings Nutrition Society*, 62: 403-411.
- Brescoll, J. and Daveluy, S. 2015. A review of vitamin B12 in Dermatology. *American Journal of Clinical Dermatology*, 16: 27-33.
- Chaerani, R. and Voorrips, R. E. 2006. Tomato early blight (*Alternaria solani*): the pathogen, genetics, and breeding for resistance. *Journal of General Plant Pathology*, 72: 335-347.
- Chandrasekaran, M., Chandrasekar, R., Chun, S. C. and Sathiyabama, M. 2016. Isolation, characterization and molecular three-dimensional structural predictions of

- metalloprotease from a phytopathogenic fungus, *Alternaria solani* (Ell. And Mart.) Sor. Journal of Bioscience and Bioengineering, 122: 131-139.
- Chandrasekaran, M., Chandrasenkar, R., Sa, T. and Sathiyabama, M. 2014. Serine protease identification (*in vitro*) and molecular structure predictions (*in silico*) from a phytopathogenic fungus, *Alternaria solani*. Journal of Basic Microbiology, 54: 210-218.
- Compant, S., Duffy, B., Nowak, J., Clément, C. and Barka, E. 2005. Use of plant growth promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Applied and Environmental Microbiology, 71(9): 4951-4959.
- Dalpati, N. N. S., Parate, R. L. and Ingle, S. T. 2010. Efficacy of some bioagents and botanicals against *Alternaria macrospora* causing leaf spot of cotton. Journal of Plant Disease Sciences, 5(1): 95-97.
- Davies, K. and Spiegel, Y. 2011. Root patho-systems nematology and biological control. In: Davies K. and Spiegel, Y. (Eds). Biological control of plant parasitic nematodes: Building coherence between microbial ecology and molecular mechanisms, Chapter 12, Springer, The Netherlands.
- Dawoud, M. E. A., Mahmoud, Z. K., Ahmed, H. E. and Farahat, M. G. 2012. Growth promotion and biocontrol of leaf spot and leaf speck diseases in tomato by *Pseudomonas* spp. Egyptian Journal of Experimental Biology, 8(1): 67-76.
- De Hoog, G. S., Guarro, J., Gene, J. and Figueras, M. J. 2000. Atlas of clinical fungi, 2nd edition, vol. 1. Centraal bureau voor Schimmel cultures, Utrecht, The Netherlands.
- Deepthi, K. P. 2006. Studies on the management of leaf blight of grape caused by *Alternaria vitis* (Cav.) Sacc. MSc Thesis, Acharya N.G. Ranga Agricultural University, PP 182.

- Deveau, A., Palin, B., Gross, H., Schnepf, M., Mehnaz, S., Leblond, P., Aigle, B. and Dorrestein, P. C. 2016. Role of secondary metabolites in the interaction between *Pseudomonas fluorescens* and soil microorganisms under iron-limited conditions. *Microbiology Ecology*, 92 (8): 1-11.
- Driks, A. 2004. The *Bacillus* spore coat. *Phytopathology*, 94: 1248-1251.
- Druzhinina, I. S., Kopchinskiy, A. G. and Kubicek, C. P. 2006. The first 100 *Trichoderma* species characterized by molecular data. *Mycoscience*, 47: 55-64.
- Ellis, J. B. and Martin, G. B. 1882. *Macrosporium solani* E & M. *American Naturalist*, 16: 1003.
- Engindeniz, S. and Ozturk, G. C. 2013. An economic comparison of pesticide application for processing and table tomatoes, a case study for Turkey. *Journal of Plant Protection Research*, 53(3): 230-237.
- Fabro, L. and Varca, M. 2011. Pesticide usage by farmers in the Pagsanjan-Lumban catchment of Laguna de bay, Philippines. *Elservier Journal on Agricultural Water Management*, 106: 27-34.
- Food and Agriculture Organization Statistics (FAOSTAT). 2019. Available at <http://www.fao.org/faostat/en/#data/QC> (accessed on 10th April, 2019)
- Foolad, M. 2007. Genome mapping and molecular breeding of tomato. *International Journal Plant Genomics*. [CrossRef] [PubMed].
- Foolad, M. R., Subbiah, P. and Ghangas, G. S. 2002. Parent-offspring correlation estimate of heritability for early blight resistance in tomato, *Lycopersicon esculentum* Mill. *Euphytica*, 126: 291-297.
- Foolad, M., Merk, H. and Ashrafi, H. 2008. Genetics, genomics and breeding for late blight and early blight resistance in tomato. *Plant Science*, 27: 75-107.

