EFFECTIVENESS OF *TRICHODERMA* SPP., *BACILLUS* SPP. AND *PSEUDOMONAS FLUORESCENS* IN THE MANAGEMENT OF EARLY BLIGHT OF TOMATOES

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DECLARATION

This thesis is my original work and has never been submitted for a degree in any other University.

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DEDICATION

To my parents Melida Byamungu and Sébastien Mufungizi and siblings Pontien Cirimwami, Benjamin Centwali and Rachel Murhimalika.

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LIST OF ABBREVIATIONS

µl	Microliter
µm	Micrometer
AEZ II	Agro-ecological zone II
AEZ III	Agro-ecological zone III
ANOVA	Analysis of Variance
AUDPC	Area Under Disease Progress Curve
BS	*Bacillus subtilis*
Cfu	Colony-forming unit
Cm	Centimeter
CRD	Completely Randomized Design
DRC	Democratic Republic of the Congo
FAOSTAT	Food and Agricultural Organization Statistics
Ha	Hectare
HCDA	Horticultural Crop Development Authority
IBM	International Business Machines
IPM	Integrated Pest Management
KALRO	Kenya Agricultural 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
Ltd	Limited
M	Meter
Ml	Milliliter
Mm	Millimeter
No	Number
PCPB	Pest Control Products Board
PDA	Potato Dextrose Agar
PDI	Percent disease index
PDS	Percent disease severity
PGI	Percent growth inhibition
RCBD	Randomized Complete Block Design
Rpm	Revolutions per minute
SPS	Specialized Products and Services
SPSS	Statistical Package for the Social Sciences
USA	United States of America
W/w	Weight for weight
WP	Wettable powder

GENERAL ABSTRACT

Tomato, a major vegetable widely used in Kenya faces a number of production challenges some of them being diseases like late blight, early blight and bacterial wilt. Chemical compounds which have environmental and health concerns are mostly used to control early blight in tomato production. In this study, two *Trichoderma* isolates, two *Bacillus* isolates and commercial *Pseudomonas fluorescens* were used in the management of early blight, a major disease of tomato. These were tested for their effectiveness in managing *Alternaria solani in vitro*. The dual culture technique was used and consisted in growing the antagonists together with the pathogen. Diameter of *A. solani* colony was measured and used to calculate the percent growth inhibition. *Trichoderma* isolates were the most effective against the radial growth of *A. solani* with percent growth inhibition of 80.9 and 82.2%. These were followed by *Bacillus* isolates with percent growth inhibition of 56.6 and 54.1%. *Pseudomonas fluorescens* also suppressed *A. solani* radial growth but with a lower percent growth inhibition of 47.6%. *Trichoderma* isolates, *Bacillus* isolates and commercial *Pseudomonas fluorescens* were also evaluated for their effectiveness in managing tomato early blight under greenhouse and field conditions. Water and Tower 72 WP® (Metalaxyl 8% and Mancozeb 64%) were used as control and standard check respectively. Data were collected on disease parameters and yield of marketable fruits. In the greenhouse, 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 from 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. At both experimental sites, on the 90th day 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 antagonists was from 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. *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas fluorescens* are effective in managing early blight under *in vitro*, greenhouse and field conditions and they are able to reduce the effects of early blight on tomato production. They should be used for a sustainable production of tomatoes.

Key words: *Trichoderma* spp., *Bacillus* spp., *Pseudomonas fluorescens*, management and early blight of tomatoes.

CHAPTER ONE: GENERAL INTRODUCTION

1.1. Background information

Tomato (*Solanum lycopersicum* L.) is a major vegetable grown worldwide (Monte *et al*., 2013). It 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 have significant amounts of potassium, ion and phosphorus (Masinde *at 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, pests and diseases (KALRO, 2005; Mizubuti *et al*., 2007; Goufo *et al*., 2008). As an example, temperature and humidity fluctuations during 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 including cotton bollworms, whiteflies, melon thrips and tomato leaf miners among others, significantly contribute to tomato yield losses (Engindeniz and Ozturk, 2013; Islam *et al*., 2013). 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 production (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 through application of a limited number of chemical compounds due to withdrawal of some effective fungicides reported to have detrimental effects on the environment and on human health (Singh *et al*., 2011).

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 malnutrition 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.

Tomato cultivars which are resistant to early blight, are of low agronomic or commercial quality. Synthetic chemicals are thereby intensively applied by most farmers to lower the intensity of early blight and the accompanying crop losses (Yadav and Dabbas, 2012). In addition, the rotation strategy is limited by the prolonged survival of *A. solani* in the soil and scarcity of land for cultivation (Foolad *et al*., 2008; Karuku *et al*., 2017). Given the high demand for tomato in Kenya and pathogen resistance, farmers increase the rate of chemical application. The required pre-harvest intervals are often not observed. This leads to increased

chemical residues in the produce and increased production costs (Waiganjo *et al*., 2006; 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. This is often due to misuse of synthetic chemicals (Sutanu and Chakrabartty, 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 and humans. These entail reduced 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 but also to interceptions of produce with excessive chemical residues at the export market (Rhoda *et al*., 2006; Wandati, 2014).

1.3. Justification of the study

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 microbial antagonists (Mamgain *et al*., 2013). These are deemed to be biodegradable, friendly to the environment and have minimal effects on humans and non-targeted organisms including 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 antagonists along with locally available formulations of microorganisms are known to be effective in managing early blight disease (Zhao *et al*., 2008). These include species of *Trichoderma*, *Bacillus*, *Pseudomonas* and *Streptomyces* genera among others. These microorganisms differ in their efficacy in managing tomato early blight (Tapwal *et al*., 2015). Integration of microbial antagonists 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 microbial antagonists in the management of early blight in the field. *Trichoderma*, *Bacillus* and *Pseudomonas* antagonists grow rapidly, have long shelf-life at room temperature and can be mass produced at lower costs. They are also deemed to be compatible with several fungicides (Pertot *et al*., 2015). In contrast, several other microbials including *Streptomyces* species have been reported to be less efficient and not compatible with many crop plants (Pertot *et al*., 2015). Integration of effective *Trichoderma* isolates, *Bacillus* isolates and *Pseudomonas fluorescens* in early blight management will contribute to a sustainable production of tomatoes through reduction of the dependence on 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

(Gupta *et al.*, 2014). This will result in reduced interceptions of tomato produce at the export market. Consumers will have access to tomato fruits that are free from chemical residues. Integration of selected antagonists in the management of tomato early blight will also contribute to a better conservation of the environment given that selected antagonists do not pollute the environment as they are biodegradable. *Trichoderma* spp. and *Bacillus* spp. used 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 *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas fluorescens* in managing early blight for sustainable production of tomatoes.

1.4.2. Specific objectives

1. To evaluate the antagonistic effects of *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas fluorescens* on growth of *Alternaria solani* under *in vitro* conditions.
2. To evaluate the effectiveness of *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas fluorescens* in managing early blight and in increasing tomato yield.

1.5. Hypotheses

1. *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas fluorescens* have significant antagonistic effects on *in vitro* growth of *A. solani*.
2. *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas fluorescens* are effective in managing early blight and in increasing tomato yield.

CHAPTER TWO: LITERATURE REVIEW

2.1. Economic importance of tomatoes

Tomato fruits have been reported to contain adequate amounts of vitamins A, B and C. Additionally, tomato fruits contain 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). Tomatoes are grown and produced for consumption 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. 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. Africa total production was estimated at 21.5 million tons in 2017 (FAOSTAT, 2020). Egypt is the biggest tomato producing country in African with a total production of approximately 6.6 million tons in 2018. Kenya is the biggest tomato producing country in the East African Community with a total production estimated at 0.6 million tons in 2018. Major tomato producing countries in the Eastern African Community are mentioned in Table 2.1. Cameroon is the biggest tomato producing country in Central Africa with a total production estimated at 1.1 million tons in 2018 (FAOSTAT, 2020). Major tomato producing countries in Central Africa are illustrated in Table 2.2.

Table 2.1: Tomato production from the major tomato producing countries in the East African Community

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)
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
2018	28,263	599,458	10,212	93,062	7,200	39,462	25,985	356,094
Total	210,368	4,539,791	83,579	1,163,512	59,828	350,084	327,507	4,106,387
Mean	21,036.8	453,979.1	8,357.9	116,351.2	5,982.8	35,008.4	32,750.7	410,638.7

Source: FAOSTAT, 2020

Table 2.2: Tomato production from the major tomato producing countries in Central Africa

Year	Cameroon		DRC		Angola		Congo	
	Harvested Area (ha)	Production (tons)	Harvested Area (ha)	Production (tons)	Harvested Area (ha)	Production (tons)	Harvested Area (ha)	Production (tons)
2009	115,677	666,607	6,600	49,000	4,617	15,036	685	3,291
2010	139,976	795,327	6,740	50,089	4,969	15,500	732	3,516
2011	69,182	853,060	6,437	48,031	5,243	15,634	755	3,641
2012	79,408	889,794	6,490	48,330	6,150	16,500	777	3,743
2013	69,903	875,700	6,515	48,536	6,200	17,000	800	3,844
2014	76,304	949,587	6,534	48,727	6,091	16,384	807	3,851
2015	82,370	1,020,601	6,598	49,144	6,444	16,270	813	3,881
2016	92,626	1,182,114	6,638	49,305	6,886	16,519	822	3,938
2017	98,910	1,127,158	6,666	49,514	7,307	16,681	834	3,994
2018	93,762	1,068,495	6,694	49,723	7,773	16,842	847	4,051
Total	918,118	9,428,443	65,912	490,399	61,680	162,366	7,872	37,750
Mean	91,811.8	942,844.3	6,591.2	49,039.9	6,168	16,236.6	787.2	3,775

Source: FAOSTAT, 2020

Kenya is one of the major tomato producing countries in Africa (FAOSTAT, 2020). Tomato is a highly consumed vegetable in Kenya (Wachira *et al*., 2014) where it is being produced for both local and international markets (Koening *et al*., 2008). The production of tomatoes in the country offers employment opportunities and increases income for many households (Koening *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 processing 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).

2.2. 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 losses (KHCP, 2011).

2.2.1. Physiological disorders

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). Excessive nitrogen makes tomato plants to grow bigger and stronger with larger branches than normal but interferes with fruit production (Newcomb, 2020). In addition, excessive nitrogen is considered as an important factor in the development of blossom end rot (Simpson, 2020).

2.2.2. Pests and weeds

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. They thus interfere with tomato production by affecting colour, flavour and consistency of the produce.

2.2.3. Diseases

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, Fusarium wilt, bacterial wilt, root-knot nematodes, yellow leaf curl virus and tomato spotted virus (KALRO, 2005; Kariuki *et al*., 2010; Singh *et al*., 2014b). Most diseases reduce tomato yield quality and quantity resulting in loss of income (Mizubuti *et al*., 2007; Goufo *et al*., 2008).

Late blight is incited by the fungus *Phytophthora infestans* and is marked by black or brown lesions that form on leaves and stems. The lesions are small and water soaked at the start of the disease but become large and necrotic with its development (Schumann and Arcy, 2000). The fungus causes infected stems and petioles to collapse at the infection area. Tomato fruits affected by the disease do not ripen but turn greasy and fall-off (Alexandrov, 2011). Late blight leads to plant vigour loss, extensive plant defoliation resulting in massive fruit loss. The pathogen can survive in tomato plant debris or in volunteer weeds of the nightshade family and be spread from one season to another. The propagation of late blight is facilitated by wind and water splashes

(Goufo *et al*., 2008). Late blight is more severe in cool humid conditions when temperatures are between 15 and 20^{0}C (Nelson, 2008). Major late blight management strategies include use of resistant varieties, application of fungicides, crop rotation and sanitation among others (Mizubuti *et al*., 2007).

Fusarium wilt is a fungal infection incited by *Fusarium oxysporum* f. sp. *lycopersici* which affects the vascular system of tomato plants. The fungus is soilborne and gains entry into tomato plants through roots (Akrami and Yousefi, 2015). The pathogen famishes plant branches and causes yellowing followed by wilting and fall-off of lower leaves. This leads to tomato plant death and massive yield losses. Dissemination of the disease can be facilitated through wind and water (Anitha and Rabeeth, 2009). Development of the disease is favoured by warm temperatures, acidic soils, drought and presence of root knot nematodes (Nelson, 2008; Anitha and Rabeeth, 2009). Crop rotation and use of resistant varieties are the most effective management strategies (Bonanomi *et al*., 2007).

