ABUNDANCE OF TRICHODERMA SPECIES IN DIFFERENT HABITATS AND THEIR EFFICACY IN THE MANAGEMENT OF BACTERIAL WILT OF TOMATO

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UNIVERSITY OF NAIROBI

2022

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This thesis is my original work and has not been presented for award of a degree at any other university.

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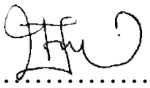
Prof. John W. Kimenju……  ……Date.25 - 08 - 2022……

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D E D I C A T I O N

To my family Mr. Edward Okinda and Mrs. Damaris Okinda, Cedric and Vincent for their relentless support financially and emotionally during the entire journey of the work.

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LIST OF ABBREVIATIONS

FAO	Food Agricultural Organization
CABI	Centre of Agricultural Biodiversity
KARI	Kenya Agricultural Research Institute
EPPO	European Plant Protection Organization
RCBD	Randomized Complete Block Design
CAN	Calcium Ammonium Nitrate
NPK	Nitrogen Phosphorous Potassium
FAME	Fatty Acid and Methyl esters
GLM	General Linear model
LSD	Least Significant Difference
PDA	Potato Dextrose Agar
CFU	Colony Forming Units
PCR	Polymerase Chain Reaction
TZC	Triphenyl Tetrazolium Chloride

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GENERAL ABSTRACT

Tomato (*Lycopersicon esculentum* L.) is a key vegetable in Kenya, listed as second most economically important in the horticultural industry. The production of tomatoes has greatly been affected by bacterial wilt caused by *Ralstonia solanacearum*. Losses to 100% have been reported in both greenhouse and open fields growing conditions. Most of the bacterial wilt management strategies in place have not provided effective, safe and sustainable solution. Therefore, this study contributes, to sustainable tomato production by the use of *Trichoderma* species as an alternative method of managing bacterial wilt. The study determined antagonistic activity of *Trichoderma* species from different habitats against *Ralstonia solanacearum in vitro* and evaluated their efficacy in managing bacterial wilt of tomato at field level.

Trichoderma species were isolated and identified from different soil habitats of Karura forest, compost, manure, coffee, and tomato fields. The dominant *Trichoderma* species were *Trichoderma harzianum* and *Trichoderma asperellum* and antagonistic check performed using dual plate technique against *Ralstonia solanacearum*. The antagonistic ability of the *Trichoderma* species was determined by measuring the growth radius as a percentage. The field experiments were further conducted in a randomized complete design (RCBD) replicated four times in three greenhouses at Naivasha, Mirera area. The treatments included; isolated *Trichoderma asperellum*, isolated *Trichoderma harzianum*, combination of isolated *Trichoderma asperellum* and *Trichoderma harzianum*, plots with no applications, commercial *Trichoderma harzianum*, commercial *Trichoderma asperellum*, combination of commercial *Trichoderma harzianum* and commercial *Trichoderma asperellum*. The isolated *Trichoderma* species were mass multiplied by growing in sterilized sorghum grains. The already infested greenhouse soil was re-inoculated with isolated *Ralstonia solanacearum* to ensure uniform pathogen levels. This was isolated from infected tomato plants and introduced one week earlier at 35 ml per pot and properly mixed to ensure uniformity. *Trichoderma* application was done at the transplanting stage of a greenhouse tomato variety Anna F1, and two more applications after every two weeks. The bacterial wilt incidence and severity assessment was then done weekly and yield data recorded based on physiological maturity of the tomato crops.

The laboratory *in vitro* work indicated that the habitats with high organic matter and fewer disturbances in terms of cultivation had high *Trichoderma* presence. The habitats had a

total of 42 *Trichoderma harzianum* isolates and nine *Trichoderma asperellum*. *Trichoderma harzianum* were 15 and four *Trichoderma asperellum* from forest habitat while three *Trichoderma asperellum* and 10 *Trichoderma harzianum* from compost habitat. The other habitats also had similar *Trichoderma* isolates with low frequency. *Trichoderma asperellum* and *Trichoderma harzianum* from the forest and compost habitats had the highest percentage inhibition *in vitro*. In greenhouse conditions, treatments with *Trichoderma asperellum* or *Trichoderma harzianum* at $P \leq 0.05$ had significant reduction of bacterial wilt incidence and severity as compared to the plots with no applications done. The *Trichoderma* species combinations treatments had no significant difference from the single *Trichoderma* species applications at $P \leq 0.05$. The incidence and severity of *Ralstonia solanacearum* were greatly reduced hence better yields in the *Trichoderma* treated plots. The results indicated that *Trichoderma harzianum* and *Trichoderma asperellum* were efficient in managing bacterial wilt in tomatoes an adoptable alternative management solution to bacterial wilt in tomatoes.

Keywords: Antagonism, Incidence, Habitats, *Ralstonia solanacearum*, Severity, *Trichoderma spp*.

CHAPTER 1:

INTRODUCTION

1.1. Background information

Tomato (*Lycopersicon esculentum*) is the second relevant vegetable crop after potatoes, at 4.85 million ha per year globally (FAOSTAT, 2019). Tomato is an annual plant originating from the South American Andes (Bedassa, Fufa, & Aga 2020; Saleem *et al*., 2013), with reports of the Netherlands and Mexico being the world's leading producers (Costa & Heuvelink, 2018). In sub-Saharan Africa, Kenya is amongest the leading countries in tomato production with 410,033 tones (Ochilo *et al*., 2019), constituting 7% of the total horticultural produce and second-leading vegetable in Kenya (Momanyi *et al*., 2019). In Africa, tomatoes are important food and economic source (Mansour *et al*., 2019; Wafula, Waceke, & Macharia 2018). In Kenya, tomato ranks as the second widely cultivated crop in value and production after potato (Mitra & Yunus, 2018). *Lycopersicon esculentum* is a vegetable grown world wide due to its numerous health benefits (Liu *et al*., 2018) such as its high lycopene content an antioxidant, additionally reduces the chances of Type two diabetes which is related to chronic (Hassan & Barde, 2020) and cardio-vascular diseases (Banihani, 2018). Tomato production has risen over the years in Kenya. However, it suffers losses from biotic and abiotic elements (Nakhungu *et al*., 2021). Biotic factors involve several fungal and bacterial diseases where annual tomato production is restricted by bacterial wilt (Boyaci *et al*., 2021). The disease is known to occur in tomatoes (Kago *et al*., 2019) and other solanaceous crops (Manda *et al*., 2020). Bacterial wilt causes severe economic impact even to the world's big solanaceous vegetables producers like India, Italy, Portugal, Spain, Brazil, Indonesia, USA, Israel, Colombia, China, Kenya, with many more vegetables cultivating countries (Costa & Heuvelink, 2018).