- Franzetti, L. and Scarpellini, M. 2007. Characterization of *Pseudomonas* spp. isolated from foods. *Annals of Microbiology*, 57(1): 39-47.
- Galloway, B. T. 1891. The new potato disease. *Garden and Field*, Adelaide, Australia 16: 158.
- Ganie, S. A., Ghani, M. Y., Nissar, Q. and U-Rehman, S. 2013. Bio-efficacy of plant extracts and bio-control agents against *Alternaria solani*. *African Journal of Microbiology Research*, 7: 4397-4402.
- George, G. M. 2001. *Bergey's Manual of Systematic Bacteriology*. Second Edition. Springer-Verlag, New York.
- Giovanelli, G. and Paradise, A. 2002. Stability of dried and intermediate moisture tomato pulp during storage. *Journal of Agriculture and Food Chemistry*, 50: 7277-7281.
- Giovannucci, E. 2002. A review of epidemiologic studies of tomatoes, lycopene and prostate cancer. *Experimental Biology and Medicine*, 227: 852-859.
- Goufo, P., Mofor, T. C., Fontem, D. A. and Ngnokam, D. 2008. High efficacy of extracts of Cameroon plants against tomato late blight disease. *Agronomy for Sustainable Development*, 28 (4): 567-573.
- Grey, N. S. 2010. Monitoring the simultaneous response of various water status indicators for use in the irrigation scheduling and drought stress detection of a greenhouse tomato crop. MSc Thesis, University of Zimbabwe, PP 150.
- Grosso, G., Bei, R., Mistretta, A., Marventano, S., Calabrese, G., Masuelli, L., Giganti, M. G., Modesti, A., Galvano, F. and Gazzolo, D. 2013. Effects of Vitamin C on health: A review of evidence. *Frontiers in Bioscience*, 1(18): 1017-1029.

- Gupta, S., Singh, G. and Sharma, N. 2014. *In vitro* screening of selected plant extracts against *Alternaria alternata*. Journal of Experimental Biology and Agricultural Sciences, 2(3): 344-351.
- Harman, G. E., Howell, C. R., Viterbo, A., Chet, I. and Lorito, M. 2004. *Trichoderma* species-opportunistic, avirulent plant symbionts. Nature Reviews Microbiology, 2: 43-56.
- Hassanein, N. M., Abou Zeib, M. A., Youssef, K. A. and Mahmoud, D. A. 2010. Control of tomato early blight and wilt using aqueous extract of neem leaves. Phytopathologia Mediterranea, 49: 143-151.
- Hendricks, P. 2012. Life cycle assessment of greenhouse tomato (*Solanum lycopersicum* L.) production in Southwestern Ontario. MSc Thesis, University of Guelph, PP 95.
- Heydari, A. and Pessarakli, M. 2010. A review on biological control of fungal plant pathogens using microbial antagonists. Journal of Biological Sciences, 10: 273-290.
- Horticultural Crop Development Authority (HCDA). 2013. Production and Marketing news Vol.6 Issue NO. 2.
- Hou, M. S. and Huang, J. B. 2006. Agricultural phytopathology. Science Press, Beijing.
- Hunziker, A. T. 2001. Genera *Solanacearum*-the genera of *Solanaceae* illustrated, arranged according to a new system. A.R.G. Gantner Verlag, Ruggell, Liechtenstein, [Google Scholar](#)
- Islam, R. M. D., Mondal, C., Hossain, I. and Meah, M. B. 2013. Organic management: An alternative to control late blight of potato and tomato caused by *Phytophthora infestans*. International Journal of Theoretical and Applied Sciences, 5(2): 32-42.

- Jaetzold, R., Schimidt, H., Hornet, Z. B. and Shisanya, C. A. 2006. Farm management handbook of Kenya. Natural conditions and farm information Vol 11/C 2nd edition ministry of Agriculture/GTZ Nairobi.
- Jagadeesh, K. S. and Jagadeesh, D. R. 2009. Biological control of early blight of tomato caused by *Alternaria solani* as influenced by different delivery methods of *Pseudomonas gladioli* B25. In II International Symposium on Tomato Diseases; Saygili, H., Sahid, F., Aysan, Y., Eds., ISHS: Kasadari, Turkey, 808: 327-332.
- Jain, A. and Das, S. 2016. Insight into the interaction between plants and associated fluorescent *Pseudomonas* spp. International Journal of Agronomy, 2016: 1-8.
- Jaria, F. 2012. Irrigation scheduling strategies for tomato production in Southwest Ontario. PhD Dissertation, McGill University, Montreal, PP 274.
- Jones, J. B. 2008. Tomato plant culture: In the field greenhouse and garden. Taylor and Francis group. USA. PP 55.
- Jones, L. R. 1893. The new potato disease or early blight. Vermont Agricultural Experimental Station Bulletin, 6: 66-70.
- Joseph, A., Igbiosa, O. B., Alori, E. T., Aluko, A. P. and Ademiluyi, B. O. 2017. Effectiveness of *Pseudomonas* species in the management of tomato early blight pathogen *Alternaria solani*. African Journal of Microbiology Research, 11(23): 972-976.
- Junior, J. B. T., Rezende, R., Itako, A. T., Freitas, P. S. L. and Frizzone, J. A. 2011. Drip fungigation in early blight control of tomato. Maringá, 33(1): 9-14.
- Kamanu, J. K., Chemining'wa, G. N., Nderitu, J. H. and Ambuko, J. 2012. Growth, yield and quality response of snap bean (*Phaseolus vulgaris* L.) plants to different inorganic fertilizers applications in Central Kenya. Journal of Applied Biosciences, 55: 3944-3952.