Root-knot nematodes are soilborne pathogens belonging to the genus *Meloidogyne*. They feed on roots of tomato and many other crop plants and lead to formation of galls or knots (Cerkauskas, 2004). Root-knot nematodes cause stunting, yellowing and an unthrifty appearance of tomato plants. Infected tomato plants also become more vulnerable to soilborne diseases including bacterial wilt and Fusarium wilt among others. Development of the disease is favoured by drought and hot environment (Seebold, 2014). In Kenya root-knot nematodes are common in tomato fields and hence are a major threat to tomato production causing massive yield losses (Kariuki *et al*., 2010). Root-knot nematodes can be managed through cultural practices, application of nematicides and use of resistant varieties. However, use of resistant varieties is

limited by the existence of many infective strains of root-knot nematodes and application of nematicides has environmental and health concerns (Alfianny *et al*., 2017).

Bacterial wilt is a soilborne and waterborne disease of tomato plants that is incited by the bacterium *Ralstonia solanacearum*. The bacterium mostly makes its way into the stem through wounds on the roots (Champoiseau and Momol, 2008). *Ralstonia solanacearum* can survive for several years in weeds. Presence of the bacterium in the stem prevents leaves from getting water and nutrients resulting in wilting of leaves and in gradual plant death. Bacterial wilt can lead to massive tomato yield losses (Champoiseau and Momol, 2008; Vanitha *et al*., 2009). Establishment of bacterial wilt is favoured by temperature exceeding 30^0C, high humidity and wet soils (Champoiseau and Momol, 2008). Use of resistant varieties has been reported to be the most effective management strategy for bacterial wilt. However, this strategy is limited by the existence of several infective strains of the pathogen. Management of the disease can also be achieved through approaches including grafting, sanitation and use of microbial based pesticides among others (Champoiseau and Momol, 2008; Vanitha *et al*., 2009).

Tomato yellow leaf curl virus is a highly damaging disease of tomato. It is incited by several species belonging to the viral genus *Begomovirus* (Verlaan *at al*., 2013). This virus has been associated with tomato yield losses reaching 100% (Abhary *et al*., 2007). Tomato yellow leaf curl virus can be disseminated from an infected plant to a healthy one by the silver-leaf whitefly. Major effects of the virus on tomato plants include chlorosis, twisting, cupping of leaves, stunting and fall-off of flowers and fruits (Abhary *et al*., 2007). Management of the disease can be achieved through cultural practices and through introgression of resistant genes from other tomato species (Abhary *et al*., 2007; Verlaan *at al*., 2013). In Kenya, tomato yellow leaf curl

virus is mainly managed through elimination of the vector through application of insecticides resulting to environmental and health concerns (Otipa *et al*., 2014).

2.3. Limitations in the management of tomato diseases in Kenya

Crop rotation is one of the most common cultural practices for managing tomatoes diseases including early and late blights, Fusarium wilt, bacterial wilt and root-knot nematodes among others. However, the effectiveness of crop rotation as a strategy to manage tomato diseases is limited by the fact that in most cases, farmers have small lands for cultivation (Sally *et al*., 2006). Management of tomato diseases is achieved in some cases by the use of resistant varieties (Alabouvette *et al*., 2006). However, introgression of resistant genes from wild tomato relatives is very challenging and thus requires expertise and high investment (Abhary *et al*., 2007; Verlaan *et al*., 2013). In addition, disease tolerant varieties do not often perform well in terms of yield (Foolad *at al*., 2002). Most efficient strategy in managing tomato diseases is application of chemical compounds to hinder establishment of pathogens or to eliminate their vectors (Foolad *et al*., 2008; Van den Berg *et al*., 2012). However, long term application of chemicals results in development of pathogen biotypes that are pesticide resistant (Rojo *et al*., 2007). Consequently, farmers increase the rates of chemical application which results in excessive chemical residues on the produce and in environmental pollution (Fabro and Varca, 2011). Use of microbial based pesticides is now regarded as a promising way to reduce frequent application of agrochemicals for managing tomato diseases (Arora *et al*., 2012; Mishra *et al*., 2014). However, only a few microbial based pesticides are available in the local market (PCPB, 2018). In addition, most of the available biopesticides are imported (Infonet-Biovision, 2020). The incorporation of biopesticides in managing tomato diseases may also be limited by the fact that the effectiveness of a microbial antagonist can vary with pathogen strains and environmental conditions among

other factors (Bailey and Gilligan, 2004; Mishra *et al*., 2014). Integration of biopesticides in managing tomato diseases requires more studies on the effectiveness of microbial antagonists against most tomato pathogens.

2.4. Description of early blight of tomatoes

2.4.1. Economic importance of early blight of tomatoes

Early blight is one of the most damaging diseases of tomato in Kenya (Mwangi *et al*., 2015) and wordwide (Abada *et al*., 2008). Fruit yield losses ranging between 50 and 86% have been recorded in early blight susceptible tomato cultivars (Mathur and Shekhawat, 1986). Losses in tomato yield reaching 78% have been reported for disease severity of 72%. An increase of 1% in disease severity reduces tomato yield by up to 1.4% (Yadav and Dabbas, 2012). Once early blight has been introduced into an area, it is very difficult to manage and fruit infection of 65% have been recorded in tomato plants as a result of the disease. This leads to increased production costs and loss of income (Fontem, 2003).

2.4.2. Description of tomato early blight causative agent

Alternaria solani hyphae are septate, branched, light brown turning darker as they mature. Rising individually or in tiny groups, *A. solani* conidiophores are septate, straight or flexous, dark coloured and measure 50 to 90µm (Ganie *et al*., 2013). *Alternaria solani* belongs 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).

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

2.4.3. Infection process of early blight in tomatoes

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 8-32^{0}C and leads to the formation of germ tubes (Kemmitt, 2002). Using their 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 stems, thereafter causing the disease (Kemmitt, 2002). It has been reported that *A. solani* invades tissues of tomato plants by producing enzymes that degrade cell walls. The pathogen also produces toxins to kill host cells and make their content available (Gulzar *et al*., 2018). Phytotoxic compounds including alternariol, altersolanol A, altertoxin, macrosporin, solanapyrone A, B, C and alternaric acid among others, have been associated with the infection of tomato plants by *A. solani*

(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 and C, the role in disease development of most of these metabolites is not well documented (Adhikari *et al*., 2017). *Alternaria solani* also secretes extracellularly a serine protease and metalloprotease which may be related to pathogenicity (Chandrasekaran *et al*., 2014; Chandrasekaran *et al*., 2016). Depending on leaf age and cultivar susceptibility, symptoms can appear within a week of infection if environmental conditions are conducive (Kemmitt, 2002).

2.4.4. 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, stem 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. Spores of the pathogen 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* causing Septoria leaf spot 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 coloured, sunken, leathery and purple lesions with

concentric rings (Chaerani and Voorrips, 2006; Junior *et al*., 2011). Symptoms on stems 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).

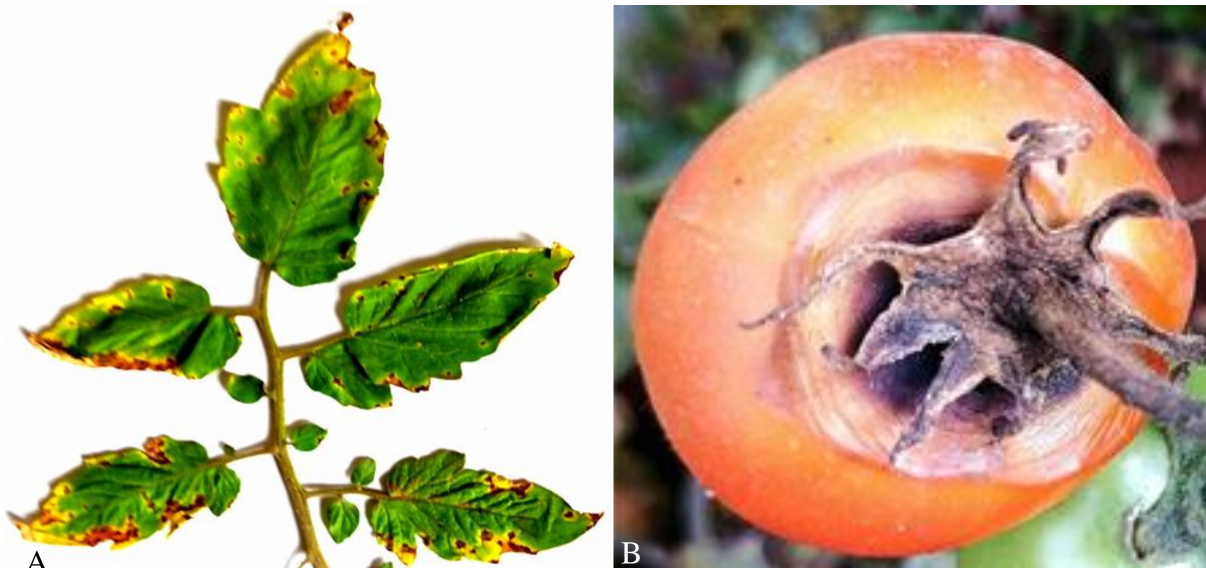

Plate 2.2: Early blight symptoms on tomato leaf and fruit

A: Early blight symptoms on tomato leaf, B: early blight symptoms on tomato fruit.

2.4.5. Source of inoculum

Although *A. solani* mainly infects potato and tomato plants, other solanaceous plants including eggplant and pepper, wild cabbage and cucumber can also be infected by the fungus and serve as source of inoculum (Schultz and Ronald, 2009). Conidial cells of the fungus are protected by a thick cell wall which enables them to withstand adverse environmental conditions (Foolad *et al*., 2008). *Alternaria solani* conidia can survive one to several years in the soil, plant debris, seed and infected seedlings. The fungus can survive in alternate hosts and weeds in its conidial or

mycelial form, which can therefore serve as primary sources of inoculum (Foolad *et al.*, 2008). *Alternaria solani* conidia can readily be disseminated by wind, rain splashes, insects and other animals including man through machinery and cause disease to healthy host plants. Early blight pathogen can complete several cycles within the same cropping season (Kemmitt, 2002).

2.4.6. Factors favoring early blight of tomatoes

The production of *A. solani* spores is induced by day light (Singh *et al.*, 2015). Prolonged periods of wetness are often needed for production of *A. solani* spores. However, 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. Varying levels of minimum, optimum and maximum temperatures for germination of *A. solani* spores have been reported. Singh *et al.* (2015) reported that an optimum temperature of 20°C is required for germination of spores of *A. solani* in a two-hour time period. Rotem (1994) reported that a minimum temperature of 10^{0}C and a maximum temperature of 35^{0}C are required for the germination of *A. solani* spores. *Alternaria solani* thrives in warm temperature (20-25^{0}C) and prolonged periods of leaf wetness resulting from high humidity (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). Early blight is also favoured by cultural practices including overhead irrigation which increases wetness, poor sanitation which maintains the pathogen available, use of infected seeds and non-rotation of crops among others practices (Chaerani and Voorrips, 2006; Neils *et al.*, 2015 ; Abuley *et al.*, 2018).

2.4.7. Management of early blight of tomatoes

Although early blight of tomatoes 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 (Foolad *et al*., 2008). Early blight of tomatoes 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, Saaf, copper oxychloride, Propiconazole, Thiophanate Methyl, Propamocarb, Azoxystrobin, Cymoxanyl, 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). However, continued application of synthetic chemicals has environmental and human health concerns and induces pathogen resistance to chemicals (Rojo *et al*., 2007).

Most common cultural practices involved in managing tomato early blight include sanitation, rotating tomatoes with non-host crops for a period of two to three years, use of pathogen-free seeds and transplants, elimination of infected plant materials from the garden and management of volunteer crops and weeds including 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

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 in early blight management (Chaerani and Voorrips, 2006; Li, 2012). Thermal treatment of infected seeds at 50^0C for 25 minutes prior to sowing is beneficial for preventing seedborne infection (Neils *et al*., 2015). However, cultural practices have not been effective in managing early blight given that the pathogen is soil borne and has many infective strains (Foolad *et al*., 2008).

Management of early blight can be achieved through use of plant extract based pesticides also known as botanicals as well as use of formulations of antagonistic microbes (Sultan, 2012; Neils *et al*., 2015). Extracts of various plants including *Cinnamomum zeylanicum*, *Ferula foetida*, *Glycyrrhiza glabra*, *Hemidesmus indicus* and *Syzygium aromaticum* among others, have been reported to induce antifungal activity against early blight (Yeole *et al*., 2014). Botanicals are beneficial to sustainable agricultural production given that they are cheaper than synthetic chemicals, biodegradable and reduce crop losses (Gurjar *et al*., 2012). However, botanicals are less effective, have shorter shelf life and are less available in formulations compared to synthetic chemicals (Gurjar *et al*., 2012).