Ralstonia solanacearum, which is a soil-borne pathogen is the causal agent for bacterial wilt (Kumar, & Sood 2021; Siregar *et al*., 2021), which occurs worldwide but is more severe in temperate, equatorial, and subequatorial areas (Yabuuchi *et al*., 1995). It affects beyond 450 species of plants, with most susceptible crops from the solanaceous family (Kurabachew & Ayana, 2017; Lebeau *et al*., 2011; Lee *et al*., 2018). Additionally, the bacterium can manifest itself in over 200 various species of plants, including tomato, eggplant, and tobacco (Hayward, 2006; Tsuchiya, 2014). The pathogen is phytopathogenic bacteria monitored globally owing to its persistence, destructiveness, extensive geographic distribution, and wide host range

(Huet, 2014). Increased soil dampness (−0.5 to −1 bar) (Jiang *et al*., 2021) and high temperature (24 °C to 35 °C) supports the survival, pathogen dispersal, and easier multiplication (Nesmith & Jenkins, 1985).

The soil is the principal origin of inoculum for the pathogen where it could persist to 40 years (Chiranjeevi & Raghavendra, 2021) with temperatures of 20 °C - 25 °C (Denny, 2007). Not with standing *Ralstonia solanacearum* fully loses its viability at 0 % soil wetness after six months. This situation does not crop up in temperate, tropical, and subtropical regions (Singh *et al*., 2015). The dispersal mode is irrigation, infected soil, latently infected weeds, use of contaminated tools, seed materials, and insect vectors (Deberdt *et al*., 2014). The pathogen easily enters plants through wounds and natural openings (Wijayanti *et al*., 2021). Invading the xylem vessels spreading to the other plant parts (Genin, 2010). It multiplies (1010 cells cm^{-1} of the stem) by developing high exopolysaccharides leading to obstruction of vessels and killing the host. Bacterial wilt invasion is also noted to occur at the root level, preceded by the occupation of the roots (Ingel *et al*., 2021). Through the intercellular spaces they access the xylem vessels where high multiplication happens, causing the wilting symptoms and finally, death (Hikichi *et al*., 2017).

In the field conditions, symptoms of the disease occur in the mature tomato plants. The leaves frequently wilt during the day and recover at night or in the early morning hours. If the weather is favorable enough, with high soil humidity and high temperatures, the disease can lead to wilting of the entire plant (Wang *et al*., 2021) and eventually death (Hong *et al*., 2011). Bacterial wilt majorly affects plants starting from vegetative to their fruiting stages. The leaves maintain their green colour, but eventually, the whole plant wilts abruptly in hot and humid climate, conducive for pathogen growth (Singh *et al*., 2015). In the progressive stages, the green nature of the leaves of the wilted plants persists (Zohoungbogbo *et al*., 2021) and the vascular tissues turn to brownish-yellow. In the field, the disease is rampant in the more damp sections nevertheless, plants indicating symptoms of the disease can be found randomly. The plants inffected by *Ralstonia solanacearum* additionally dwarf as a result of insufficient water and inadequate nutrient take-up (Hong *et al*., 2011).

The present integrated management strategies involve; resistant cultivars and germplasm (Ravishankar *et al*., 2021; Pandey *et al*., 2020), soil sterilization (Enfinger *et al*., 1979; Ganiyu *et al*., 2020), crop rotation (Michel *et al*., 1996), grafting techniques (Kaushal *et al*., 2020). Use of coco-peat as growing media (Black *et al*., 2003; Singh *et al*., 2015), irrigation using seawater (Elsas *et al*., 2001) and screening of antagonists (Lwin &

Ranamukhaarachchi, 2006). Planting of pathogen-free transplants (Pradhanang *et al*., 2005), with other crop protection methods. Although, these strategies have demonstrated to be insubstantial because of the complicated nature of soil-borne pathogens, expansive host range, extensive distribution of *Ralstonia Solanacearum* (Hayward, 2006). The development of resistant cultivars has been limited to averagely tolerant cultivars which are defined by location, climate, and resistance to strains of the pathogen (Adhikari *et al*., 2020; Chaudhary *et al*., 2021). Transplants can reduce the dispersal of the bacterium, but because it is a soil-borne pathogen, majority of the crops in the field can still be infected. Use of crop rotation can be complicated owing to the diverse host range of *Ralstonia solanacearum* strains, and that the pathogen can live or colonize various types of weeds (Hayward, 2006).

The management of bacterial wilt is hence challenging from the methods suggested (Mamphogoro *et al*., 2020) and that are widely used to manage the disease. The insubstantial effectiveness of the current management strategies warrants alternative methods to manage the disease (Aguk *et al*., 2018). Vast studies have therefore commenced on the use of biological control agents in managing plant disease. Biological control agents are soil microorganisms that occur naturally whose mode of action include initiation of host resistance through the release of plant growth stimulating hormones (Haidar *et al*., 2016). Additionally, they use competition of nutrients, parasitism, antibiosis and cell wall degrading enzymes. Numerous studies have been carried on the use of BCAs in management of plant diseases these include the use of *Bacillus spp, Trichoderma spp* and many more (Al-Ani, 2017; Konappa *et al*., 2018; Wang *et al*., 2021). Therefore the need to evaluate the performance of *Trichoderma spp* from different native habitats against *Ralstonia solanacearum*.

1.2. Problem statement

The production of tomatoes is challenged by various factors worldwide, including living and non-living factors (Gharbi *et al*., 2017; Zhou *et al*., 2019). In Kenya, biotic factors have a major economic impact on tomato production, consisting of pests, fungal, bacterial, and viral diseases (Ochilo *et al*., 2019) where bacterial wilt is of concern. Bacterial wilt has been observed to be endemic in different areas in Kenya, including Kirinyaga, Kiambu, Bomet, Kajiado areas (Kago *et al*., 2019; Kones *et al*., 2020) Nakuru, Muranga, and Nyandarua counties. The disease can lead to up to 100% loss of the whole crop (Kamuyu,

2017; Mbaka *et al*., 2013; Rivard & Louws, 2008). These resulting in low income from the growing of tomatoes due to reduced productivity of the crop (Onduso, 2014).

Most of the growers of tomatoes have resorted to abandoning their greenhouses and fields stopping farming activities of growing tomatoes and crops susceptible to the disease for a long time (Kamuyu, 2017; Mbaka *et a*l., 2013). There are limited conventional solutions for management of bacterial wilt as they have been banned due to their non-biodegradability in the environment (Aguk *et al*., 2018) such as Methyl bromide. The other methods used in managing bacterial wilts such as grafting, crop rotation and other cultural practices have additionally not given satisfactory results. This therefore warrants the need for sustainable, effective, and safe methods to be utilized in the management of bacterial wilt of tomatoes. These issues have greatly affected all those involved within the production and consumption chain of tomatoes. Additionally, this impacts negatively on the economical aspect of the society at large and Kenya's food security.