- Kannangara, S., Dharmarathna, R. M. and Jayarathna, D. L. 2016. Isolation, identification and characterization of *Trichoderma* species as a potential bio-control agent against *Ceratocystis paradoxa*. *Journal of Agricultural Sciences*, 12(1): 51-62.
- Karuku, G. N., Kimenju, J. W. and Verplancke, H. 2017. Farmers' perspectives on factors limiting tomato production and yields in Kabete, Kiambu County, Kenya. *East African Agricultural and Forestry Journal*, 82(1): 70-89.
- Kemmitt, G. 2002. Early blight of potato and tomato. In *The Plant Health Instructor*. The American Phytopathological Society (APS): St Paul, MN, USA.
- Kenya Agriculture and Livestock Research Organization (KALRO). 2005. KALRO annual report 2005. Nairobi, Kenya.
- Kenya Horticultural Competitiveness Project (KHCP). 2011. Fruits and vegetables retail tracker June-August 2011 report. USAID. http://www.fintrac.com/cpanelx_pu/Kenya%20khcp/10_00_3937_KHCP_Horticulture%20Retail%20Audit_August_August%202011.pdf. (Accessed on 10th September, 2018).
- Kimura, S. and Sinha, N. 2008. Tomato (*Solanum lycopersicum*): A model fruit-bearing crop. *Cold Spring Harbor Protocols*, doi:10.1101/pdb.emo105
- Knapp, S. 2002. *Solanum* section Geminata. *F1 Neotrop* 84:1-405.
- Knapp, S. and Peralta, I. E. 2016. The tomato (*Solanum lycopersicum* L., *Solanaceae*) and its botanical relatives. Books.google.com
- Koenig, T., Blatt, J., Brakel, K., Kloss, K., Nilges, T. and Woellert, F. 2008. Market-driven development and poverty reduction: A value chain analysis of fresh vegetables in Kenya and Tanzania (SLE series). Humboldt Universitat zu Berlin, Center for Advanced Training in Rural Development.

- Kumar, P. 2017. Studies on *Alternaria solani* causing early blight disease in tomato (*Lycopersicon esculentum* Mill.). MSc Thesis, Indira Gandhi Krishi Vishwa Vidyalaya, Raipur (Chhattisgarh), PP 171.
- Kumar, V., Kumar, A., Srivastava, M. and Pandey, S. 2015. *Trichoderma harzianum* (Th. azad) as a mycoparasite of *Fusarium* and growth enhancer of tomato in glasshouse conditions. *Journal of Pure and Applied Microbiology*, 10(2): 1463-1468.
- Lengai, W. M. G. 2016. Efficacy of plant extracts and antagonistic fungi as alternatives to synthetic pesticides in management of tomato pests and diseases. MSc Thesis, University of Nairobi, PP 99.
- Li, Y. H. 2012. Early blight of tomato. The Connecticut Agricultural Experiment Station (www.ct.gov/caes).
- Liu, Y., Tao, J., Yan, Y. J., Li, B., Li, H. and Li, C. 2011. Biocontrol efficiency of *Bacillus subtilis* SL-13 and characterization of an antifungal chitinase. *Chinese Journal of Chemical Engineering*, 19: 128-134.
- Loeffler, W., Tschen, J. S. M., Vanittanakom, N., Kugler, M., Knorpp, E., Hsieh, T. F. and Wu, T. G. 1986. Antifungal effects of bacilysin and fengymycin from *Bacillus subtilis* F-29-3: A comparison with activities of other *Bacillus* antibiotics. *Journal of Phytopathology*, 115(3): 204-213.
- Lugtenberg, B. and Kamilova, F. 2009. Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology*, 63(1): 541-556.
- Mamgain, A., Roychowdhury, R. and Tah, J. 2013. *Alternaria* pathogenicity and its strategic controls. *Research Journal of Biology*, 1: 1-9.

- Martinez, S. P., Snowdon, R. and Pons-Kuhnemann, J. 2004. Variability of Cuban and international populations of *Alternaria solani* from different hosts and localities: AFLP genetic analysis. *European Journal of Plant Pathology*, 110: 399-409.
- Masinde, A. O. A., Kwambai, K. T. and Wambani, N. H. 2011. Evaluation of tomato (*Lycopersicon esculentum* L.) 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.
- Maurya, M. K., Singh, R. and Tomer, A. 2014. *In vitro* evaluation of antagonistic activity of *Pseudomonas fluorescens* against fungal pathogen. *Journal of Biopesticides*, 7(1): 43-46.
- Meera, T. and Balabaskar, P. 2012. Isolation and characterization of *Pseudomonas fluorescens* from rice fields. *International Journal of Food, Agriculture and Veterinary Sciences*, 2(1): 113-120.
- Mishra, V. 2012. Effect of fungicides and plant extracts in management of *Alternaria* blight of tomato. *Annual of Plant Protection Science*, 20(1): 243-244.
- Mishra, V., Lal, A. A. and Simon, S. 2017. Efficacy of botanicals and bio-control agents against powdery mildew disease of garden pea (*Pisium sativum* L.). *Journal of Pharmacognosy and Phytochemistry*, 6(4): 1125-1126.
- Mizubuti, G. S. E., Junior, V. L. and Forbes, G. A. 2007. Management of late blight with alternative products, *Pest Technology*, 1(2): 106-116.
- Mobisa, O. 2002. Studies on control of French bean rust (*Uromyces appendiculatus* (Pers.) Unger var. *appendiculatus*) using fungal and bacterial metabolites. MSc Thesis, University of Nairobi, PP 92.