2.4.8. Management of early blight using microbial antagonists

Microbial antagonists are friendly to the environment and have minimal effects on non-target organisms, including humans, animals and host plants. Microbial antagonists have been reported to have varied activities which hinder growth and development of many plant pathogens (Alabouvette *et al*., 2006). The mechanisms of action through which microbial antagonists protect plants from pathogen attack are numerous and differ from one antagonist to another (Alabouvette *et al*., 2006). Microbial antagonists have been associated with one or 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 (Alabouvette *et al*., 2006; Heydari and Pessarakli, 2010). Most reported microbial antagonists used in managing early blight include *Bacillus subtilis*, *Pseudomonas aeruginosa*, *P. putida*, *P. cepacia, P. gladioli, P. fluorescens*, *Trichoderma viride* and *T. harzianum* (Heydari and Pessarakli, 2010). High variability of the capability of antagonistic microorganisms in controlling one or another plant pathogen is the main limitation in incorporating microbial based pesticides in the management of plant diseases (Sundin *et al*., 2009). The effectiveness of microbial antagonists against plant pathogens is influenced by varied factors including temperature, relative humidity, nature of the host plant and nature of the plant pathogen among other factors (Sundin *et al*., 2009).

2.4.8.1. Modes of action for *Bacillus subtilis*

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) and bacitracin among others (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). Volatile compounds secreted by *B. subtilis* are known to induce activities that enhance plant

growth (Compant *et al*., 2005). These include peroxidase, polyphenol oxidase and superoxide dismutase among others (Neils *et al*., 2015).

Abdelmoteleb *et al*. (2017) evaluated *B. subtilis* strain coded 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 coded EPCO16 of *B. subtilis* against *Fusarium oxysporum* f. sp. *lycopersici* and reported production of antifungal metabolites including bacillomycin, fengycin, iturin and bacilysin. In addition, they reported formation of 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 in plant treated with the antagonist compared to control plants.

2.4.8.2. 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 fluorescens* is a saprophytic bacterium mostly found in soil, water (Nepali *et al*., 2018), aquatic environments (Nagel *et al*., 2012) and 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 25-35^{0}C (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, ion chelating siderophores, hydrogen cyanide and lytic enzymes among others. Antibiotic compounds produced by *P. fluorescens* include 2,4-diacetyl phloroglucinol, phenazine, pyoluteorin and biosurfactant antibiotics (Angayarkanni *et al*., 2005). Iron chelating siderophore compounds produced by the antagonist include salicylic acid, pyochelin and pyoveridine (Ramanujam *et al*., 2015) while lytic enzymes include 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 and 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. brassiceae* and *Colletotrichum gloeosporioides in vitro* and on chilli seeds and reported

concurrent production of siderophore, indole-3-acetic acid, hydrogen cyanide, phosphate solubilization among others as mechanisms used by the antagonist to hinder growth of plant pathogens and promote plant growth.

2.4.8.3. Modes of action of *Trichoderma* species

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. Interestingly, these modes of action are often synergistically employed by *Trichoderma* spp. rending *Trichoderma* spp. more efficient against plant pathogens (Vinale *et al.*, 2008; Saba *et al.*, 2012; Vinale *et al.*, 2014). *Trichoderma* species have been reported to produce compounds that promote growth of host plants and improve productivity. These include ethylene or terpenoid and phytoalexins among others (Alabouvette *et al.*, 2006).

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, isocyanide 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 from 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.

2.4.8.4. Description and modes of action of *Streptomyces* species

Streptomyces species are filamentous gram-positive, aerobic bacteria that are found in soil and water. *Streptomyces* species form a mycelium that releases chains of spores when it matures (Anderson and Wellington, 2001). Several *Streptomyces* species are involved in decomposition of soil organic matter and thus improve soil fertility (Golińska and Dahm, 2013). They also produce secondary metabolites that act as plant growth promoters. These include gibberellin, indole-3-acetic acid and siderophores among others (Sadeghi *et al.*, 2012; Goudjal *et al.*, 2015). *Streptomyces* species have been associated with the production of several antibiotics which are used to hinder establishment and growth of other microorganisms including plant pathogens. These include oligomycin A, kanosamine, zwittermicin A and xanthobactin among others (Compant *et al.*, 2005). *Streptomyces* species have also been reported to stimulate host plant defense system against plant pathogens (Fujita *et al.,* 2017). However, incorporation of

Streptomyces species in agricultural production as antagonistic microbes is challenging due to their low sporulation ability, their moderate antagonistic activity and the short shelf life of their formulations (Vurukonda *et al*., 2018).

2.5. Use of microbial based pesticides in plant disease management

Microbial based pesticides are referred to as pesticides whose active ingredients are typically microorganisms. Microbial pesticides are formulations of either the spores or the cells of bacteria, fungi, protozoans, algae among others (Clemson, 2007; Gupta and Dikshit, 2010). The active ingredient in a microbial based pesticide can also be a virus (Clemson, 2007). Naturally occurring or genetically modified microorganisms can serve as source of active ingredients for microbial based pesticides (Clemson, 2007). These are known to be environmentally friendly, inexpensive and effective against an array of plant pathogens (Bailey and Gilligan, 2004). Microbial based pesticides are efficient even when applied slightly and degrade rapidly after usage (Arora *et al*., 2012). However, success of microbial based pesticides is limited by the inconsistency of their efficiency, the shortness of their shelf life and the complexity of their handling (Mishra *et al*., 2014).

Microbial active ingredients are applied on plants as living or dead cells or spores but living microorganisms are mostly used (Mishra *et al*., 2014). The modes of action of microbial based pesticides depend on the pesticidal microorganism and on the target pathogen (Bailey and Gilligan, 2004; Clemson, 2007). Microbial pesticides can impede development of plant pathogens by secreting toxic compounds which are harmful to target pathogens. Several other modes of action including competition for nutrients and space can be used by microbial pesticides (Clemson, 2007). Microbial pesticides encompass biofungicides, biobacteriocides, bioinsecticides and bioherbicides among others (Gupta and Dikshit, 2010). Bacterial based

biopesticides are the most common and account for 74% of the global market while fungal biopesticides, viral biopesticides and other biopesticides account for 10%, 5% and 11% respectively (Thakore, 2006). Species of the genus *Bacillus* are the most commonly used in bacterial based biopesticides (Fravel, 2005). They include *B. subtilis*, *B. amyloliquefaciens*, *B. licheniformis* among others. These have been reported to be active against a wide range of fungal plant pathogens, especially oomycetes (Arrebola *et al.*, 2010). Several biopesticides are based on formulations of species belonging to the group of pseudomonads. Most common species of the pseudomonad group used for formulating biopesticides include *Pseudomonas fluorescens*, *P. aeruginosa* and *P. syringae* among others (Berg, 2009). Pseudomonad species based biofungicides have been reported with activities which improve plant growth and productivity. These biopesticides have been reported to be active against a range of plant pathogens including *Alternaria* spp., *Fusarium* spp., *Macrophomina* spp. and *Rhizoctonia* spp. among others. Other bacterial species formulated as biopesticides include *Agrobacterium radiobacter* and *Streptomyces lydicus* among others (Chunxue *et al.*, 2010).

Fungal based biopesticides can be used to impede development of an array of air- and soil- borne plant pathogens including *Rhizoctonia* spp., *Pythium* spp., *Fusarium* spp. and *Alternaria* spp. among others (Bailey and Gilligan, 2004). However, effectiveness of fungal based biopesticides requires a narrow range of environmental conditions including temperature and soil moisture (Bailey and Gilligan, 2004). Most fungal species used in biopesticide formulations include *Trichoderma harzianum* and *T. viride* among others (Harman, 2005). Efficiency of *Trichoderma* based biopesticides is associated with the capability of *Trichoderma* species to degrade cell walls and use cell contents of target plant pathogens to produce their own spores (Domingues *et al.*, 2000). Several viruses referred to as bacteriophages are known to antagonize bacterial plant

pathogens. These can be used to formulate viral based biopesticides (Gill *et al.*, 2007). Several viral based biopesticides have been availed and are effective against plant pathogens including *Xanthomonas campestris* pv. *vesicatoria* and *P. syringae* pv. *tomato* among others (Schofield *et al.*, 2012). However, production of viral based biopesticides is limited by the complexity of virus culturing and handling techniques (Mishra *et al.*, 2014). Major microbial based pesticides used in Kenya are as shown in Table 2.3.

Table 2.3: Major microbial based pesticides used to manage plant diseases in Kenya

Trade name	Active ingredient	Distributor	Target disease(s)
REAL Trichoderma Granule®	*Trichoderma asperellum* TRC900 ($1.7x10^9$ cfu/gram)	The Real IPM Company (K) Ltd.	Root knot nematodes
TRICHOTECH®	*Trichoderma asperullum* ($8x10^9$ spores/gram)	Flamingo Horticulture (K) Ltd.	Rhizoctonia Root Rot and Fusarium wilt
TRIANUM –P 11.5 WP®	*Trichoderma harzianum* Rifai strain KRL-AG2 (T-22) ($1x10^9$ cfu/gram)	Koppert Biological Systems (K) Ltd.	Rhizoctonia Root Rot and Fusarium wilt
ROOTGARD®	*Trichoderma harzianum* strain 21 ($2x10^9$ spores/gram)	Juanco SPS Ltd.	Root fungal diseases
REAL BACILLUS SUBTILIS Aqueous solution®	*Bacillus subtilis* BS-01 ($1x10^{10}$ cfu/ml)	Real IPM Company (K) Ltd, Thika	Powdery mildew
BIOCURE B 1.75®	*Pseudomonas fluorescens* 1.75% w/w	Osho Chemical Industries Ltd.	Gray mold and Early blight
BIO NEMATON®	*Paecilomyces lilacinus* 1.5% ($1x10^8$ cfu/ml)	Osho Chemical Industries Ltd.	Root knot and Cyst nematodes
MYTECH WP®	*Paecilomyces lilacinus* ($1x10^{10}$ cfu/gram)	Dudutech (Division of Flamingo Horticulture (K) Ltd.	Root knot nematodes

Source: PCPB (2018).

CHAPTER THREE: EFFECTS OF *TRICHODERMA* SPP., *BACILLUS* SPP. AND *PSEUDOMONAS FLUORESCENS* ON RADIAL GROWTH OF *ALTERNARIA SOLANI*

3.1. Abstract

The fungus *Alternaria solani* causes early blight which is a common threat to tomato production worldwide. Chemical compounds which have environmental and human health concerns are frequently used to manage the disease. *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas fluorescens* are friendly to the environment and have few effects on human health. They can be used for managing early blight to minimize the dependence on chemical compounds. The aim of this study was to evaluate inhibition activities of *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas fluorescens* on *A. solani*. The dual culture technique was followed and consisted of plating the pathogen together with the antagonists. Diameter of *A. solani* colony was measured and percent growth inhibition was calculated. Selected antagonists 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 distinct 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 *P. fluorescens* with a percent growth inhibition of 47.6%. *Bacillus* isolates exhibited distinct growth inhibition zones while *P. fluorescens* exhibited faint growth inhibition zones. *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas fluorescens* are effective against *A. solani* under *in vitro* conditions and further evaluation under greenhouse and field conditions needs to be done.

3.2. Introduction

The fungus *Alternaria solani* causes early blight which is a major challenge to tomato production in Kenya and all over the world (Hou and Huang, 2006; Mwangi *et al*., 2015). *Alternaria solani* leads to massive losses when it is not managed both pre and post-harvest. Synthetic chemicals are most commonly used for early blight management as they quickly control of 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 (Nderitu *et al*., 2007; Rojo *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 microbial antagonists have been reported to have inhibitory activity over growth of plant pathogens (Zhao *et al*., 2008). Microbial antagonists 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 microbial antagonists (Alabouvette *et al*., 2006). Understanding interactions between plant pathogens and microbial antagonists is necessary in integrating microbial antagonists in the management of plant diseases (Ngoc, 2013). *In vitro* studies are useful in evaluating inhibitory activities of microbial antagonists on pathogen growth. This study was carried out to evaluate the inhibitory effects of *Trichoderma* spp., *Bacillus* spp. and *P. fluorescens* on growth of *A. solani* under *in vitro* conditions.

3.3. Materials and methods

3.3.1. Isolation and identification of *Alternaria solani*

Leaves of tomato plants exhibiting early blight symptoms were obtained from tomato plants. Using sterile blades, diseased leaves were chopped into small pieces, which were surface sterilized for 60 seconds in 1% sodium hypochlorite. Surface sterilized tissues were rinsed in four changes of sterile distilled water and dried on sterile paper tissues and transferred to Petri plates containing sterilized PDA medium (Narayanasamy, 2011). The plates were incubated at 28^0C for seven days for growth and sporulation of the pathogen (Vaghabhai, 2016). 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). *Alternaria 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 at power 40 magnification.