1.3. Justification of the study

In tomato production, bacterial wilt is the commonest disease for both open field and greenhouse setup (Kago *et al*., 2019). The management of *Ralstonia solanacearum* has been difficult as the pathogen has proven to persist for duration in the soils and wide geographical distribution (Mihovilovich *et al.,* 2017). The strategies in managing bacterial wilt over the years have included the use of disease-free planting materials or tolerant varieties; crop rotation, and chemical use. Disease-free planting materials involving grafting to more tolerant tomato varieties have been reported to manage bacterial wilt (Alividza, 2019), although a costly method to a small-scale farmer. Tolerant tomato varieties that have been tried still show different levels of susceptibility to the pathogen (Michael *et al*., 2020), not giving the farmer proper tolerance against the disease. Crop rotation has been observed to be ineffective as the pathogen can endure and survive in the soils for a long time (Jiang *et al*., 2017; Mihovilovich *et al*., 2017; Yang-Xian *et al*., 2015). Chemicals are known to affect bacterial wilt however, they are very few (Aguk *et al*., 2018) and have become ineffective due to overuse (Shiva *et al*., 2018). Therefore, chemicals as a management strategy are insufficient (Namisy *et al*., 2019) and not sustainable. Increased use of chemical techniques involving bactericides have been reported (Marian *et al*., 2018) but have been seen to cause negative consequences on the surroundings and human health (Satapute *et al*., 2019). Reports have also shown that certain chemical molecules pose a high risk to the environment once

they start undergoing the degradation process (Kumar *et al*., 2020; Sharma *et al*., 2020), whose results are detrimental. They are greatly being placed in reduced microbial life due to microbial degradation (Tudi *et al*., 2021) and pollution in the environment (Warra & Prasad, 2020). The handlers and users who are the farmers are also at risk as some of the pesticide formulations have heavy metals that are lethal to human health (Dhananjayan *et al*., 2020). Therefore the need for alternative safe, sustainable, and effective methods in controlling bacterial wilt of tomatoes (Morais *et al*., 2019). According to Kumar (2017), (BCAs) have been used as antagonistic plant pathogenic agents. These BCAs exhibit characteristics that involve self-sustaining ability, reduced input of non-replenishable resources, scattered across after the first establishment, and provision of continuous disease suppression (Whipps, 2007). Research has shown that combining BCAs like *Trichoderma species* and *Bacillus* in the management of *Ralstonia solanacearum* gives promising results (Kariuki *et al*., 2020; Konappa *et al*., 2018). Therefore, the need for use microbial-based pesticides, which are deemed more sustainable and safer, as an alternative solution in managing the disease (Todorović, 2017). This ensures the farmers' safety during handling and application of the biological control agents in regards to their health (Abd-Elgawad, 2020), is cost-effective (Bhusal & Mmbaga 2020; Messmer *et al*., 2021), and safe to environmental microbial life (Kumari *et al*., 2020) hence this study.

1.4. Objectives

The general objective of the study was to contribute to effective management of bacterial wilt of tomato by the using *Trichoderma* species.

The specific objectives were

i. To determine the abundance of *Trichoderma* species from different habitats and their antagonistic activity against *Ralstonia solanacearum in vitro*.
ii. To evaluate efficacy of *Trichoderma* species in managing bacterial wilt of tomatoes.

1.5. Hypothesis

The following hypotheses were to be investigated in this study.

i. Native *Trichoderma* species are not abundant in soils and have no antagonistic activity against *Ralstonia solanacearum in vitro.*

ii. Native *Trichoderma* species have no effects on bacterial wilt incidence and severity in field conditions.

CHAPTER 2:

LITERATURE REVIEW

2.1. History of tomato production in Kenya

The available data shows, tomato as the second vegetable after potato in Kenya, accounting for 14% of the total production of vegetables (Mwangi *et al*., 2020) and globally the second important commercial vegetable crop (Costa & Heuvelink, 2018). Tomato production in Kenya has also increased to over 410,033 tones (FAOSTAT, 2019), with Kenya among the leaders in its production in sub-Saharan Africa. Tomato production over the years in Kenya has been in the open field, but this has changed with the small-scale farmers adopting of producing the crop. This is done in protected environments (Sanzua *et al*., 2018). 85% of production is from open fields, while greenhouse technology covers up to 15% of tomato production and still growing (Wafula *et al*., 2021).

Tomato (*Solanum lycopersicum L. syn. Lycopersicon esculentum* Mill.) is in the Solanaceous family, which contains many important food crops, including potatoes (Quinet *et al*., 2019). The crop is generally a perennial, although some regions are grown as annuals (Waheed *et al*., 2020). In Kenya, production is both for local and export (Chemeltorit *et al*., 2018) due to increasing demand for fresh consumption and processing to maintain livelihood (Orwa *et al*., 2019). Tomatoes in Kenya are generally grown in areas with altitudes ranging between 1150 and 2000m above sea level (Akoko *et al*., 2020), with Kirinyaga leading in production. There is currently a wide variety of tomatoes being grown (Costa & Heuvelink 2018; Enciso *et al*., 2019).

The types of tomatoes grown in Kenya are the determinate type characterized by bushy appearance with flowers produced at almost every internode until terminal buds are formed and mainly grown in the open fields (Kubai, 2017). The indeterminate types are characterized by continuous growth, almost indefinitely producing flowers at every third internode (Maina, 2020; Ochilo, 2019), and require staking and pruning. The establishment of tomatoes can be done by seeds or transplants (Finch-Savage, 2020; Pill, 2020), depending on the farmers' preference (Kithome, 2019). The most commonly grown indeterminate varieties in Kenya include Tylka F1, Anna F1, Money maker, Corazon F1 while the determinate varieties are Cal J, Rio Grande, Kilele F1, Shanty F1, Assila F1, Eden F1 and Rambo F1 (JICA, 2016).

2.2. Biotic challenges to tomato production in Kenya

The production of tomato crop faces several challenges, including pests and diseases (Wayua *et al*., 2020). Tomatoes are also affected by climatic conditions (Samuel & Orji, 2015), with prevalence experienced in Kiambu, Kajiado, Laikipia, and Kirinyaga counties (Odoyo, 2016). The major insect pests on tomatoes are African bollworm (*Helicoverpa armigera*), the red spider mites (*Tetranychus spp*), whiteflies (*Bemisia tabaci*), thrips (*Ceratothrip oidesbrunneus*), and *Tuta absoluta* (Zeist *et al*., 2018) where yield losses from the same pests are pretty high (Dent & Binks, 2020). Research has shown that tomato production greatly suffers from soil-dwelling pathogens (Manickam *et al*., 2019). The diseases identified to reduce production are late, early blight by fungus *Phytophthora infestans* and *Alternaria solani* (Blancard 2019; Fuentes *et al*., 2017), and bacterial wilt positioned as second most destructive amidst the species of bacteria to solanaceous plants (Mansfield *et al*., 2012).