- Moges, M. M., Val Selvaraj, T. and Jebessa, M. T. 2012. Influence of some antagonistic bacteria against early blight (*Alternaria solani* (Ell. and Mart.) Jones and Grout.) of tomato (*Lycopersicon esculentum* Mill.). The African Journal of Plant Science and Biotechnology, 6(1): 40-44.
- Monsanto website. 2013. Tomato Anna F1 Hand book. http://www.monsantoafrica.com/pdfs/tomato_anna_f1_growers_handbook.pdf. 1. (Accessed on 27th, August 2018).
- Monte, A. J., Carvalho, D. F., Medici, O. L., Silva, L. B. D. and Pimentel, C. 2013. Growth analysis and yield of tomato under different irrigation depths. Revista Brasileira de Engenharia Agricola e Ambiental, 17(9): 926-931.
- Montemurro, N. and Visconti, A. 1992. *Alternaria* metabolites-chemical and biological data. In: Chelkowski, J. and Visconti, A. (eds) *Alternaria* biology, plant disease and metabolites. Elsevier, Amsterdam, PP: 449-558.
- Muiru, W. M. 2000. Isolation of soil actinomycetes, characterization and screening of their antibiotics against economically important plant pathogens. MSc Thesis, University of Nairobi, PP 171.
- 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.
- Nagel, K., Schneemann, I., Kajahn, I., Labes, A., Wiese, J. and Imhoff, F. 2012. Beneficial effects of 2,4-diacetylphloroglucinol producing pseudomonads on the marine alga *Saccharina latissima*. Aquatic Microbial Ecology, 67: 239-249.

- Naika, S., De Jeude, J. V. L., De Goffau, M., Hilmi, M. and Van Dam, B. 2005. Cultivation of tomato, production, processing and marketing, Agromisa Foundation and CTA, Wageningen, NL.
- Nandakumar, R., Babu, S., Radjacommar, R., Raguchander, T. and Samiyappan, R. 2002. *Pseudomonas fluorescens* mediated antifungal activity against *Rhizoctonia solani* causing sheath blight in rice. *Phytopathologia mediterranea*, 41: 109-119.
- Narayanasamy, P. 2011. Microbial plant pathogens-detection and disease diagnosis: Fungal Pathogens, Vol. 1, DOI 10.1007/978-90-481-9735-4-2.
- Nashwa, S. M. A. and Abo-Elyousr, K. A. M. 2012. Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. *Plant Protection Science*, 48: 74-79.
- Nderitu, J., Kasina, M., Nyamasyo, G. and Oronje, M. 2007. Effects of insecticide application on sunflower (*Heliantus annuus* L.) pollination in Eastern Kenya. *World Journal of Agricultural Sciences*, 3(6): 731-734.
- Neergaard, P. 1945. Danish species of *Alternaria* and *Stemphylium*: taxonomy, parasitism, economic significance. Oxford University Press, London, PP: 260-287.
- Nepali, B., Bhattarai, S. and Shrestha, J. 2018. Identification of *Pseudomonas fluorescens* using different biochemical tests. *International Journal of Applied Biology*, 2(2): 27-32.
- Ngoc, N. K. 2013. Management of early blight of tomato caused by *Alternaria solani* (Ellis and Martin) Jones and Grout. MSc Thesis, University of Agricultural Sciences, Bengaluru, PP 134.
- Neils, A., Salamanca, L. R. and Hausbeck, M. K. 2015. Organic management of early blight of tomato. Michigan State University Extension, PP 1.

- Noling, J. W. 2013. Nematode management in tomatoes, peppers and eggplant. IFAS extension publication #ENY-032, University of Florida.
- Nusret, O. and Steven, E. N. 2004. Biological control with *Trichoderma* spp. with emphasis on *Trichoderma harzianum*. Pakistan Journal of Biological Science, 7(4): 478-484.
- Onduso, J. N. 2014. Management of bacterial wilt of tomato by use of resistant rootstock. MSc Thesis. University of Nairobi, PP 84.
- Ozbay, N. and Newman, S. E. 2004. Effect of *Trichoderma harzianum* Strains to Colonize Tomato Roots and Improve Transplant Growth. Pakistan Journal of Biological Sciences, 7: 253-257.
- Pandey, A., Palni, L. M. S. and Trivedi, P. 2006. *In vitro* evaluation of antagonistic properties of *Pseudomonas corrugata*. Microbiological Research, 163 (3): 329-336.
- Pandey, K. K., Pandey, P. K., Kalloo, G. and Banerjee, M. K. 2003. Resistance to early blight of tomato with respect to various parameters of disease epidemics. Journal of General Plant Pathology, 69: 364-371.
- Papadopoulos, A. P. 1991. Growing greenhouse tomatoes in soil and in soilless media. Agriculture Canada Publication 1865/E.
- Papavizas, G. C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for bio-control. Annual Review of Phytopathology, 23: 23-54.
- Patel, P. K. 2013. Correlation and path analysis in tomato (*Solanum lycopersicum* Mill.), MSc Thesis, The Indira Gandhi Krishi Vishwavidyalaya, Raipur, PP 119.
- Pelletier, J. R. and Fry, W. E. 1989. Characterization of resistance to early blight in three potato cultivars: Incubation period, lesion expansion rate, and spore production. Phytopathology, 79: 511-517.