3.3.2. Isolation and identification of *Trichoderma* spp.

Isolation of *Trichoderma* spp. was done following the serial dilution technique. 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 medium using a sterile glass rod and incubated at 28^0C 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* isolate identification. The morphological keys of *Trichoderma* genus developed by Watanabe (2010) were used for identification. The percent isolation frequency was calculated for each isolate follows:

$\text{PIF} = \frac{\text{NS}}{\text{TS}} * 100$; where PIF=Percent isolation frequency, NS=Number of samples where the isolate was present and TS=Total number of samples.

3.3.3. Isolation and identification of *Bacillus* spp.

Bacillus spp. were isolated from local soils and identified at the Department of Plant Science and Crop Protection, University of Nairobi.

3.3.4. Preparation of *Pseudomonas fluorescens*

Bio-cure® is a water soluble liquid containing $1\text{x}10^9$ bacterial cells/ml of the rhizobacterium *P. fluorescens*. It was acquired from Osho Chemical Industries Ltd and was 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*.

3.3.5. Preparation of *Alternaria solani* inoculum

Ten ml of sterile distilled water were poured on 14 day old single spore PDA cultures of *Alternaria solani*. 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 $3\text{x}10^6$ spores/ml (Hassanein *et al*., 2010).

3.3.6. Pathogenicity test of *Alternaria solani*

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^{0}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.

Pathogenicity test of *A. solani* was carried out under greenhouse conditions following Koch's postulates. Using a hand sprayer, 40 day old seedlings were sprayed with 20 ml of *A. solani* conidial suspension at $3x10^{6}$ spores/ml prepared as described in section 3.3.5 (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).

3.3.7. *In vitro* activity of the antagonists against *Alternaria solani*

Microbial antagonists were evaluated for their inhibitory activities against *A. solani* under *in vitro* conditions following the dual culture technique. Using a sterile cork borer, five ml diameter discs 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). Due to its slow growth, *A. solani* discs were plated three days before bacterial antagonists. Colonies of five mm diameter from respective bacterial antagonists 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). 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 28^0C. Plates were arranged in a Completely Randomized Design (CRD). Diameter of the pathogen colony was measured (in mm) on a daily basis commencing on the third day after plating the antagonists until the thirteenth day when no increase in pathogen colony 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 the various antagonists was compared to diameter of *A. solani* in the control. Percent *A. solani* radial growth inhibition was calculated as demonstrated by Whipps (1997):

PGI $= \frac{C-T}{C} * 100$; 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).

Activity of the antagonists over the radial growth of *A. solani* was categorized using a rating scale modified from Bell *et al*. (1982) as follows: ‘‘very low’’ when the percent growth inhibition ranged between 0-25%, ‘‘low’’ when it ranged between 26-50%, ‘‘moderate’’ when it ranged between 51-75% and ‘‘high’’ when it ranged between 76-100%.

3.3.8. Screening of *Trichoderma* isolates and *Bacillus* isolates

Since the number of microbial antagonists was high (19 *Bacillus* and 10 *Trichoderma* isolates), screening 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 cock borer. Colonies from each isolate were plated in four different Petri plates. 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. The radius of pathogen colony was measured (in mm) from the center of Petri plates to the antagonist colony. Measurements were done on a daily basis for seven days. The antagonists which recorded the lowest mean pathogen radius were considered as most promising and were used for further evaluation.

3.3.9. Data analysis

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

3.4. Results

3.4.1. Morphological features of isolated *Alternaria solani* and early blight symptoms on tomato plants inoculated with *Alternaria solani* conidia

The hyphae of isolated *A. solani* 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).

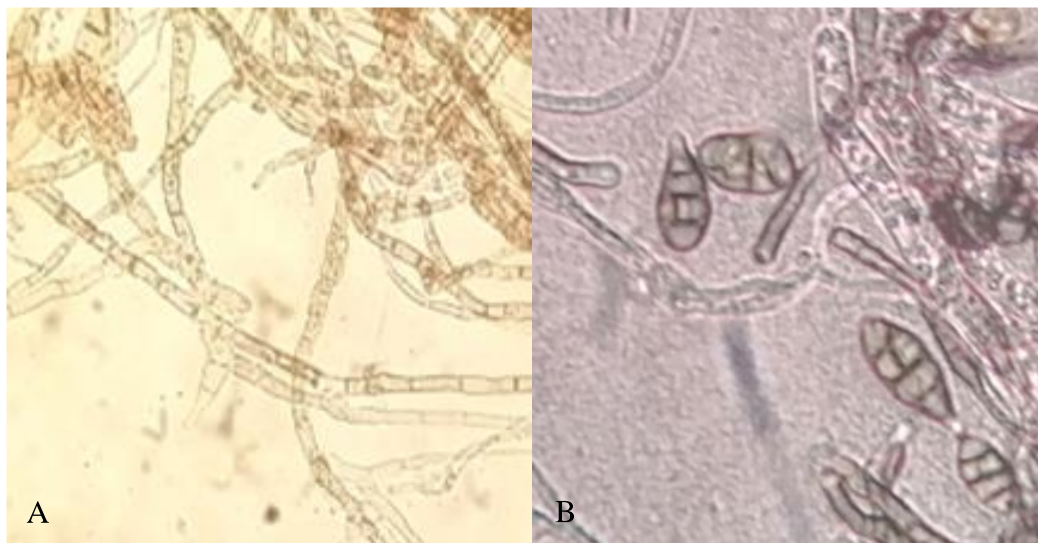

Plate 3.1: Morphological characteristics of isolated *Alternaria solani*

A: *Alternaria solani* hyphae (x40), B: *Alternaria solani* conidia (x40).

Tomato plants inoculated with *A. solani* conidia were observed with early blight symptoms. 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). Single spore colonies of re-isolated pathogen exhibited morphological features which were comparable to those of the original pathogen. Observed conidial, hyphal features were comparable to those of the original pathogen.

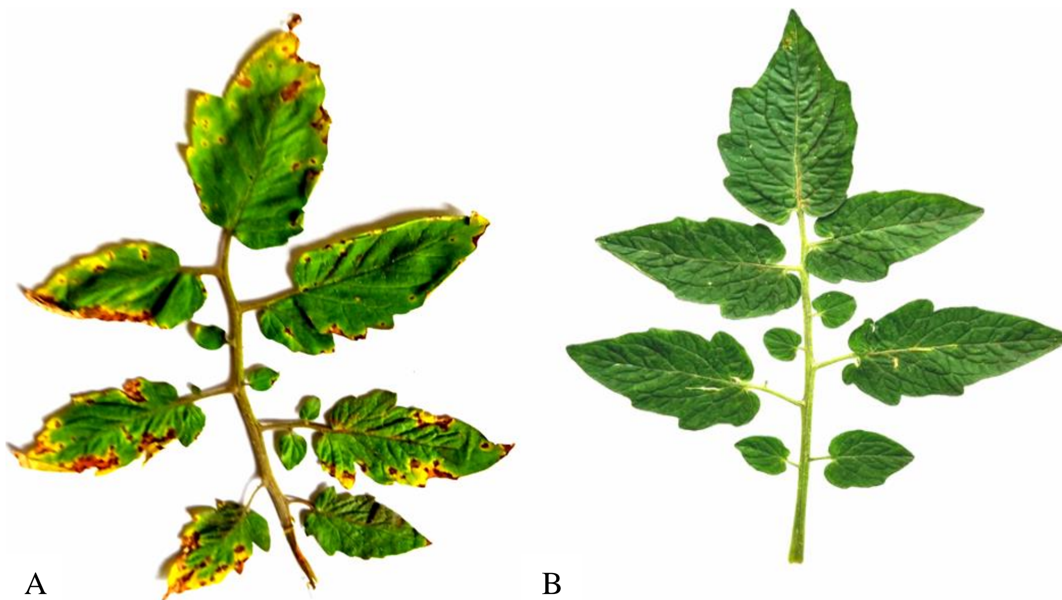

Plate 3.2: Early blight symptoms on infected tomato leaf (A) and healthy tomato leaf (B)
A: Tomato leaf infected with *Alternaria solani*, B: Healthy tomato leaf.

3.4.2. Morphological features and isolation frequency of *Trichoderma* isolates

Trichoderma spp. were isolated from agricultural soils and pure cultures were obtained as described in section 3.3.2. 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. The morphological features and isolation frequency of the isolated *Trichoderma* spp. are mentioned in Table 3.1 and shown in Plate 3.3.

Table 3.1: Isolation frequency and morphological features of *Trichoderma* isolates

Isolate code	% Isolation frequency	Morphological features
Tricho 1, Tricho 4, Tricho 5, Tricho 6, Tricho 7, Tricho 8 and Tricho 10	40	Colonies forming scattered patches appearing in concentric rings, greyish on the back side of the plates and greenish on the front side. Septate, hyaline and green couloured hyphae. Septate, hyaline, branched conidiophores forming verticillate phialides. Small sized, single celled, ovate, green couloured conidia released at the top of phialides in masses.
Tricho 2, Tricho 3 and Tricho 9	20	White and green couloured colonies on the front side of the plates and light brownish on the back side and displaying no scattered patches. Septate, hyaline and green couloured hyphae. Septate, hyaline, branched conidiophores forming verticillate phialides. Small sized, single celled, ovate, green couloured conidia released at the top of phialides in masses.

%: Percent

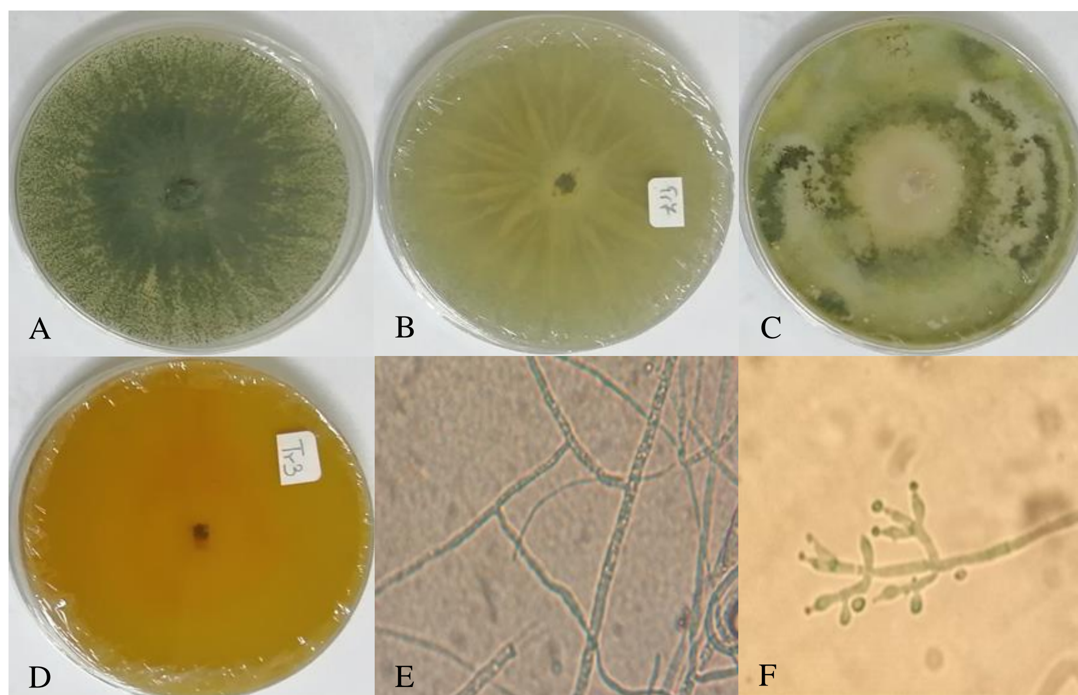

Plate 3.3: Morphological and cultural characteristics of *Trichoderma* isolates

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).

3.4.3. Effects of *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas fluorescens* on the radial growth of *Alternaria solani*

3.4.3.1. Screening of *Bacillus* isolates against *Alternaria solani*

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.