Bacterial wilt in Kenya has been endemic in different areas, including Kirinyaga, Kiambu, Bomet, and Kajiado (Kones *et al*., 2020). The pathogen has persisted in the soils and with a wide geographical distribution (Mihovilovich *et al*., 2017), making its management difficult (Jiang *et al*., 2017). This has affected tomato production in greenhouses and outdoors setup (Ireri *et al*., 2019). In Kenya while indoor production of tomatoes ensures continuous supply throughout, the losses from bacterial wilt have been reported up to 100% in greenhouses and 64% in the fields (Mbaka *et al*., 2013), this attributed to the provision of optimal conditions for swift multiplication of pathogens (Buschermohle & Grandle, 2002).Earlier, bactericides like streptomycin were known to be effective against bacterial wilt, which is no longer the case since high quantities were required for effectiveness (Xue *et al*., 2009). The conventional methods that were thought to be effective have lost their effectiveness over time (Aguk *et al*., 2018), making its management more challenging. Other challenges countered by the growers are the uncoordinated and unorganized marketing, exploitation from middlemen, and poor production planning causing oversupply and thus low prices (Mutwiri, 2019).

2.3. Requirements for tomato production in Kenya

Tomato grows best in warm temperatures with much light. In Kenya, the growing altitude ranges from 1150 to 2000m above sea level (Anastacia *et al*., 2011). Tomatoes require deep medium-textured sandy loam or loam soils that are well-drained and fertile for

optimum growth (Drost, 2020). They are usually produced in well-drained soils with high organic matter and pH of 5 to 7.5 (Wiersinga & de Jager, 2008). They require up to 4 months of clear and warm temperatures 21°C to 27°C (Coolong & Boyhan, 2017) to maintain proper fruit set this can vary to 22°C – 25°C (Shamshiri *et al*., 2018). Temperatures that are lower than 15°C or higher than 35°C and night temperatures above 21°C, are damaging to fruit setting, inhibiting the color formation and ripening (Laxman *et al*., 2018). The water requirement ranges from about 400mm to 600mm evenly distributed over the growing period (Coolong & Boyhan, 2017) as too much water results in damping-off and too little water affect growth.

Planning how this continuous watering is done is vital for the final crop yield (Du *et al*., 2018). Balanced fertilizer regimes are required during the growth of this crop to ensure maximum yield incorporated with inorganic manure (Mallory *et al*., 2020). Tomatoes are mainly grown in the open field, but lately, the adoption of greenhouse technology has increased production indoors (Geoffrey *et al*., 2014). The pest and disease management by using synthetic chemicals has resulted in increased cost of production, are environmentally unsafe and resistance build up (Husin 2017). Therefore need to adopt alternative pest and diseases management strategies which are environmentally friendly.

2.4. Bacterial wilt in tomatoes

2.4.1. The occurrence of bacterial wilt in Kenya

Ralstonia solanacearum has a vast host range with affected crops of commercial value in Kenya involving potato and tomato (Iderawumi & Yusuff, 2020). In Kenya, the bacterial wilt endemic areas include Kiambu, Kajiado, Kirinyaga and Bomet (Kones *et al*., 2020). Additionally, the disease is also rampant in the main potato growing areas of Meru, Nakuru, Narok, Trans Nzoia, Uasin Ngishu and Nyandarua in Kenya (Moses *et al*., 2021). The disease has resulted in crop losses of 50-100% in Embu and Mau Narok in potatoes (Iraboneye *et al*., 2021), moreover in tomatoes similar losses of 33-99% in Nyandarua (Oluoch *et al*., 2021), Kirinyaga and other areas in the Kenyan highlands with 100% tomato crop loss (Kago *et al*., 2016) with Kiambu and Bomet recording 100% crop loss (Aoko *et al*., 2021). The symptoms associated with the disease being wilting and death (Manda, Addanki, & Srivastava 2020; Nayiga 2021; Yang *et al*., 2021) of the crop before attaining maturity, with no harvestable yield or poor fruits (Sadashiva, 2020; Sood *et al*., 2021). In open fields continuous cropping of plants of the same family is normally done resulting in the

accumulation of *Ralstonia solanacearum* in the soil, which with no proper management causes bacterial wilt disease to manifest in the cultivated crops (Zheng *et al*., 2020). The low soil acidity levels in open fields, influences the occurrence of bacterial wilt (Tafesse *et al*., 2021). The highest losses in production are however experienced in greenhouses due to limited knowledge of bacterial wilt (Aloyce, Ndakidemi, & Mbega 2019; Wayua *et al*., 2020) and poor identification and implementation of the bacterial wilt protocols in greenhouses (Manda *et al*., 2020). Bacterial wilt spreads faster due to suitable environment (Mwaniki *et al*., 2017) limiting crop production once the pathogen infestation occurs.

2.4.2. The causal agent of bacterial wilt of tomatoes

Ralstonia solanacearum is the pathogen causing bacterial wilt. The bacterium is classified as the world's most critical phytopathogenic bacteria (Lu *et al*., 2016). It is gram-negative on the KOH test (Álvarez *et al*., 2021) and a nonsporing aerobic plant pathogen (Hayashi *et al*., 2019) which is rod shaped with polar tuft flagella. *Ralstonia solanacearum* single colonies characteristics when plated on Triphenyl tetrazolium chloride have round shaped colonies with pink to red centers (Balamurugan *et al*., 2020; Seleim *et al*., 2014). On Kings B medium *Ralstonia solanacearum* is non-fluorescent and forms cream colonies on yeast extract dextrose-calcium carbonate medium a major characterization method (Álvarezet *et al*., 2021). In the species-specific primers, where molecular markers are used the *Ralstonia solanacearum* proteins in the CSIs position have high homologues having multiple sequence alignment. Their positions flank on both sides at 5 - 6 conserved positions attached to this bacterium (Etminani *et al*., 2020). Further molecular identification is by PCR amplification which clearly shows the hrpB gene of *Ralstonia solanacearum* differentiating it from other bacteria (Hossain *et al*., 2021).

Radhi *et al*. (2016) and Hayward, (1994) reported that the pathogen causes tomato yield losses of nearly 100% globally and distinctly in tropical and subtropical areas and warm temperate regions according to Du *et al*., 2018 and Kelman (1998). Globally it has posed a threat to food security due to severe crop loss (Ravelomanantsoa *et al*., 2018). The pathogen affects over 450 species belonging to 54 different families, with the impact higher on the solanaceous crop (Kurabachew & Ayana, 2017). This affects different varieties of tomatoes (Afroz *et al*., 2009) and other hosts which include; *Capsicum annum* (sweet pepper), *Solanum tuberosum* (potato), *Solanum melongena* (Brinjals), *Nicotiana tabacum* (tobacco), *Arachis hypogaea* (groundnut), *Musa paradisiaca* (banana) and *Heliconia* spp (plantain) (Lopes and

Rossato, 2018; Lowe-Power *et al*., 2018). The pathogen is grouped in different races; Race one has a wide host range that is endemic to Africa, South America, Asia, and the United States. Race two is mainly found in Central America and Southeast Asia and has been known to attack bananas. Race three has its distribution worldwide, affecting potatoes. Race four in Asia and Hawaii are affecting ginger, finally, Race five in China mainly affecting mulberry (Denny, 2007). Alternatively, the pathogen is classified as (I): Asia (II): America (III): Africa (IV): Pacific (Lowe-Power *et al*., 2018). *R solanacearum* has further been classified taxonomically into infra-sub specific classification (Zou *et al*., 2017).