- Peralta, I. E., Spooner, D. M. and Knapp, S. 2008. Taxonomy of wild tomatoes and their relatives (*Solanum* sections *Lycopersicoides*, *Juglandifolia*, *Lycopersicon*; *Solanaceae*). Monographs in Systematic Botany, 84: 1-186.
- Piazza, P., Sjiniski, S. and Tsiantis, M. 2005. Evolution of leaf developmental mechanisms. *New Phytologist*, 167: 693-710.
- Ramanujam, J. R. Kulothungan, S., Prabhu, S. S., Kumaran, E., Shanmugaraju, V. and Arun, P. 2015. A study on synergism between *Pseudomonas fluorescens* and *Parthenium hysterophorus* as biocontrol agents to *Alternaria alternata* (leaf spot) in *Lycopersicon esculentum* (Tomato). *Scrutiny International Research Journal of Microbiology and Bio Technology*, 2(1): 25-38.
- Ramyabharathi, S. A. and Raguchander, T. 2014. Efficacy of secondary metabolites produced by *Bacillus subtilis* EPCO16 against tomato wilt pathogen *Fusarium oxysporum* f.sp. *lycopersici*. *Journal of Mycology and Plant Pathology*, 44(2): 148-153.
- Ramyasmruthi, S., Pallavi, O., Pallavi, S., Tilak, K. and Srividya, S. 2012. Chitinolytic and secondary metabolite producing *Pseudomonas fluorescens* isolated from *Solanaceae* rhizosphere effective against broad spectrum fungal phytopathogens. *Asian Journal of Plant Science and Research*, 2(1): 16-24.
- Rani, S., Singh, R. and Gupta, S. 2017. Development of integrated disease management module for early blight of tomato in Jammu. *Journal of Pharmacognosy and Phytochemistry*, 6(2): 268-273.
- Rhoda, B., Freyer, B. and Macharia, J. 2006. Towards reducing synthetic pesticides imports in favour of locally available botanicals in Kenya, Conference on International Agricultural Research for Development. Held From 11th to 13th October 2006, Bonn, Germany.

- Rojo, F. G., Reynoso, M. M., Sofia, M. F. and Torres, A. M. 2007. Biological control by *Trichoderma* species of *Fusarium solani* causing peanut brown root rot under field conditions. *Crop Protection*, 26: 549-555.
- Rotem, J. 1994. The Genus *Alternaria*: biology, epidemiology and pathogenicity. The American Phytopathological Society: St. Paul, MN, USA, Volume 326, PP 48.
- Rukmini, M., Sahoo, D., Dalei, J. and Ray, R. 2015. Production, purification and characterization of bacitracin from *Bacillus subtilis*. *The Pharma Innovation Journal*, 3(12): 77-82.
- Saba, H., Vibhash, D., Manisha, M., Prashant, K. S., Farhan, H. and Tauseef, A. 2012. *Trichoderma*-a promising plant growth stimulator and biocontrol agent. *Mycosphere*, 3(4): 524-531.
- Schultz, D. and Ronald, D. 2009. Early blight of potatoes and tomatoes. Texas AgriLife Extension System, 009-01.
- Selim, M. E. 2015. Effectiveness of *Trichoderma* biotic applications in regulating the related defense genes affecting tomato early blight disease. *Essential oil. Journal of Plant Pathology and Microbiology*, 6: 311.
- Shahzaman, S., Inam-UI-Haq, M., Bibi, S., Sufyan, M., Altaf, A., Mehmood, U. and Ahmed, R. 2016. Bio-efficacy of *Pseudomonas fluorescens* isolated from chickpea fields as plant growth promoting rhizobacteria. *International Journal of Biosciences*, 9(4): 138-146.
- Sigei, K. G., Ngeno, K. H., Kibe, M. A., Mwangi, M. M. and Mutai, C. M. 2014. Challenges and strategies to improve tomato competitiveness along the tomato value chain in Kenya. *International Journal of Business and Management*, 9(9): 230-245.