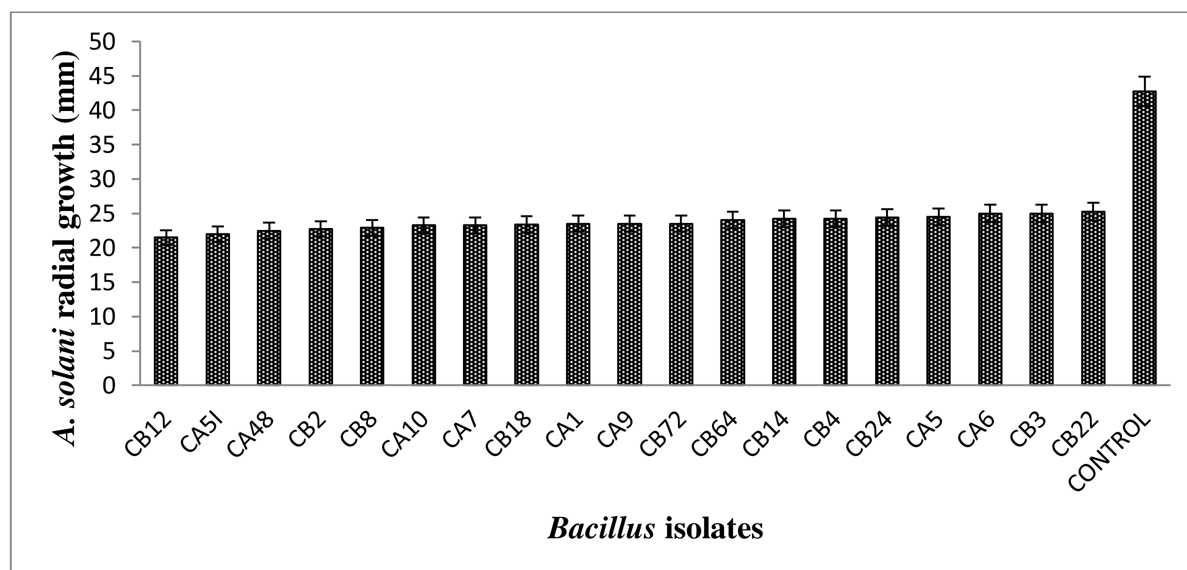

Figure 3.1: Mean radius of *Alternaria solani* colony in the presence of *Bacillus* isolates

Bars indicate standard errors.

3.4.3.2. *In vitro* activity of *Bacillus subtilis* isolates and *Pseudomonas fluorescens* against *Alternaria solani*

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 moderate percent growth inhibition of 56.6 and 54.1% respectively, over the radial growth of *A. solani. Pseudomonas fluorescens* recorded lower

percent growth inhibition of 47.6% (Table 3.2). 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 antagonists. Those zones later (4-5 days) reduced in size measuring 2-3 mm. Plates treated with *P. fluorescens* exhibited faint growth inhibition zones.

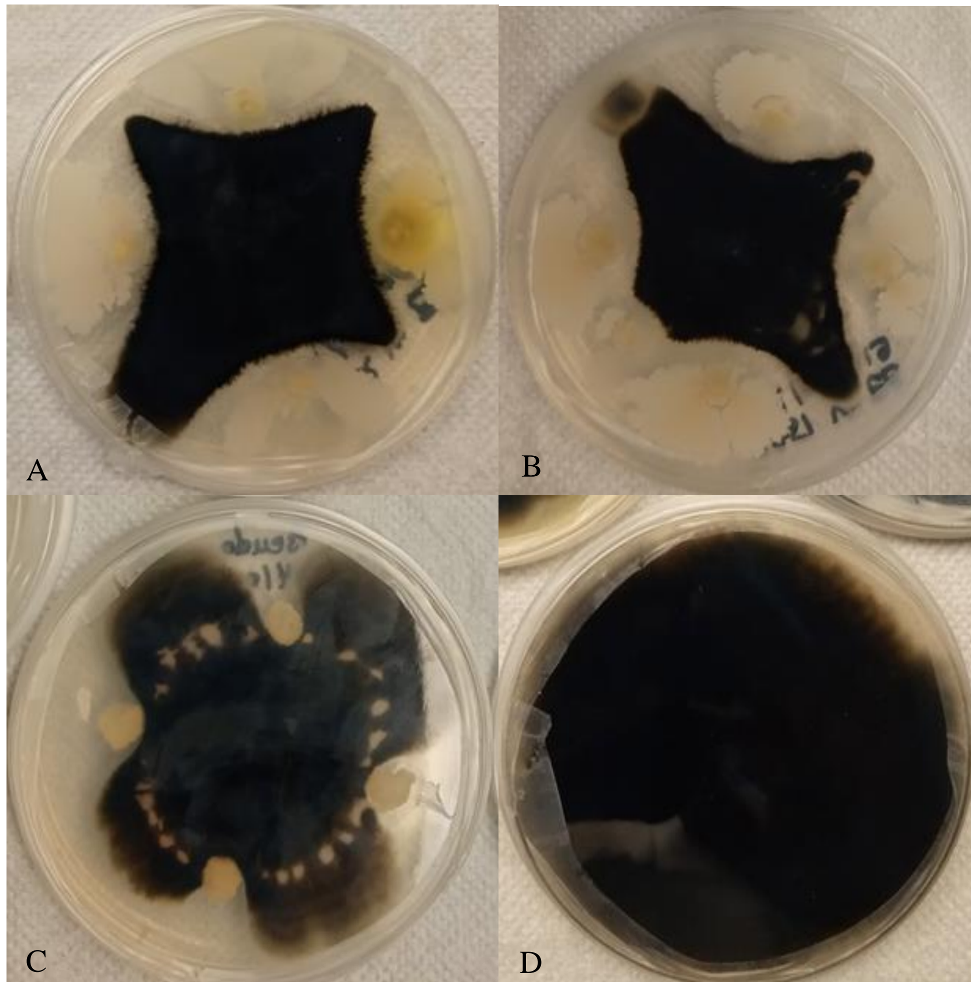

Plate 3.4: *Alternaria solani* colony in the presence of *Bacillus subtilis* isolates and *Pseudomonas fluorescens* and *Alternaria solani* colony in the control

A: *Alternaria solani* colony in presence of CA51, B: *Alternaria solani* colony in presence of CB12, C: *Alternaria solani* colony in presence of *Pseudomonas fluorescens*, D: *Alternaria solani* colony in the Control. CA51 and CB12: *Bacillus subtilis* isolates.

Table 3.2: Mean diameter of *Alternaria solani* colony in the presence of *Bacillus subtilis* and *Pseudomonas fluorescens* and mean diameter of *Alternaria solani* colony in the control

Treatments	Days after plating bacterial antagonists					
	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
Pseudomonas	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 followed by the same letter (s) in the same column are not significantly different (at 5%). Means represent diameter of *Alternaria solani* colony 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.

3.4.3.3. Screening of *Trichoderma* isolates against *Alternaria solani*

All the *Trichoderma* 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.

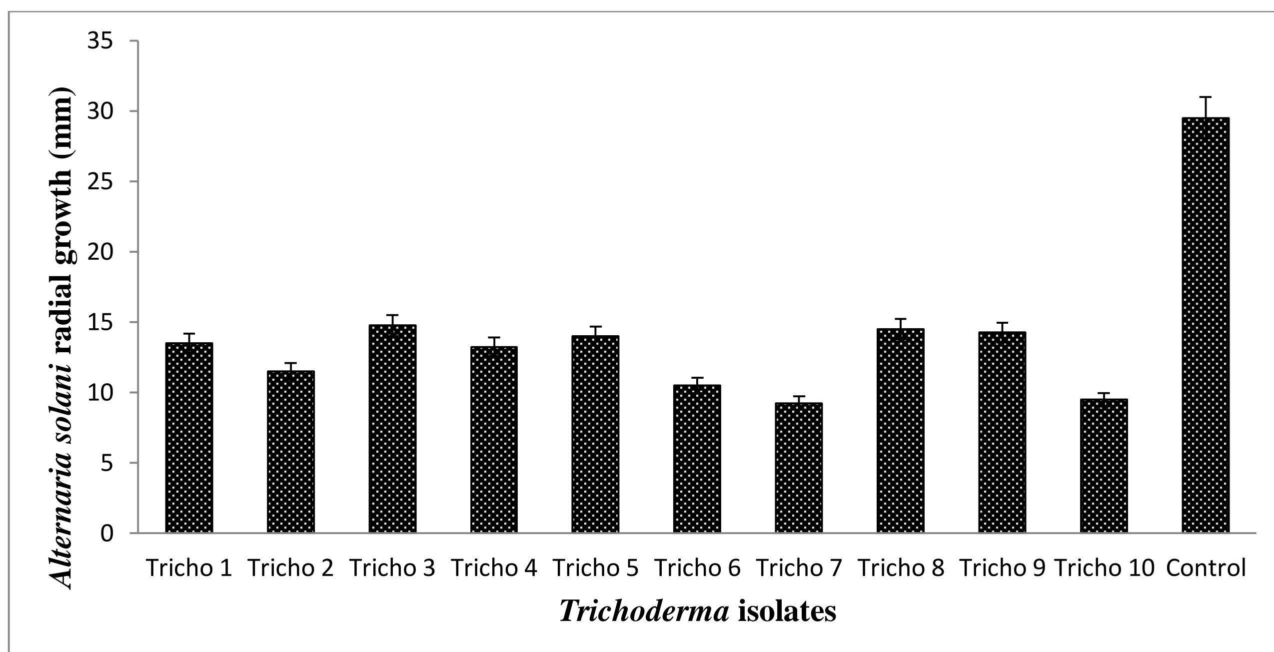

Figure 3.2: Mean radius of *Alternaria solani* colony in the presence of *Trichoderma* isolates
Bars indicate standard errors.

3.4.3.4. *In vitro* activity of *Trichoderma* isolates against *Alternaria solani*

Selected *Trichoderma* isolates from screening experiments namely Tricho 7 and Tricho 10 were evaluated for their activity over *A. solani* radial growth. Both *Trichoderma* isolates significantly inhibited *A. solani* radial growth from the third day after they were plated until the thirteenth day with percent growth inhibition of 80.9 and 82.2% for Tricho 7 and Tricho 10 respectively (Table 3.3). 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 and measured 2 to 5 mm on the thirteenth day.

Table 3.3: Mean diameter of *Alternaria solani* colony in the presence of *Trichoderma* isolates and mean diameter of *Alternaria solani* colony in the control

Treatments	Days after plating fungal antagonists					
	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 followed by the same letter (s) in the same column are not significantly different (at 5%). Means represent diameter of *Alternaria solani* colony in mm. LSD: Least significant difference, % CV: Percent of coefficient of variation, Tricho 7 and Tricho 10: *Trichoderma* isolates.

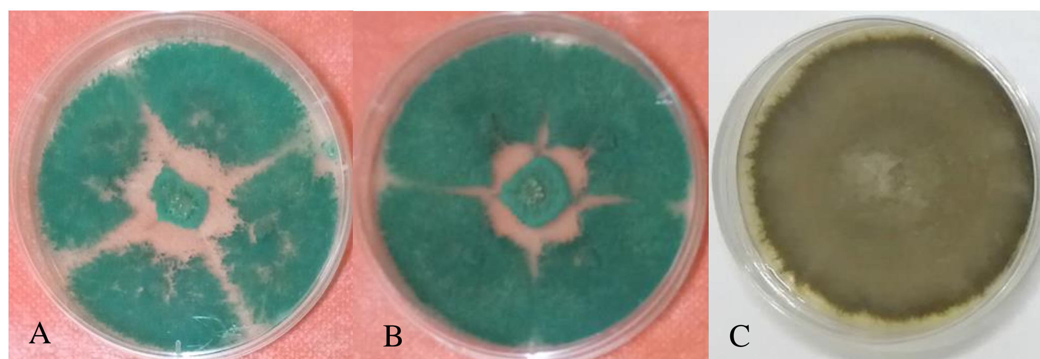

Plate 3.5: *Alternaria solani* colony in the presence of *Trichoderma* spp. and *Alternaria solani* colony in the control

A: *Alternaria solani* colony in the presence of Tricho 7, B: *Alternaria solani* colony in the presence of Tricho 10, C: *Alternaria solani* colony in the Control. Tricho 7 and Tricho 10: *Trichoderma* isolates.

3.5. Discussion

Trichoderma isolates were isolated from agricultural soil samples with an isolation frequency ranging between 20 and 40%. These findings are in accordance with those of Okoth *et al*. (2009) who isolated *Trichoderma harzianum* from maize plantation and indigenous and planted forests and recorded isolation frequencies ranging between 16 and 36%.