In Africa, it is found in Angola, Burkina Faso, Burundi, Cameroon, Congo, Ethiopia, Gabon, Gambia, Kenya, Madagascar, Malawi, Mauritius, Mozambique, Nigeria, Rwanda, Senegal, Sierra Leone, Seychelles, Somalia, South Africa, Swaziland, Tanzania, Tunisia, Zaire, Zambia, Zimbabwe and Uganda (PHYLOTYPE II, 2017). The bacterium can survive in contaminated plants, susceptible weed hosts, volunteer crops, and infested soil (Hayward, 1994). Bacterial wilt causal pathogen under the Agricultural terrorism Act of 2002 is a quarantine pest (Pal *et al*., 2019). Pathogen isolation can be done from infected plant parts, soil, and waste materials (Alamer *et al*., 2020; Gutarra *et al*., 2017).

Once the bacterium infects a plant through root wounds or natural openings (Xue *et al*., 2020), it initiates very fast establishment, especially at the plant's root system, before it becomes systemic, with the typical shoot symptoms (Lowe-Power *et al*., 2018). The plant begins to show wilt symptoms by wilting the youngest leaves as the first symptoms, but the leaves remain green, usually during the day's hottest periods (Jiang *et al*., 2017). The stem near the affected root produces many adventitious roots, and freshly cut sections obtained from infected stems exude milky white substance with vascular discoloration (Swanson *et al*., 2007) with browning colours (Genin, 2010; Zinnat *et al*., 2018). The change in colour of the vascular system from light yellow to brown is also observed (Harveson *et al*., 2015). When there is an excessive infestation of the cortex by the pathogen, permanent wilting occurs, and the plant dies in three to four days (Mihovilovich *et al*., 2017).

2.4.3. Epidemiology of bacterial wilt of tomatoes

The pathogen infection is initiated through natural openings of wounds, on the roots, these are usually formed during lateral root emergence, while other wounds result from root damage caused by organisms in the soil (Lowe-Power *et al*., 2018), transplanting, stem injuries at cultivation or insects (Tahat & Sijam, 2010). The pathogen is dispersed through

infected plants, which include vegetative propagated plant material where the pathogen survives over two to three years within the vegetative organs (Coutinho, 2005), inactively infected planting material, and contaminated irrigation water (Hayward, 1994). The pathogen sources of inoculum are from different soils where it persist for years subject to soil type, cultural practices, moisture content, amendments (Nion & Toyota, 2015), water (Fajinmi & Fajinmi, 2010) since it can also survive in drain water (Stevens *et al*., 2018), irrigation water that has come into contact with bacteria from plant roots becomes a source of inoculum (Saddler, 2005) and human or machinery contact (Choudhary *et al*., 2018).

Ralstonia solanacearum is a flagellated bacterium aiding its mobility. The pathogen on gaining access to plant roots through stimuli penetrates through the natural wounds and further attaches itself to the root extension zones. The pathogen produces enzymes damaging the plant cell walls enabling it to inhabit the intercellular cell regions and feed. Once the bacterium gets to the root cortex it results in the formation of large intercellular pockets (Álvarez *et al*., 2021). On permeating the endodermis, the vascular bundles through the secondary roots' axils are invaded (Rakha *et al*., 2020). The parenchymal cells are then damaged and the pathogen moves to the apex of the plant (Rakha *et al*., 2020). It then multiplies inhibiting the xylem fluids movements resulting in clogging further wilting and death of the plant (Nguyen & Ranamukhaarachchi, 2010). After the death of the plant the pathogen lives saprophytically until contacts another host (Nguyen & Ranamukhaarachchi, 2010).

Ralstonia solanacearum still spreads to neighbouring plants through root contact which it is also considered as alternate hosts (Wenneker *et al*., 1999). The pathogen other plant hosts include tobacco (*N tabacum*) (García-Rodríguez & Thiessen, 2020), bananas (*Musa paradisiaca)* (Ocimati *et al*., 2018), ginger (*Zingiber officinale*) , beans (*Phaseolus vulgaris*) and nearby plant weeds (Osdaghi *et al*., 2020; Prameela & Suseela Bhai, 2020) The pathogen is usually common in the hot and humid regions of the world, surviving a wide range of temperatures 15- 37°C and cannot survive in less than 10°C (Elsas *et al*., 2001). The pathogen grows within a pH range of 5.2 - 7.4 and prefers mainly acidic to slightly alkaline soils (Satyaprakash *et al*., 2020).

2.4.4. Management of bacterial wilt in tomatoes

Various approaches have been adopted over the years for the management of bacterial wilt in tomatoes. Introduction of bacterial wilt resistance tomato rootstocks has been

researched and observed to give resistance in soils that had high bacterial wilt (Suchoff *et al*., 2019). The tomato varieties of choice are grafted on hybrid bacterial wilt-resistant rootstocks enabling them to grow on infested soil and give good yields (Ganiyu *et al*., 2020). The technique of grafting susceptible tomato varieties on resistant tomato rootstocks according to (Nakaho, 2021) yields good results in managing the disease. The use of eggplant rootstocks in grafting tomatoes has also given good results in manageming bacterial wilt in tomatoes (Manickam *et al*., 2021; Rakha *et al*., 2020). The eggplant has been used as a resistant rootstock has been widely researched making grafting a good management alternative in managing bacterial wilt (Kumbar *et al*., 2021). Research work done by Pandey *et al*. (2020) also supports good results in managing bacterial wilt in tomatoes by grafting on a resistant rootstock this concurs with Mamphogoro *et al*. (2020) and Shweta *et al*. (2021), although a slightly expensive method to farmers it is a good management method (Maurya *et al*., 2019). More resistant tomato varieties are coming up from the continuous work done by breeders more (Ramesh *et al*., 2021; Thies 2021).

The use of crop rotation has also been adopted as a bacterial wilt management strategy. This involves the planting of plants from different families in the same land after a tomato cropping season (Li *et al*., 2019). Crop rotation when done for a period of more than 2 years can reduce bacterial wilt incidences (Gonçalves *et al*., 2021). However, the pathogen can still persist (Ramesh *et al*., 2021) in the soil for more than 10 years. Proper farm hygiene minimizes the pathogens inoculum from spreading. The disinfection of farm implements should be adhered to reducing the pathogens introduction during cultural activities (Haile *et al*., 2020). This additionally is achieved by removal of crops showing disease symptoms, burying or burning of plant residues in one place (Belete *et al*., 2021). Further studies have indicated that strains of *Pseudomonas solanacearum* that are avirulent isolated from *Sterizia reginae* have reduced bacterial wilt spread (Moon *et al*., 2021). There are possibilities of *Pseudomonas solanacearum* having anatagonistic activity on the pathogen *Ralstonia solanacearum* (Moon *et al*., 2021; Nguyen *et al*., 2021).