- Sikora, R. A., Schäfer, K. and Dababat, A. A. 2007. Modes of action associated with microbially induced in planta suppression of plant-parasitic nematodes. *Australasian Plant Pathology*, 36: 124-134.
- Singh, D. and Singh, A. 2002. Evaluation of fungicides against early blight of potato in spring crop. *Research on crops*, 3(1): 204-206.
- Singh, D., Yadav, D. K., Sinha, S. and Choudhary, G. 2014b. Effect of temperature, cultivars, injury of root and inoculum load of *Ralstonia solanacearum* to cause bacterial wilt of tomato. *Phytopathology and Plant Protection*, 47(13): 1574-1583.
- Singh, P. C., Kumar, R., Singh, M., Rai, A. and Singh, M. C. 2011. Identification of resistant sources against early blight disease of tomato. *Indian Journal of Horticulture*, 68: 516-521.
- Singh, S., Singh, D. R., Kumar, K. and Birah, A. 2014a. Eco-friendly management modules for bacterial wilt (*Ralstonia solanacearum*) of tomato for protected cultivation in a tropical island ecosystem. *Biological Agriculture and Horticulture*, 30(4): 219-227.
- Singh, V., Yadav, S. M. and Chand, R. 2015. Mass sporulation of *Alternaria solani* causing early blight of tomato. *Indian Phytopathology*, 68(1): 83-86.
- Slepecky, R. A. Hemphill, H. E. 2006. The genus *Bacillus*-nonmedical. *Prokaryotes*, 4: 530-562.
- Soesanto, L., Mugiastuti, E. and Rahayuniati, R. F. 2011. Morphological and physiological features of *Pseudomonas fluorescens* P60.
- Spring, S., Scheuner, C., Göker, M. and Klenk, H. P. 2015. A taxonomic framework for emerging groups of ecologically important marine gammaproteobacteria based on the reconstruction of evolutionary relationships using genome-scale data. *Frontiers in Microbiology*, 6(281): 1-17.

- Srivastava, R. S. 2008. Antifungal Activity of *Pseudomonas fluorescens* against different plant pathogenic fungi. Internet Journal of Microbiology, 7: 8-10.
- Suleiman, A. S., Simon, S. and Babychan, M. 2017. Effects of bioagents and their consortia in the management of early blight disease on potato. International Journal of Agriculture Innovations and Research, 5(3): 495-498.
- Sultan, M. 2012. Biological control of leaf pathogens of tomato plants by *Bacillus subtilis* (strain FZB24): antagonistic effects and induced plant resistance. PhD Thesis, Rheinischen Friedrich-Welhems University, PP 155.
- Sundaramoorthy, S. and Balabaskar, P. 2013. Biocontrol efficacy of *Trichoderma* spp. against wilt of tomato caused by *Fusarium oxysporium* f. sp. *lycopersici*. Journal of Applied Biology and Biotechnology, 1(3): 36-40.
- Sutano, M. and Chakrabartty, P. K. 2014. Bio-control of bacterial wilt of tomato caused by *Ralstonia solanacearum* by isolates of plant growth promoting rhizobacteria. Australian Journal of Crop Science, 8(2): 208-214.
- Tahat, M. M. and Sijam, K. 2010. *Ralstonia solanacearum*: the bacterial Wilt causal Agent. Asian Journal of Plant Sciences, 9: 385-393.
- Tamez-Guerra, P., Zuñiga-Sanchez, J. O., Orozco-Flores, A. A., Valadez-Lira, J. A., Rodriguez-Padilla, C., Cañizares-Villanueva, R. O. and Gomez-Flores, R. 2017. Prevalence of Proteobacteria and Armatimonadetes phyla in a protobioreactor under Carbon- and Nitrogen-free production process. Fermentation Technology, 6(2): 1-8.
- Tapwal, A., Tyagi, A., Thakur, G. and Chandra, S. 2015. *In vitro* evaluation of *Trichoderma* species against seed borne pathogens. International Journal of Chemical and Biochemical Sciences, 1 (10): 14-19.

- Thomma, B. 2003. *Alternaria* spp.: From general saprophyte to specific parasite. *Molecular Plant Pathology*, 4: 225-236.
- Torres, M. J., Brandan, C. P., Petroselli, G., Erra-Balsells, R. and Audisio, M. C. 2016. Antagonistic effects of *Bacillus subtilis* subsp *subtilis* and *B. amyloliquefaciens* against *Macrophomina phaseolina*: SEM study of fungal changes and UV-MALDI-TOF MS analysis of their bioactive compounds. *Microbiological Research*, 182: 31-39.
- Udhav, B. S. 2013. Studies on epidemiology and integrated management of early blight of potato caused by *Alternaria solani* (Ellis and Mart.) Jones and Grout. MSc Thesis, Marathwada Agricultural University, PP 135.
- Vaghabhai, C. C. 2016. Investigation on early blight (*Alternaria* sp.) of tomato (*Solanum lycopersicum* L.) under South Gujarat condition. MSc Thesis, Navsari Agricultural University, PP 99.
- Van der Waals, J. E., Korsten, L. and Aveling, T. A. S. 2001. A review of early blight of potatoes. *African Plant Protection*, 7(2): 91-102.
- Van der Waals, J. E., Korsten, L. and Slippers, B. 2004. Genetic diversity among *Alternaria solani* isolates from potatoes in South Africa. *Plant Disease*, 88: 959-964.
- Verma, P. K., Gandhi, S. K. and Singh, S. 2008. Biological control of *Alternaria solani*, the causal agent of early blight of tomato. *Journal of Biological Control*, 22(1): 67-72.
- Verma, A., Kumar, S., Shina, H. A. and Jaiswal, S. 2018. Evaluate the efficacy of bio-control agents and botanicals against early blight of tomato caused by *Alternaria solani*. *The Pharma Innovation journal*, 7(3): 28-30.
- Vinale, F., Ghisalberti, E. L., Sivasithamparam, K., Marra, R., Ritieni, A., Ferracane, R., Woo, S. and Lorito, M. 2009. Factors affecting the production of *Trichoderma harzianum*

secondary metabolites during the interaction with different plant pathogens. The Society for Applied Microbiology, Letters in Applied Microbiology, 48 (6): 705-711.