The antagonists used in this study showed varied levels of efficacy in inhibiting the *in vitro* growth of *A. solani*. *Trichoderma* isolates coded Tricho 7 and Tricho 10 had higher inhibitory effects over radial growth of the pathogen, while *B. subtilis* isolates coded CA51 and CB12 and *P. fluorescens* had moderate and low inhibitory effects respectively. The superiority of *Trichoderma* isolates could be associated with the fact that *Trichoderma* species employ varied mechanisms of action to impede development of phytopathogens (Nusret and Steven, 2004; Vinale *et al*., 2009) in addition to their fast growing ability (Harman *et al*., 2004; Waghunde *et al*., 2016). This corroborates the results of Meena (2015) who evaluated *Trichoderma viride*, *T. harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* against *Alternaria alternata* and reported that both *Trichoderma* antagonists had higher percent growth inhibition over the pathogen compared to both bacterial antagonists. Similarly, Dalpati *et al*. (2010) 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, Koley *et al*. (2015) evaluated the effects of *B. subtilis*, *P. fluorescens*, *T. viride* and *T. harzianum* over the radial growth of *A. solani* and reported that *B. subtilis* had the highest percent growth inhibition followed by *P. fluorescens*. *Trichoderma* species had lower percent growth inhibition. These results are in contrast with the results recorded in this study. This suggests that the efficacy of microbial antagonists can be influenced by pathogen strains. 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. These findings are in agreement with those of Yendyo *et al*. (2018) who evaluated *Trichoderma* spp., *Bacillus subtilis* and *P. fluorescens* against *Ralstonia* spp. causing bacterial wilt of tomato and found that the antagonists had varied activity over the *in vitro* growth of the pathogen. However, in their study, *P. fluorescens* recorded higher effects over the pathogen radial growth compared to *Trichoderma* and *B. subtilis* isolates. This difference could be explained by the fact that they tested the antagonists against a different pathogen. It has been reported that, the susceptibility of a plant pathogen to microbial antagonists can vary with the antagonists (Tapwal *et al*., 2015).

Findings from this study recorded presence of growth inhibition zones between *Trichoderma* spp. colonies and those of *A. solani*. Growth inhibition zones were also recorded between *A. solani* colonies and those of *B. subtilis*. 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, koninginins, viridins, azaphilones, butenolides, diketopiperazines and peptaibols among others (Vinale *et al*., 2009). Secondary metabolites produced by *Bacillus* isolates and which hinder growth of plant pathogens 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 Meena (2015) who tested *Trichoderma viride*, *T. harzianum* and *Bacillus subtilis* against *Alternaria alternata in vitro* and recorded growth inhibition zones between the pathogen colonies and those of selected antagonists. Similar finding were reported by Abdalla *et al*. (2014) who evaluated the effects of *Bacillus* strains on the radial growth of *Alternaria alternata* isolated from tomato and reported formation of growth inhibition zones between *A. alternata* colonies and those of *Bacillus*. 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. Similarly, Meena (2015) tested *Trichoderma viride* and *T. harzianum* against *Alternaria alternata* and reported that both *Trichoderma* antagonists grew over the pathogen colonies.

In this study faint growth inhibition zones were noticed between *P. fluorescens* colonies and those of *A. solani*. These results were in partial agreement with 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. In contrast, Meena (2015) evaluated the effects of *Pseudomonas fluorescens* on the radial growth of *Alternaria alternata* and recorded formation of

distinct growth inhibition zones between the pathogen colonies and those of the antagonist. This suggests that the effects of a microbial antagonist on the growth of plant pathogens can vary with pathogen species.

Trichoderma isolates coded Tricho 7 and Tricho 10, *Bacillus subtilis* isolates coded CA51 and CB 12 and *P. fluorescens* were effective against *A. solani* under *in vitro* conditions.

CHAPTER FOUR: EFFECTIVENESS OF *TRICHODERMA* SPP., *BACILLUS SUBTILIS* AND *PSEUDOMONAS FLUORESCENS* IN THE MANAGEMENT OF EARLY BLIGHT OF TOMATOES

4.1. Abstract

Integrating microbial antagonists 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 microbial antagonists with significant inhibitory effects over *A. solani* under *in vitro* conditions, were selected and evaluated for their effectiveness in managing early blight in tomatoes. These included two *Trichoderma* isolates, two *Bacillus subtilis* isolates and one *Pseudomonas fluorescens* strain. Water and Tower 72 WP® were used as control and standard check respectively. Each treatment was replicated thrice. Data were taken on disease parameters. Marketable fruits were selected from harvested fruits and weighed. Percent disease incidence was significantly lower in all treatments compared to control treatment at both experimental sites. The percent disease incidence recorded for Tower 72 WP® was comparable to percent disease incidence recorded for most selected antagonists. The percent disease severity and the percent disease index were significantly lower in all treatments compared to control treatment. 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 and 53.1%. All treatments recorded significantly higher quantities of marketable fruits compared to the control. However, Tower 72 WP® recorded higher quantities compared to all selected antagonists at both experimental sites. In the greenhouse, Tower 72 WP® and both *Trichoderma* isolates recorded higher quantities. *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas fluorescens* are effective in managing early blight in tomatoes and minimize yield losses caused by early blight.

4.2. Introduction

The fungus *Alternaria 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 application of synthetic chemicals leads 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). Microbial antagonists 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 microbial antagonists to hinder the growth and development of plant pathogens (Benítez *et al*., 2004). This study was conducted to evaluate five promising isolates of microbial antagonists selected from *in vitro* evaluation for their effectiveness in managing tomato early blight in the greenhouse and in the field. These included two

Trichoderma isolates coded Tricho 7 and Tricho 10, two *Bacillus* isolates coded CA51 and CB12 and one commercial *P. 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 Kenya Agricultural and Livestock Research Organization (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 above sea level and receives approximately 1,000 mm of rainfall annually with annual maximum temperature being 28^0C, average temperature at 23^0C and minimum going up to 13^0C. 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 is under AEZ II, has a bimodal distribution of rainfall and it is characterized 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 15.6^0C to 28.6^0C and an average of 22^0C. Mwea is in the Lower Midland 4 (LM4) and falls under an altitude of 1,159 m above 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. Preparation of culture filtrates from the antagonists

4.3.2.1. Preparation of cultures filtrates from *Bacillus subtilis* isolates

Culture filtrates from *B. subtilis* isolates were produced in Tschen's liquid medium as described by Loeffler *et al*., (1986). Composition of the medium was as follow: 15 gram glucose, 15 ml glycerol, five gram $5(NH_4)_2SO_4$, 15 gram soybean meal, one gram yeast extract, five gram NaCl and five gram $CaCO_3$ in 1,000 ml distilled water (pH, 7.5). One hundred ml of the medium were prepared in 250 ml of Erlenmeyer flasks. Sterilization of the medium was done at 121^0C at one bar pressure for 15 minutes. The medium was cooled after sterilization. Ten day old colonies of *Bacillus subtilis* sub-cultured on nutrient agar in plastic Petri dishes were flooded 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 Tschen'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 $(24\pm2)^0$C 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 revolutions per minute (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 4^0C.

4.3.2.2. Preparation of culture filtrates from *Trichoderma* isolates

Culture filtrates from *Trichoderma* isolates were produced in Czapek Dox Broth medium. Composition of the medium was as follow: 30.00 grams sucrose, 3.00 grams sodium nitrate, 1.00 gram of dipotassium hydrogen phosphate, 0.50 gram magnesium sulphate, 0.50 gram potassium chloride, 0.01 gram ferrous sulphate in 1,000 ml distilled water (final pH at 25^0C: 7.3 ± 0.2).

Approximately 14.00 grams of the medium were weighed and transferred into 1,000 ml conical flasks. Four hundred ml distilled water were added and the blend was shaken to dissolve. The mixture was sterilized by autoclaving at 121^0C at one bar pressure for 15 minutes. The medium was then cooled before use. Seven day old colonies from *Trichoderma* isolates sub-cultured in plastic petri dishes containing PDA medium 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 $(24\pm2)^0$C 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 4^0C.

4.3.3. Design and set up of the greenhouse experiment

Preparation of the potting medium, raising of tomato seedlings and inoculation of plants with *A. solani* conidia were done as described in section 3.3.6. Inoculation of plants with *A. solani* conidia was done one week after the first application of the treatments. A total of seven treatments were applied. These included two *Trichoderma* isolates coded Tricho 7 and Tricho 10, two *Bacillus subtilis* isolates coded CA51 and CB12, Bio-cure® which is a commercial formulation of the rhizobacterium *P. fluorescens*, one standard check (Tower 72 WP®; 64% Mancozeb and 8% Metalaxyl) and one negative control which entailed spraying with water only. Fertilization, insecticide application, irrigating and weeding were carried out as per standard agronomic practices. The experiments were laid in CRD with four replicates.

4.3.4. Design and set up of the field experiments

Microbial antagonists selected from the *in vitro* tests were evaluated for their effectiveness in managing tomato early blight in the field. 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. Thirty days after sowing, resultant seedlings were transplanted in plots of 2 x 1.5 meter (Baka and Rashad, 2016). 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. Plot to plot distance was one meter. The same treatments were used as described in section 4.3.3. Fertilization, insecticide application, irrigating and weeding were done as described in section 4.3.3. The experimental design was a Randomized Complete Block Design (RCBD) in triplicates.

4.3.5. Application of the treatments

Treatments were applied on a 10 day interval commencing 50 days after sowing in the greenhouse and 20 days after transplanting in the field. One thousand ml of culture filtrates from isolates of selected antagonists prepared as described in sections 4.3.2.1 and 4.3.2.2 were thoroughly mixed with one ml acquawet to allow it to stick on the leaf surface. The treatments were 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 72 WP® and Bio-cure® were applied according to the manufactures' guidelines. A total of 6 sprays were done for the whole cropping season.

4.3.6. Assessment of early blight

Tomato early blight was assessed on a 10 day interval commencing 50 days after sowing in the greenhouse and 20 days after transplanting in the field until the end of harvesting at 120 days of sowing and 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 selected from the 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 (Lengai, 2016). Assessment of disease severity was done on five plants randomly selected from the 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 follow: 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 (PDS) was calculated from data on disease severity as follow:

$$\text{PDS} = \frac{Severity\ score}{5} * 100$$

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

$$\text{PDI}= \frac{Distribution\ score+Incidence\ score+Severity\ score}{Cumulative\ maximum\ disease\ score\ (8)} *100$$ (Lengai, 2016).

In the greenhouse, data were not collected on disease distribution. The percent disease index was thus calculated as follow:

$$\text{PDI} = \frac{Incidence\ score + Severity\ score}{Cumulative\ maximum\ disease\ score\ (6)} * 100$$

The AUDPC was calculated using the data on percent disease severity as demonstrated by Simko and Piepho (2012):

AUDPC=$\Sigma[(X_{i+1}+X_i)/2]\ [t_{i+1}-t_i]$; where Σ=Sum total of the disease, X_i= Disease measure 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).

4.3.7. Assessment of tomato yield

Tomato fruits at pink or ripe stage were harvested per treatment on a weekly basis. Marketable fruits were selected from harvested fruits and weighed. For greenhouse experiment, the yield of marketable fruits was converted to Kg per plant (Kg/plant). For field data, the quantity of marketable fruits from each plot was extrapolated to tons per hectare (Tons/ha) as follow:

$\text{Yh} = \frac{10*\text{Yp}}{3}$; where Yh=Yield per hectare and Yp=Yield per plot.

4.3.8. Data analysis

Data were analyzed as described in section 3.3.9. Correlations between tomato early blight measurement parameters and tomato yield were tested by the two-tailed correlation coefficient of Pearson using IBM® SPSS® statistics 20.

4.4. Results

4.4.1. Percent disease incidence for early blight in tomato plants treated with the various antagonists

In the greenhouse and at both experimental sites, the percent disease incidence did not significantly ($p>0.05$) differ among treatments on the 20th day after transplanting. Significant ($p\leq0.05$) increases were recorded for the percent disease incidence in all the treatments with time. However, all the treatments significantly ($p\leq0.05$) reduced the percent disease incidence compared to control treatment over time (Appendix 1). In the greenhouse, on the 60th, 70th, 80th and 90th days after transplanting, Tower 72 WP® and isolates coded Tricho 10 and CB12 significantly ($p\leq0.05$) reduced the percent disease incidence compared to control treatment. On the 90th day after transplanting, percent disease incidence ranged from 6.9 to 16.2% for all treatments while for control treatment, percent disease incidence was 17.9%. At Kabete Field Station, 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 ($p>0.05$) differences were recorded over time in reducing the percent disease incidence between the various antagonists and the standard chemical (Tower 72 WP®). At KALRO Mwea, on the 90th day after transplanting, percent disease incidence was between 12.2 and 15.5% for all treatments while for control treatment, a percent disease incidence of 35.4% was recorded. Over time, effects recorded for Tower 72 WP® in reducing the percent disease incidence were comparable ($p>0.05$) to all selected antagonists except for the isolate CB12 which had lower effects compared to Tower 72 WP® (Table 4.1).