Solarization of bacterial wilt infested soil, improves the structure of the soil allowing for plant growth (Jibat & Alo, 2020). It has also been used in combination with fumigants for better results (Panth *et al*., 2020). Unfortunately, the fumigants have had notable negative impacts on the environment these include methyl bromide and chloropicrin (Shen *et al*., 2021). Solarization solely dependent on the sun thus can time consuming for a farmer but with good results as reported by Manda *et al*. (2020) and Mamphogoro *et al*. (2020) in managing bacterial wilt in tomatoes (Iraboneye *et al*., 2021). This technique greatly relies on

the climatic conditions which would allow for solarization to occur (Dai *et al*., 2020). The incorporation of bio fertilizer has been used as a management method on bacterial wilt (Dong *et al*., 2020). These have been able to amend the soil nutrient content thus suppressing the disease (Zheng *et al*., 2020). The soil nutrient balance has also been incorporated with soil fumigations to give better results (Deng *et al*., 2020).

These have been done in form of organic amendments from composting matter (He *et al*., 2020) that increases the soil pH and EC which further improve nutrient uptake (Chen *et al*., 2020; Gao *et al.,* 2019; Gutarra *et al*., 2017). The use of chemicals that are less lethal like silver nanoparticles, magnesium oxide nanoparticles has indicated managing bacterial wilt in tomatoes (Santiago *et al*., 2019). A larger category of the available chemical solutions are pollutants and harmful to human health but are able to manage the disease (Ali *et al*., 2021; Li *et al*., 2021; Ravikumar *et al*., 2021). This has brought the need for sustainable, safe and sustainable solutions hence further research on biological control agents as an alternative solution (He *et al*., 2021; Singh & Kesharwani 2021).

2.5. Biological control agents used in crop protection

2.5.1. The biological control agents of pests and diseases

The biological control of plant diseases involves suppressing plant pathogen populations by living organisms (Brodeur *et al*., 2018). These BCAs are also described as living organisms that can reduce plant pathogen density (O'Brien, 2017). This has increased due to the need for environmentally friendly alternatives to chemicals (Rahman *et al*., 2018) and has become an essential part of sustainable agriculture (Niu *et al*., 2020). Research has shown that biological control assesment involve use of antagonistic fungal and bacterial agents to control pests and diseases (Mandal *et al*., 2017). Their antagonistic nature has been exploited (Konappa *et al*., 2018) on crops in the solanaceous family (Kumar, 2017) against bacterial wilt. Biological control agents used against plant diseases act more as antagonists dwelling in various parts of the plants, causing positive effects (Stack *et al*., 2020).

Plant disease suppression by biological control agents typically comes from the antifungal compounds they produce and their competitive colonization (Li *et al*., 2013). Research has shown that biological control of plant diseases is possible by using the agents controlling plant diseases (Meena, 2018). Several antagonistic biological control agents have been studied in the management of wilt disease in many crops, mainly *Bacillus subtillis*, *Trichoderma* species (Geoffrey *et al*., 2014; Sundaramoorthy & Balabaskar 2013). Other

biological control agents that have shown effects on plant disease include *Pseudomonas fluorescens, Pseudomonas. Bacillus* species like *Bacillus amyloliquefaciens, B. coagulans, Bacillus spp, B. licheniformis, B. pumilus, B. subtilis* and *B. vallismortis* (Nguyen & Ranamukhaarachchi, 2010; Mai *et al*., 2011).

Use of nonpathogenic *Fusarium spp, Petriella spp, Aspergillus spp, Gliocladium spp, Enterobacter spp, Lysobacter spp, Streptomyces spp* and *Pantoea spp* have been noted as key biological control agents of diseases (Arjona-Girona & López-Herrera, 2018; Stenberg *et al*., 2021).The BCAs classified as endophytes also have a wide fungal diversity whose mode of action is by either growing locally, systemically or into the host without causing visible symptoms of the disease existing in every habitat. Among them is *Sarocladium strictum* which has been observed to reduce sporulation and hyphal growth of *Helminthosporium solani*. Additionally leaf necrosis caused by *Phytophthora spp* have also been reduced on cocoa seedlings on inoculation with endophytes (De Silva *et al*., 2019; Wijekoon & Quill, 2021).

Sclerotinia homoeocarpa causing dollar spot disease has been reduced by an endophyte *Epichloe festucae* on tuff grass (Fernando *et al*., 2021).*Trichoderma* species have controlled plant diseases (Al-Ani, 2017) bacterial wilt included (Yuan *et al*., 2016). *Trichoderma* species such as *Trichoderma viride* has been used in suppressing rhizome rot of ginger (Tripathi & Singh, 2021). *Trichoderma harzianum* and *asperellum* have been used to suppress fusarium wilt in tomatoes, French beans and capsicum (Kumar *et al*., 2022). *Trichoderma aggressivum f. europaeum* suppresses the growth of *Fusarium solani f. cucurbitae, Sclerotinia sclerotiorum, Botrytis cinerea, Mycosphaerella melonis, Pythium aphanidermatum* and *Rhizoctonia solani* (Sánchez-Montesinos *et al*., 2021) hence key biological control agents (Vinale *et al*., 2008) with good effects (Yedidia *et al*., 2003) on plant diseases.

2.5.2. *Trichoderma* species in management of plant diseases

Trichoderma species are among the biological control agents used against soil borne pathogens (Elshahawy *et al*., 2017). This has been attributed to their capability to compete for resources and space, antibiosis effect, and mycoparasitism (Verma *et al.*, 2018). These unique characteristics have resulted in more work on *Trichoderma* species to manage plant diseases (Gupta *et al*., 2014; Konappa et al., 2020). *Trichoderma* species are abundant in the soil (De

Medeiros *et al*., 2017) as part of their natural habitat (Jangir *et al*., 2018). According to Sharma *et al*. (2019), species of the *Trichoderma* genus are cosmopolitan (Jiang *et al*., 2017) in soils, herbaceous litter, and decaying wood from which they can be isolated (Howell, 2003). Their abundance in the soil is attributed to diverse metabolic capabilities and aggressive competitiveness (Elad, 2000). They are also known to be fast-growing saprophytes hence comprising 3.1 and 15% of total fungal propagules from forest and pasture soils, respectively (Hagn *et al*., 2003; Kubicek, 2012). The biocontrol agents of plant pathogens are also known as Plant Growth Promoting Fungi (Demain & Fang, 2000; Tucci *et al*., 2011). They have therefore been broadly researched for their capability to enhance plant growth, provide wide antagonistic activities against various soil-borne pathogens, while stimulating plant disease resistance against pathogens (Benítez *et al*., 2004; Gottel *et al*., 2011).