Vinale, F., Krishnapillai, S., Emilio, L., Ghisalberti, R. M., Sheridan, L. and Woo, M. L. 2008. *Trichoderma* plant-pathogen interactions. Soil Biology and Biochemistry, 40: 1-10.

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Woo, S. L., Nigro, M., Marra, R., Lombardi, N., Pascale, A., Ruocco, M., Lanzuise, S., Manganiello, G. and Lorito, M. 2014. *Trichoderma* secondary metabolites active on plants and fungal pathogens. The Open Mycology Journal, 8: 127-139.

Viterbo, A., Ramot, O., Chernin, L. and Chet, I. 2002. Significance of lytic enzymes from *Trichoderma* ssp. in the biocontrol of fungal plant pathogens. Antonie von Leewenhoek, 81: 549-556.

Wachira, M. J., Mshenga, M. P. and Saidi, M. 2014. Comparison of profitability of small scale greenhouse and open-field tomato production systems in Nakuru North District, Kenya. Asian Journal of Agricultural Sciences, 6(2): 54-61.

Waghunde, R., Shelake, R. M. and Sabalpana, A. N. 2016. *Trichoderma*: A significant fungus for agriculture and environment. African Journal of Agricultural Research, 11(22): 1952-1965.

Waiganjo, M. M., Wabule, N. M., Nyongesa, D., Kibaki, J. M., Onyango, I., Wepukhulu, S. B. and Muthaka, N. M. 2006. Tomato production in Kirinyaga district Kenya, a baseline survey report. KALRO/IPM CRSP, Nairobi, Kenya 1-43.

Wandati, Z. 2014. "Scientists battle deadly tomato pests", Daily Nation.

- Wang, S. L. and Yeh, P. Y. 2008. Purification and characterization of a chitosanase from a natto-kinase producing strain *Bacillus subtilis* TKU007. *Process Biochemistry*, 43: 132-138.
- Wang, X. Q., Zhao, D. L., Shen, L. L., Jing, C. L. and Zang, C. S. 2018. Application and mechanisms of *Bacillus subtilis* in biological control of plant disease. Meena, V. S. (Eds.), *Role of Rhizospheric Microbes in Soil*, 225-250
- Watanabe, T. 2010. *Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species*. 3rd ed, ISBN 978-1-4398-0419-3.
- Wener, J. N. 2000. Guide to tomato production in home gardens. Online, Available from: www.agrisupportonline.com (Accessed on 23rd October 2009).
- Wilkerson, T. M., Weaver, L., Hovius, C. and Zandstra, J. W. 2007. Nutritional and health benefits of fresh vegetables-Past, Present and Future: A Literature Review, PP 51.
- Woudenberg, J. H. C. 2015. Restyling *Alternaria*. PhD Thesis, Wageningen University, Wageningen, NL, PP 251.
- Xie, C., Shao, Y., Li, X. and He, Y. 2015. Detection of early blight and late blight diseases on tomato leaves using hyperspectral imaging. *Scientific Reports*, 5: 16564.
- Yadav, O. P. and Dabbas, M. R. 2012. Efficacy of fungicides in the management of early blight of tomato (*Alternaria solani*). *International Journal of Plant Protection*, 5 (2): 413-416.
- Yadav, R. S. 2014. Biocontrol potential of *Trichoderma harzianum* and *Pseudomonas fluorescens* with organic amendments against tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*. *International journal of Microbial Resource Technology*, 2(2): 1-5.

- Yeole, G. J., Teli, N. P., Kotkar, H. M. and Mendki, P. S. 2014. *Cinnamomum zeylanicum* extracts and their formulations in control of early blight of tomato. *Journal of Biopesticides*, 7(2): 110-123.
- Zegeye, E. D., Santhanam, A., Gorfu, D., Tessera, M. and Kassa, B. 2011. Bio-control activity of *Trichoderma viride* and *Pseudomonas fluorescens* against *Phytophthora infestans* under greenhouse conditions. *Journal of Agricultural Technology*, 7(6): 1589-1602.
- Zhao, Y., Tu, K., Shao, X. F., Jing, W., Yang, W. and Su, Z. P. 2008. Biological control of post-harvest pathogens *Alternaria solani*, *Rhizopus stolonifer* and *Botrytis cinerea* on tomato fruit by *Pichia guilliermondii*. *Journal of Horticultural Science and Biotechnology*, 83(1): 132-136.
- Zheng, M., Shi, J. Y., Shi, J., Wang, Q. G. and Li, Y. H. 2013. Antimicrobial effects of volatiles produced by two antagonistic *Bacillus* strains on the anthracnose pathogen in postharvest mangoes. *Biological Control*, 65: 200-206.