Table 4.1: Percent disease incidence for early blight in tomato plants treated with the various antagonists

Experimental site	Treatments	Time after transplanting (Days)							
		20	30	40	50	60	70	80	90
Greenhouse	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
	Pseudomonas	4.8a	6.7ab	19.5b	14.4b	11.2c	13.1b	13.2bc	16.2bc
	Tower 72 WP®	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
Kabete Field Station	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
	Pseudomonas	10.4a	9.4b	21.0a	33.0a	40.6a	36.5a	30.4a	20.5ab
	Tower 72 WP®	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
KALRO Mwea	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
	Pseudomonas	8.4a	8.8a	18.8ab	20.4b	13.6ab	11.2a	12.6a	13.8a
	Tower 72 WP®	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 followed by the same letter (s) in the same column and experimental site are not significantly different (at 5%). KALRO: Kenya Agricultural and Livestock Research Organization, 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 72 WP®: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

4.4.2. Percent disease severity for early blight in tomato plants treated with the various antagonists

In the greenhouse and at both experimental sites, the percent disease severity was comparable ($p>0.05$) among all the treatments on the 20th day after transplanting. Over time, significant ($p\leq0.05$) increases were recorded for the percent disease severity in all the treatments. However, over time, all the treatments significantly ($p\leq0.05$) reduced the percent disease severity compared to control treatment (Appendix 2). In the greenhouse, on the 90th day after transplanting, percent disease severity 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 72 WP® in reducing the percent disease severity were comparable ($p>0.05$) to the various antagonists except for commercial *P. fluorescens* which had a lower effect. At Kabete Field Station, on the 90th day after transplanting, percent disease severity ranged from 40.5 to 51.5% for all treatments while for control treatment, a percent disease severity of 83.7 was recorded. Over time, performance recorded for Tower 72 WP® in reducing the percent disease severity were comparable ($p>0.05$) to the various antagonists except for commercial *P. fluorescens* which recorded lower effect. No significant ($p>0.05$) differences were recorded between bacterial and *Trichoderma* antagonists over time. At KALRO Mwea, 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 ($p\leq0.05$) lower in plots treated with Tower 72 WP® compared to those treated with the various antagonists except for *B. subtilis* isolates which recorded percent disease severity that was not significantly ($p>0.05$) different from Tower 72 WP® (Table 4.2).

Table 4.2: Percent disease severity for early blight in tomato plants treated with the various antagonists

Experimental site	Treatments	Time after transplanting (Days)							
		20	30	40	50	60	70	80	90
Greenhouse	CA51	3.7a	6.3a	11.8a	16.3a	17.1a	25.4a	32.3a	34.0a
	CB12	3.6a	6.1a	11.7a	16.6a	16.7a	22.2a	28.5a	32.6a
	Tricho 7	3.2a	6.4a	11.3a	15.7a	20.0ab	24.3a	28.3a	32.1a
	Tricho 10	3.4a	6.2a	15.9a	18.0a	25.3b	30.8ab	32.1a	36.3a
	Pseudomonas	3.5a	5.2a	13.3a	22.1a	26.2b	35.1b	42.1b	48.2b
	Tower 72 WP®	3.3a	5.6a	12.2a	17.0a	21.1ab	25.1a	30.6a	35.4a
	Water	3.5a	12.2b	30.1b	44.0b	57.6c	68.3c	71.2c	74.4c
	LSD ($p \leq 0.05$)	5.2	5.6	10.6	7.2	6.2	8.5	7.6	7.2
	% CV	66.1	55.1	42.8	47.3	36.2	47.5	33.7	39.6
Kabete Field Station	CA51	10.1a	16.3abc	29.3b	38.7a	38.9a	36.8a	40.0ab	48.8b
	CB12	10.4a	16.8bc	29.6b	40.3a	41.3a	35.7a	35.7a	46.7ab
	Tricho 7	10.1a	15.2ab	21.6a	40.5a	40.3a	38.1a	34.4a	44.3ab
	Tricho 10	9.9a	14.9ab	23.2a	36.5a	41.3a	38.7a	34.1a	45.9 ab
	Pseudomonas	10.4a	18.1c	35.7c	42.7a	45.1a	39.5a	46.4b	51.5b
	Tower 72 WP®	11.5a	14.1a	21.1a	40.0a	41.6a	34.1a	36.5a	40.5a
	Water	10.9a	37.6d	50.9d	79.5b	82.1b	78.9b	73.9c	83.7c
	LSD ($p \leq 0.05$)	2.3	2.4	5.6	11.3	10.1	7.1	9.8	7.4
	% CV	12.2	7.0	10.4	13.9	12.1	9.3	12.8	8.0
KALRO Mwea	CA51	9.6a	12.5a	22.7ab	24.3ab	28.3b	25.1a	33.9bc	34.7bc
	CB12	10.1a	20.3b	23.5 ab	26.1b	24.0ab	26.7a	30.4ab	29.1ab
	Tricho 7	9.6a	20.8b	22.4 ab	26.9b	22.7a	26.7a	39.2de	34.9 bc
	Tricho 10	9.4a	17.3ab	25.9b	26.4b	25.3ab	27.5a	36.0cd	29.6ab
	Pseudomonas	9.6a	20.5b	20.8a	26.7b	29.1b	28.0a	40.0e	39.5c
	Tower 72 WP®	11.2a	14.9ab	19.2a	21.3a	22.1a	26.1a	30.1a	24.0a
	Water	9.6a	29.3c	50.7c	53.6c	64.3c	61.3b	67.2f	62.9d
	LSD ($p \leq 0.05$)	2.0	6.0	5.0	3.3	5.4	6.2	3.7	8.8
	% CV	11.5	17.5	10.6	6.3	9.9	11.1	5.2	13.6

Means followed by the same letter (s) in the same column and experimental site are not significantly different (at 5%). KALRO: Kenya Agricultural and Livestock Research Organization, 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 72 WP®: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

4.4.3. Percent disease index for early blight in tomato plants treated with the various antagonists

In the greenhouse and at both experimental sites, the percent disease index did not significantly ($p>0.05$) differ among treatments on the 20th day after transplanting. Over time, significant ($p\leq0.05$) increases in the percent disease index were recorded in all the treatments. However, over time, all treatments significantly ($p\leq0.05$) reduced the percent disease index compared to control treatment (Appendix 3). In the greenhouse, on the 90th day after transplanting, percent disease index ranged between 28.4 and 42.7% for all treatments while for control treatment, the percent disease index was 64.7%. Effects recorded for Tower 72 WP® in reducing the percent disease index, were not significantly ($p>0.05$) different from the various antagonists except for commercial *P. fluorescens* with a lower effect. At Kabete Field Station, 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 72 WP® in reducing the percent disease index did not significantly ($p>0.05$) differ from the antagonists except for commercial *P. fluorescens* with a lower effect compared to Tower 72 WP®. Compared to each other, bacterial and *Trichoderma* antagonists recorded comparable ($p>0.05$) effects in reducing the percent disease index. At KALRO Mwea, 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. Over time, Tower 72 WP® recorded a significantly ($p\leq0.05$) higher effect in reducing the percent disease index compared to the various antagonists. The various antagonists recorded comparable ($p>0.05$) effects in reducing the percent disease index (Table 4.3).

Table 4.3: Percent disease index for early blight in tomato plants treated with the various antagonists

Experimental site	Treatments	Time after transplanting (Days)							
		20	30	40	50	60	70	80	90
Greenhouse	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
	Pseudomonas	3.3a	5.3a	14.8a	20.9b	23.4c	31.3b	37.2b	42.7b
	Tower 72 WP®	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
Kabete Field Station	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
	Pseudomonas	14.9a	16.8a	36.1d	44.6a	48.2a	45.5a	49.9c	53.1c
	Tower 72 WP®	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
KALRO Mwea	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
	Pseudomonas	14.1a	23.9ab	25.4ab	33.0b	34.4c	33.5a	41.2d	41.0c
	Tower 72 WP®	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 followed by the same letter (s) in the same column and experimental site are not significantly different (at 5%). KALRO: Kenya Agricultural and Livestock Research Organization, 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 72 WP®: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

4.4.4. Area under disease progress curve for early blight in tomato plants treated with the various antagonists

In the greenhouse and at both experimental sites, significant reductions in the AUDPC for tomato early blight were recorded ($p \leq 0.05$) for all treatments compared to control treatment. In the greenhouse, 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 ($p > 0.05$) differences were recorded in reducing the AUDPC between Tower 72 WP® and the various antagonists except for commercial *P. fluorescens* which recorded a lower effect. However, no significant ($p > 0.05$) differences were recorded between commercial *P. fluorescens* and the isolate Tricho 10. At Kabete Field Station, AUDPCs ranging between 2,192 and 2,636 were recorded for all treatments while for control treatment, the AUDPC was 4,557. No significant ($p > 0.05$) differences were recorded in reducing the AUDPC between Tower 72 WP® and the various antagonists except for commercial *P. fluorescens* which recorded a lower effect. No significant ($p > 0.05$) differences were recorded between bacterial or *Trichoderma* antagonists. At KALRO Mwea, 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 72 WP® recorded a significantly ($p \leq 0.05$) higher effect in reducing the AUDPC compared to the various antagonists except for *B. subtilis* isolates which had comparable effects. Commercial *P. fluorescens* recorded a significantly ($p \leq 0.05$) lower effect in reducing the AUDPC compared to the other antagonists except for Tricho 7 with a comparable effect (Figure 4.1).

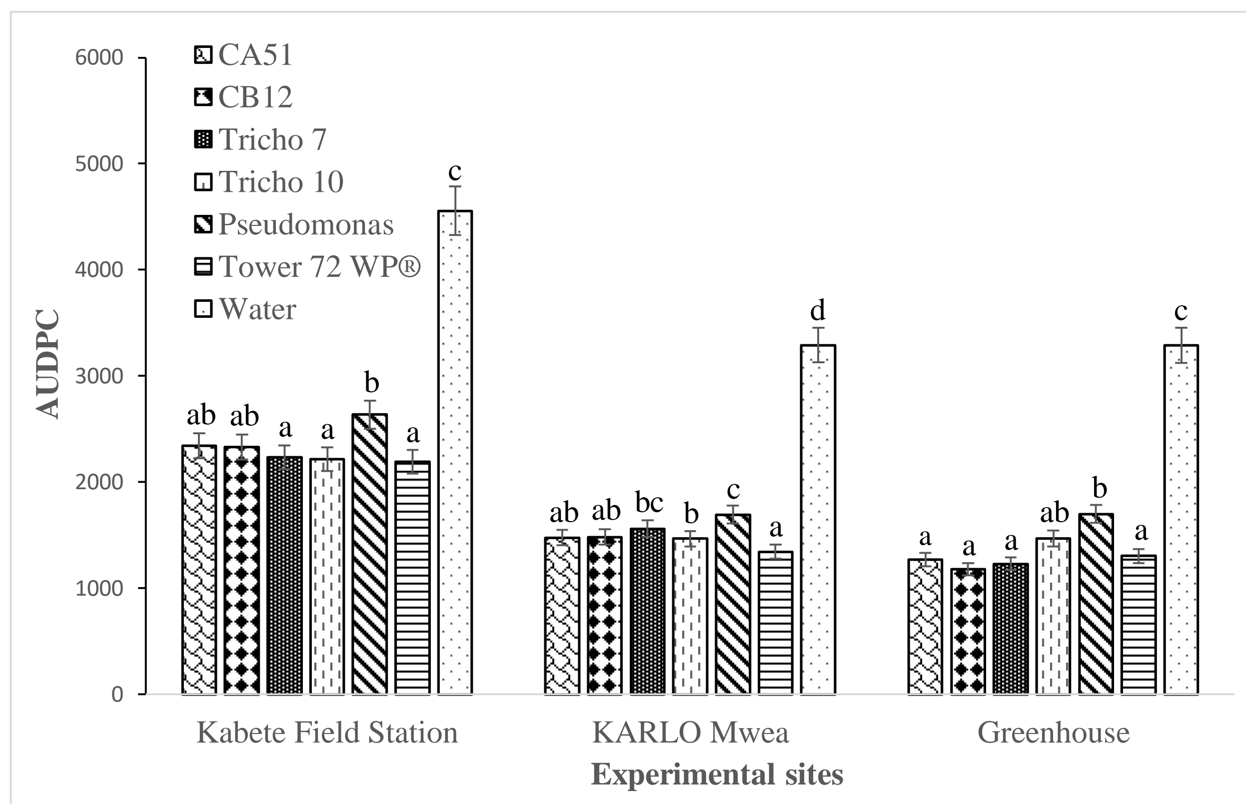

Figure 4.1: Area under disease progress curve (AUDPC) for early blight in tomato plants treated with the various antagonists

Treatments with the same letter (s) in the same experimental site are not significantly different (at 5%). Bars indicate standard errors. KALRO: Kenya Agricultural and Livestock Research Organization, CA51 and CB12: *Bacillus subtilis* isolates, Tricho 7 and Tricho 10: *Trichoderma* isolates, *Pseudomonas*: *Pseudomonas fluorescens* (from commercial Bio-cure®), Tower 72 WP®: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

4.4.5. Fruit yield for tomato plants treated with the various antagonists

In the greenhouse and at both experimental sites, quantity of marketable tomato fruits was significantly ($p \leq 0.05$) higher in all treatments compared to control treatment. In the greenhouse, total quantities of marketable fruits ranged between 0.68 and 1.06 kg/plant for all treatments while for control treatment, total quantity of 0.31 kg/plant was recorded. Compared to the bacterial antagonists both *Trichoderma* isolates and Tower 72 WP® recorded significantly ($p \leq 0.05$) higher quantities of marketable tomato fruits. At Kabete Field Station, total quantities

of marketable fruits ranging between 33.8 and 50.7 tons/hectare were recorded for all the treatments while for control treatment, the total quantity of marketable fruit was 18.8 tons/hectare. Tower 72 WP® recorded significantly ($p \leq 0.05$) higher quantity of marketable tomato fruits compared to the various antagonists. No significant differences were recorded ($p > 0.05$) in the quantity of marketable fruits between *Bacillus* or *Trichoderma* antagonists. At KALRO Mwea, total quantities of marketable fruits ranging between 40.3 and 58.7 tons/hectare were recorded for all the treatments while for control treatment, total quantity of 16.2 tons/hectare was recorded. The quantity of marketable fruits was significantly ($p \leq 0.05$) higher with Tower 72 WP® treatment compared to the various antagonists. *Trichoderma* isolates recorded significantly ($p \leq 0.05$) higher quantities of marketable tomato fruits compared to the bacterial antagonists (Table 4.4).