Fusarium oxysporum in Kenya affects many crops which include tomatoes, sweet potatoes, bananas, capsicum, legumes and melons. It results in chlorosis, wilting, necrosis, stunting, premature leaf drop, damping-off and browning of the vascular system. In these crops this has been managed by the application of *Trichoderma harzianum*, *Trichoderma asperellum* and *Trichoderma piluliferum* (Monda, 2002). The use of these *Trichoderma* species has been adopted in Kenya, Ethiopia, Egypt and Nigeria as management method for the disease (Doley *et al*., 2019; Gatahi, 2020; Olowe *et al*., 2022). Late blight in potato has been managed by the application of *Trichoderma asperellum* on the seeds (Agong, 2021; Kilonzi *et al*., 2020). Additionally, *Trichoderma viride* and *Trichoderma harzianum* have been used in managing the disease (Monjil *et al*., 2021; Purwantisari *et al*., 2018). Fusarium wilt in strawberry has also been managed by the use of *Trichoderma harzianum* and *asperellum* (Sonkar, 2019). The grey mold (*Botrytis cinerea*) of strawberries has additionally been managed by application of *Trichoderma harzianum* (Macharia, 2022) and *Trichoderma asperellum* (Wambui, 2021). Tomato late blight management has been achieved through application of *Trichoderma asperellum* while fusarium wilt on the same crop has been managed by *Trichoderma harzianum* (Kilonzi *et al*., 2020; Mbuthia *et al*., 2019).

Over the years, the application of *Trichoderm*a species has been used in controlling a wide range of soil-borne and foliar diseases in vegetables and industrial crops (Ha, 2010; Tran, 1998), mainly *Trichoderma harzianum, Trichoderma asperellum* and *Trichoderma atroviride*. Various factors have caused the difference in their abundance in various soil and geographical regions; microclimate, substrate availability, and complex ecological interaction

(Hoyos-Carvajal & Bissett, 2011; McMullin *et al*., 2017; Oskiera *et al*., 2017). *Trichoderma atroviride* is a common component of Biocontrol formulations used in plant production (Coninck *et a*l., 2020; Macías-Rodríguez *et al*., 2018). The main commercial strains in Kenya are *Trichoderma harzianum* (Kiriga *et al*., 2018; Mbuthia *et al.*, 2019) and *Trichoderma asperellum* (Kilonzi, Mafurah & Nyongesa 2020; Mutuku *et a*l., 2021) have shown ability in managing various plant pathogens. However, the need for isolation of native *Trichoderma* is required due to better colonization and adaptation of the local isolates to the local environment (Chen *et al*., 2019). The *Trichoderma* species also survive as chlamydospores in unfavorable conditions, making them relatively resistant to the commonly available fungicides (Peccatti *et al*., 2019). This, therefore, necessitates the need for the evaluation of native *Trichoderma* isolates in managing bacterial wilt caused by *Ralstonia solanacearum* in tomatoes.

CHAPTER 3:

ABUNDANCE OF ANTAGONISTIC *TRICHODERMA* SPECIES IN SOILS FROM DIFFERENT HABITATS

Abstract

Trichoderma species are filamentous group of fungi universally found in soils with decomposing residues and plant roots. They have high mycoparasitic activity and competitiveness enabling them to inhabit different habitats. *Trichoderma* species modify environmental conditions to their favour compete for space and nutrients, exhibit plant defensive mechanisms which promote plant growth. Bacterial wilt is responsible for the loss of yield in solanaceous crops reducing production of the tomato crop thus the need for more alternative methods to manage the disease. The research determined *Trichoderma* species antagonistic activity of from different habitats, *in vitro* against *Ralstonia solanacearum*. The three undisturbed habitats included forest, compost, and manure soils with 2 disturbed habitats that included coffee plantation and tomato field. Using grid soil sampling technique, each of the five sites was subdivided into four quadrants in RCBD. Representative samples of individual soils underwent tenfold serial dilution and pour plate technique used to culture on potato dextrose agar. Cultural and morphological *Trichoderma* spp identification was done by color observation, microscopic distinction of the phialides and conidia shapes and their Colony forming units (CFUs) calculated. *Ralstonia solanacearum* was isolated from infected tomato plant and cultured on 2, 3, 5-triphenyl tetrazolium chloride (TZC) media. *In vitro* screening for antagonism was done by dual plate method on PDA media and incubated at 18°C – 23°C. Total of fifty- one *Trichoderma* spp were isolated from the collected soil samples with *Trichoderma harzianum* and *T asperellum* dominating. *Trichoderma* spp populations were highest in the undisturbed habitats at 5.9×10^5 CFU/g of soil in compost and 5.5×10^5 CFU/g of the forest soil sample as compared to the disturbed habitats of coffee at 3.6×10^5 CFU/g of soil and tomato 2.25×10^5 CFU/g of soil. *Trichoderma* spp frequency was highest in forest and compost soils with *Trichoderma harzianum* and *Trichoderma asperellum* being the most common species. Among the *Trichoderma* spp isolated, the most antagonistic on *Ralstonia solanacearum* were *Trichoderma harzianum* and *Trichoderma asperellum* from the undisturbed habitats. *Trichoderma* species were recommended for further evaluation in the field set up.

Keywords: Bacterial wilt, Habitats, Populations, *Ralstonia solanacearum*, *Trichoderma*

3.1. Introduction

Tomato (*Lycopersicon esculentum*, Mill.) is consumed globally, with a production of 4.85million Ha (FAOSTAT, 2019) and has adversely been affected by bacterial wilt *Ralstonia solanacearum* (Vasconez *et al*., 2020). The scope of losses in greenhouses and open field setup has been relatively high (Mamphogoro *et al*., 2020; Rodrigues *et al*., 2018). The losses having negatively affected tomato production, have adversely resulted in low income from the growing of tomatoes (Onduso, 2014), which is deemed one of Kenya's sources of income.

Over the years, various management practices have been carried out, including pathogen-free transplants, use of crop rotation and resistant cultivars (Svetlana *et al*., 2017; Stella *et al*., 2020). The chemical management strategy has been widely researched but has been observed to have adverse effects on the environment (Tudi *et al*., 2021), human health (Dhananjayan *et al*., 2020), high-cost implications and ineffective with overuse (Aguk *et al*., 2018). These necessitated new strategies for management of bacterial wilt currently involving, use of biological control agents as part of crop protection regimes (Konappa *et al*., 2020; Mohammed, Oloyede & Odeseye 2020). The use of actinobacteria have shown great potential in the management of bacterial wilt, for example, *Bacillus subtilis* in controlling bacterial wilt in tomatoes (Peng *et al*., 2017). Additionally under greenhouse conditions *Trichoderma asperellum* against *Ralstonia solanacearum* and other *Trichoderma* spp (Guo *et al*., 2021; Konappa *et al*., 2018; Kouabenan *et al*., 2020). Various studies indicate that bacterial wilt can be managed using a consortium of biocontrol agents (Sood *et al*., 2021).