APPENDICES

Appendix 1: Analysis of variance for the percent disease incidence at Kabete Field Station

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	F
Replication	2	134.3	67.2	0.7	
Treatment	6	18,026.8	3,004.5	30.6	<.001
Residual	12	1,176.9	98.07	1.9	
Time	7	28,650.9	4,093.0	80.0	<.001
Time*Treatment	42	6,352.4	151.3	3.0	<.001
Residual	98	5,011.4	51.1		
Total	167	59,352.7			

Appendix 2: Analysis of variance for the percent disease incidence at KALRO Mwea

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	F
Replication	2	44.3	22.133	0.7	
Treatment	6	6,121.3	1,020.209	32.9	<.001
Residual	12	372.4	31.03	3.6	
Time	7	4,315.0	616.431	72.0	<.001
Time*Treatment	42	1,555.0	37.023	4.3	<.001
Residual	98	838.7	8.558		
Total	167	13,246.6			

Appendix 3: Analysis of variance for the percent disease incidence in the greenhouse

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	F
Replication	3	79.8	26.6	0.41	
Treatment	6	1,452.6	242.1	3.75	0.013
Residual	18	1,162.6	64.6	4.31	
Time	7	1,111.0	158.7	10.59	<.001
Time*Treatment	42	677.1	16.1	1.08	0.397
Residual	147	2,203.7	15.0		
Total	223	6,686.6			

Appendix 4: Analysis of variance for the percent disease severity at Kabete Field Station

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	F
Replication	2	39.8	19.9	0.4	
Treatment	6	19,451.7	3,242.0	65.1	<.001
Residual	12	597.8	49.8	3.8	
Time	7	32,182.3	4,597.5	348.0	<.001
Time*Treatment	42	4,267.4	101.6	7.7	<.001
Residual	98	1,294.8	13.2		
Total	167	57,833.8			

Appendix 5: Analysis of variance for the percent disease severity at KALRO Mwea

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	F
Replication	2	17.8	8.9	0.5	
Treatment	6	13,311.4	2,218.6	113.1	<.001
Residual	12	235.4	19.6	2.3	
Time	7	13,431.6	1,918.8	227.1	<.001
Time*Treatment	42	3,559.5	84.8	10.0	<.001
Residual	98	828	8.5		
Total	167	31,383.7			

Appendix 6: Analysis of variance for the percent disease severity in the greenhouse

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	F
Replication	3	280.8	93.6	1.3	
Treatment	6	19,251.7	3,208.6	44.9	<.001
Residual	18	1,287.7	71.5	3.9	
Time	7	39,270.8	5,610.1	301.9	<.001
Time*Treatment	42	7,071.7	168.4	9.1	<.001
Residual	147	2,731.5	18.6		
Total	223	69,894.21			

Appendix 7: Analysis of variance for the percent disease index at Kabete Field Station

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	F
Replication	2	134.3	67.2	0.7	
Treatment	6	18,026.8	3,004.5	30.6	<.001
Residual	12	1,176.9	98.1	1.9	
Time	7	28,650.9	4,093.0	80.0	<.001
Time*Treatment	42	6,352.4	151.3	3.0	<.001
Residual	98	5,011.4	51.1		
Total	167	59,352.7			

Appendix 8: Analysis of variance for the percent disease index at KALRO Mwea

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	F
Replication	2	17.0	8.5	1.2	
Treatment	6	12,477.4	2,079.6	287.9	<.001
Residual	12	86.7	7.2	1.9	
Time	7	13,134.2	1,876.3	497.4	<.001
Time*Treatment	42	3,085.9	73.5	19.5	<.001
Residual	98	369.7	3.8		
Total	167	29,170.8			

Appendix 9: Analysis of variance for the percent disease index in the greenhouse

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	F
Replication	3	223.3	74.4	1.6	
Treatment	6	14,227.2	2,371.2	49.8	<.001
Residual	18	857.4	47.6	3.2	
Time	7	28,331.8	4,047.4	275.3	<.001
Time*Treatment	42	5,241.3	124.8	8.5	<.001
Residual	147	2,161.0	14.7		
Total	223	51,042.1			

Appendix 10: Analysis of variance for site interaction for area under disease progress curve

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	F
Treatment	6	21,282,487	3,547,081	184.5	<.001
Replication	2	14,046	7,023	0.4	0.697
Environment	1	7,328,379	7,328,379	381.2	<.001
Treatment*Environment	6	324,911	54,152	2.8	0.03
Residual	26	499,825	19,224		
Total	41	29,449,649			

Appendix 11: Analysis of variance for site interaction for the percent disease incidence

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	F
Treatment	6	6,373.76	1,062.3	30.2	<.001
Replication	2	24.25	12.1	0.34	0.712
Environment	1	1,589.9	1,589.9	45.2	<.001
Treatment*Environment	6	1,002.9	167.1	4.75	0.002
Residual	26	914.57	35.2		
Total	41	9,905.3			

Appendix 12: Analysis of variance for site interaction for the quantity of marketable fruits

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	F
Treatment	6	195.7	32.6	73.3	<.001
Replication	2	1.5	0.7	1.6	0.215
Environment	1	21.0	21.0	47.3	<.001
Treatment*Environment	6	9.6	1.6	3.6	0.01
Residual	26	11.6	0.4		
Total	41	239.4			