Table 4.4: Fruit yield for tomato plants treated with the various antagonists

Site / Treatments	Greenhouse		Kabete Field Station		KALRO Mwea	
	Total (Kg/plant)	Mean (Kg/plant)	Total (Tons/ha)	Mean (Tons/ha)	Total (Tons/ha)	Mean (Tons/ha)
CA51	0.68b	0.14b	36.0bc	7.2bc	40.3b	8.1b
CB12	0.75b	0.15b	33.8b	6.8b	46.3c	9.3c
Tricho 7	0.78c	0.21c	40.9bc	8.1bc	51.4d	10.3d
Tricho 10	0.98c	0.19c	41.7c	8.3c	52.6d	10.5d
Pseudomonas	0.97b	0.16b	35.4bc	7.1bc	41.2 b	8.2b
Tower 72 WP®	1.06c	0.20c	50.7d	10.1d	58.7e	11.7e
Water	0.31a	0.06a	18.8a	3.8a	16.2a	3.2a
LSD (≤0.05)	0.15	0.03	7.1	1.4	5.0	1.0
% CV	13.1	13.1	10.9	10.9	6.4	6.4

Means followed by the same letter (s) in the same column are not significantly different (at 5%). KALRO: Kenya Agricultural and Livestock Research Organization, Ha: Hectare, Kg: Kilogram, 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 72 WP®: Synthetic fungicide (Mancozeb 64% + Metalaxyl 8%).

4.4.6. Correlations between tomato early blight parameters and tomato yield

No significant correlations were recorded between different parameters at 5% at both experimental sites. At both experimental sites, tomato yield was significantly ($p \leq 0.01$) and negatively correlated with percent disease incidence, percent disease severity, percent disease index and area under disease progress curve. Percent disease incidence was significantly ($p \leq 0.01$) and positively correlated with percent disease severity, percent disease index and area under disease progress curve. Percent disease severity was significantly ($p \leq 0.01$) and positively correlated with percent disease index and area under disease progress curve. Percent disease index was significantly ($p \leq 0.01$) and positively correlated with area under disease progress curve (Table 4.5).

Table 4.5: Correlations between tomato early blight parameters and tomato yield at both experimental sites

Experimental site	Parameters	% disease incidence	% disease severity	% disease index	AUDPC	Tomato yield
Kabete Field Station	% disease incidence	-				
	% disease severity	0.857**	-			
	% disease index	0.902**	0.992**	-		
	AUDPC	0.856**	0.974**	0.979**	-	
	Tomato yield	-0.820**	-0.837**	-0.858**	-0.802**	-
KALRO Mwea	% disease incidence	-				
	% disease severity	0.736**	-			
	% disease index	0.824**	0.976**	-		
	AUDPC	0.872**	0.924**	0.974**	-	
	Tomato yield	-0.766**	-0.919**	-0.942**	-0.901**	-

**: Significant at $p \leq 0.01$, KALRO: Kenya Agricultural and Livestock Research Organization, AUDPC: Area under disease progress curve, No: number, %: percent.

4.5. Discussion

Findings from this study reported significant reductions of early blight intensity with all the selected antagonists compared to control treatment, in the greenhouse and at both experimental sites. Thus, applications of the antagonists were effective in protecting tomato leaves from being infected by *A. solani*. Applications of the antagonists, also 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 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. Similarly, Suleiman *et al*. (2017) reported that *B. thuringiensis*, *P. fluorescens* and *T. viride* significantly reduced the percent disease incidence for early blight on potatoes under field conditions. Similar findings were reported by Udhav (2013) who evaluated *B. subtilis*, *P. fluorescens* and *T. viride* against *A. solani* on potatoes under field conditions and recorded significant reductions in the percent disease incidence by the antagonists. 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 selected microbial antagonists. Similarly, Verma *et al*. (2018) evaluated *T. harzianum*, *T. viride* and *P. fluorescens* against *A. solani* on tomato in the greenhouse and reported significant reductions in disease severity for all the selected microbial antagonists. Similar findings were reported by Pute (2016) who evaluated *B. subtilis* against *A. solani* on tomato and recorded significant reduction of AUDPC by the bacterium compared to the untreated control.

In this study, no significant differences were recorded for the percent disease index between Tower 72 WP® and most microbial antagonists 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. Similarly, Zegeye *et al*. (2011) evaluated *P. fluorescens* and *T. viride* against *Phytophthora infestans* and recorded significant reductions of AUDPC by both antagonists. In their experiment, no significant differences were recorded in reducing the AUDPC between *T. viride* and Mancozeb (a synthetic chemical).

In the greenhouse and at KALRO Mwea, Tower 72 WP® recorded a significantly higher effect in reducing the percent disease index for tomato early blight, compared to the various antagonists. 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. and *P. fluorescens*. Similar findings were reported by Udhav (2013) who evaluated *B. subtilis*, *P. fluorescens* and *T. viride* against *A. solani* on potato in open field and reported effectiveness for all the selected antagonists but noted a superior effect for Mancozeb compared to all the selected microbial antagonists. 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.

Similar findings were reported by Sundaramoorthy and Balabaskar (2013) who found that, Carbendazim (0.1%) was most effective in reducing tomato wilt in the greenhouse.

In this study, no significant differences were recorded in the percent disease incidence for all treatments compared to the control in the greenhouse from the 20th to the 50th day after transplanting. These findings were in partial agreement with those of Deepthi (2006) who tested *T. harzianum*, *T. viride* and *P. fluorescens* against *Alternaria vitis* and reported that the antagonists were not effective when they were applied 7 days after plant inoculation with the pathogen. However, compared to the control, all the treatments significantly reduced percent disease incidence in the greenhouse over the cropping season. These findings are in accordance with those reported by Moges *et al*. (2012) who evaluated *P. fluorescens* and *B. subtilis* against *A. solani* on tomato in the greenhouse and recorded significant reductions in the disease incidence by both antagonists.

In this study, the yield of marketable fruits were significantly higher in all treatments compared to control treatment, in the greenhouse and at both experimental sites. Thus, application of culture filtrates from the antagonists on tomato plants minimizes the effects of *A. solani* over tomato plants and thus reduces tomato 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 selected antagonists 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. virid*e 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.

The antagonists used in this study gave better results at KALRO Mwea than at Kabete Field Station. This could be due to the variability in environmental conditions between the two experimental sites. It has been reported that, the activity of microbial antagonists is influenced by the environmental conditions along with temperature, pH, water content, soil texture among others (Kredics *et al*., 2003; Benítez *et al*., 2004). Temperatures recorded during the experimental period were higher at KALRO Mwea than at Kabete Field Station (Appendix 4). The findings of this study are in accordance with those of Petrișor *et al*. (2016) who evaluated the effects of temperature on the antagonism capacity of two *Trichoderma* isolates against *Rhizoctonia solani* and recorded that the isolate coded *Trichoderma* Td85 was more effective against the pathogen at 30^0C than at 25^0C. In contrast, Moore *et al*. (2013) evaluated the influence of temperature on the activity of a biosurfactant produced by the *Bacillus subtilis* isolate coded BSB3 on the growth of *Staphylococcus* strains and reported a higher activity of the biosurfactant at 30^0C than at 37^0C. Similarly, Humair *et al*. (2009) evaluated the effect of temperature on the expression of typical biocontrol factors by the *Pseudomonas fluorescens* coded CHAO and reported that the expression of antibiotic compounds was higher at 30^0C than at 34^0C. This suggests variability in temperature requirement for activity of microbial antagonists against plant pathogens (Sundin *et al*., 2009).

Trichoderma isolates coded Tricho 7 and Tricho 10, *B. subtilis* isolates coded CA51 and CB12 and *P. fluorescens* were effective in managing tomato early blight under greenhouse and field conditions. Applications of commercial *P. fluorescens* and culture filtrates from *B. subtilis* and *Trichoderma* spp. reduced the intensity of tomato early blight under greenhouse and field conditions. Applications of the antagonists were beneficial to tomato fruit production as they increased the quantity of marketable fruits. Integrating *Trichoderma* isolates coded Tricho 7 and Tricho 10, *B. subtilis* isolates coded CB12 and CA51 in the management of tomato early blight will be beneficial for sustainable production of tomatoes as microbial antagonists have fewer negative effects on the environment and human health.

CHAPTER FIVE: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. General discussion

This study demonstrated effectiveness of *B. subtilis* isolates, *P. fluorescens* and *Trichoderma* isolates against the radial growth of *A. solani*. All the selected microbial antagonists were effective in managing tomato early blight under greenhouse and field conditions. Thus, in this study, a strong correlation between the effectiveness of the antagonists *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 antagonists 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 selected 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 microbial antagonists *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 antagonists 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 the various antagonists. Growth inhibition zones appearing between most antagonist colonies and *A. solani* colony were recorded. *Trichoderma* colonies grew over *A. solani* colony. It has been reported that, microbial antagonists 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 greenhouse and field conditions. The culture filtrates contain cells or spores and the metabolites from the various antagonists. 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 were applied together with their metabolites (Sultan, 2012).

In this study, increased tomato yields were recorded for plants treated with the various antagonists compared to water treated plants. The antagonists minimized the effects of *A. solani* on tomato production through reduction of the disease intensity. Microbial antagonists have been

reported to have activity that 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 been 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

Trichoderma isolates coded Tricho 7 and Tricho 10, *B. subtilis* isolates coded 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 selected antagonists were effective in reducing the percent disease index for tomato early blight under greenhouse and field conditions. All the selected antagonists significantly reduced the spread of tomato early blight infection from infected leaves to healthy ones. All the selected antagonists 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 all the antagonists.

Applications of all the selected antagonists suppressed the effects of early blight on tomato production through reduction of disease severity. The quantities of marketable fruits were higher in plots treated with the various antagonists compared to those treated with water. However, the synthetic chemical recorded the highest quantities of marketable fruits compared to the various antagonists under field conditions 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:

I. *Trichoderma* isolates coded Tricho 7 and Tricho 10 and *B. subtilis* isolates coded CA51 and CB12 be integrated in the management of tomato early blight under greenhouse and field conditions.

II. Further studies be done on making formulations from *Trichoderma* isolates coded Tricho 7 and Tricho 10 and *B. subtilis* isolates coded CA51 and CB12 for ease of storage and application and for prolonging shelf life.

III. Further studies be done for eliciting the mechanisms of action for *Trichoderma* isolates coded Tricho 7 and Tricho 10 and *B. subtilis* isolates coded CA51 and CB12.

IV. Further studies be done for more accurate identification of *Trichoderma* isolates coded Tricho 7 and Tricho 10.

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APPENDICES

Appendix 1: Analysis of variance for the percent disease incidence

Experimental site	Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	F
Greenhouse	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			
Kabete Field Station	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			
KALRO Mwea	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			

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Appendix 2: Analysis of variance for the percent disease severity

Experimental site	Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	F
Greenhouse	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			
Kabete Field Station	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			
KALRO Mwea	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			

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Appendix 3: Analysis of variance for the percent disease index

Experimental site	Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance ratio	F
Greenhouse	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			
Kabete Field Station	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			
KALRO Mwea	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			

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Appendix 4: Temperature readings during field experimental period in 2019

Month	Kabete Field Station		KALRO Mwea	
	Maximum	Minimum	Maximum	Minimum
April	22.4	16.5	31.3	18.0
May	26.2	15.7	29.0	17.7
June	20.4	14.0	26.2	17.3

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