In Kenya, different microbial fungi are available commercially and have been reported helpful in managing plant pathogens; these include *Trichoderma asperellum* and *Trichoderma harzianum* (Kariuki *et al*., 2020; Patkowska *et al*., 2020). These exist in several different formulations commercially within the country. The need to isolate native *Trichoderma* spp was key due to their adaptability and colonization characteristics for the Kenyan environment (Tegene *et al*., 2021). This study assessed the efficacy of isolated *Trichoderma* from different soil habitats for their antagonistic activity in management of bacterial wilt of tomatoes. Further testing the hypothesis that native *Trichoderma* species have no antagonistic activity against *Ralstonia solanacearum in vitro*.

3.2. Materials and methods

3.2.1. Characteristics of habitats sources of the *Trichoderma* isolates

The soil samples were collected from Swani Coffee Estate, Karura forest, University of Nairobi Kabete field station a tomato field, Grace Rock Ranch Rironi from compost, and manure soils.

Swani coffee estate in Muranga -1.0318°S, 37.1674° E is characterized by Acrisols soils (Njoroge *et al*., 2018), where coffee has been cultivated for several decades (Reetsch *et al*., 2020). This area had Mexican marigold, black jack and gallant soldier as the main weeds growing in the plantation. While *Grevillea robusta* tree species are widely grown on the boarders of Swani Coffee Estate. These provide shade, protecting the coffee bushes from extreme rainfall and winds and cooling while enabling the plants obtain sufficient light. The main soil amendments that had been performed on this area are liming. This performed, as a pH reduction (4.9 - 5.6) technique due to high soil acidity in Swani area. Both secondary and low tillage are always performed. The former done to achieve finer soil tilth for the coffee rows while the latter performed using herbicides for weed control (Reetsch *et al*., 2020).

Karura forest in Nairobi County at -1.2402°S, 36.8302° E, characterized by a black cotton soil (Macharia, 2014), vast ecosystem comprising of trees such as Mubariti (*Grevillea robusta),* Blue gum *(Eucalyptus saligna),* Cypress *(Cupressus lusitanica),* Ngong'ngong' (*Croton megalocarpus),* Muthiga tree (*Warburgia ugandensis*),Cedar tree *(Juniperus procera) and* Pine (*Araucaria cunninghamii)* shrubs include Lantana *(Lantana camara*), Sage bush (*Buddleja salviifolia*) and Sand forest poison rope (*Strophanthus petersianus).* Conservation tillage done, whereby 30% of vegetation residues are left on the soil surface (Madarász *et al*., 2021). Primary tillage performed when increasing vegetation cover to loosen the soils for tree planting

Kabete Field Station University of Nairobi Kenya -1.2483°S, 36.7411°E, characterized by loam soil (Macharia, 2014) with tomato crop in the previous growing season. The tomato field incorporates both primary and secondary tillage. Primary tillage performed after the previous harvest when the soil moisture content is adequate to allow ploughing. Secondary tillage subsequently done to give soil finer tilth during fertilizer incorporation, control weeds and to level the farm surface. Therefore, the tomato field involves intensive tillage which leaves less than 15 % crop residue cover (Naseri *et al*., 2021).

The two sites for soil sampling compost and manure habitats from Grace Rock farm in Rironi -1.1598° S, 36.6429° E Kiambu, had accumulation of vegetable waste on the composting land while livestock manure collection was done on a separate section of the land. Conservation tillage involving 100% ground cover performed in this area hence, no crops are grown on these lands (Carr *et al*., 2020).

3.2.2. Sampling and collection of soil

Grid soil sampling technique was used, whereby the sites were subdivided into four quadrants (Mallarino 2001; Mallory *et al*., 2020). Using sisal twine, zigzag patterns were drawn on the four quadrants per site and samples extracted from each cell using a soil auger from. The plant residues were removed from the spots for sampling using a shovel and soil auger driven into the spots collecting soils to depths of 30 cm. The obtained top and sub soils were then mixed for homogeneity. Using a shovel, the samples were packed into labelled brown khaki bags (one kilogram) into a cool box and transported to Plant Pathology Laboratory at the University of Nairobi, Kenya.

3.2.3. Isolation and identification of *Trichoderma* species from the soil

The representative five grams of each of the soil samples were weighed into five conical flasks containing 100 ml of sterile distilled water. To attain uniformity the mixture was placed in sterile test tubes and for 25 minutes subjected to a rotary machine. Serial dilutions of the representative soil samples were then conducted from 10^0 to 10^7(Tkacz et al., 2018) using sterile pipette one milliliter of the suspension was drawn and dispensed on PDA media by pour plate technique (Kale et al., 2018).The plates were incubated at 18°C - 23°C, to allow fungal growth (Maji *et al*., 2019). Identification of *Trichoderma* isolates was carried out by use of microscopic and morphological characteristics (Mallory *et al*., 2020; Samuels *et al*., 2004; Yadav *et al*., 2020). Colony forming units were further calculated for each soil habitat (Equation 3.1).

$$\frac{CFU}{g} = \frac{\text{Number of } Trichoderma \text{ colonies} \times \text{Dilution facto}}{\text{volume of culture per plate}} \qquad (3.1)$$

The total numbers of colonies and the total number of *Trichoderma* colonies were counted per plate at day three, day six, and day nine (three days interval). From which the *Trichoderma* frequency (% *Tr* freq) of each soil sample was calculated using Equation 3.2.

$$\%Tr\text{freq} = \frac{\text{Total colonies} - \text{Total } Trichoderma \text{ colonies}}{\text{Total number of colonies}} \times 100 \qquad (3.2)$$

3.2.4. Isolation of *Ralstonia solanacearum* and pathogenicity test

Tomato plants were collected from infected greenhouse in Mirera, Naivasha for isolation of *Ralstonia solanacearum*. The stems were chopped into two cm pieces and surface sterilized with 2 % sodium hypochlorite for two minutes then rinsed with sterile distilled water. In a universal bottle with sterile distilled water, the stems were mashed using a sterile glass rod (Aley & Elphinstone, 1995; Jeong *et al*., 2007). Sterile wire loop was inserted into the obtained suspension and streaked onto a plate with TZC agar (Kelman, 1954). Kelman's TZC media was prepared by first making TZC stock solution involving 1g of Triphenyl tetrazolium chloride dissolved into 100ml of sterile distilled water, basal medium containing a mix of; dextrose 10g, peptone 10g, casamino acids one gram, agar 18g, and sterile distilled water 1000 ml and autoclaved. Incubation was done at 18°C - 23°C and pathogeny identification carried out by morphological and cultural characteristics (Mallory *et al*., 2020; Yadav *et al*., 2020).The bacterial suspensions obtained from the isolation was stored and used for pathogenicity validating Koch's postulate (Byrd *et al*, 2016; Khasabulli *et al*., 2017). Anna F1 30 day's old seedlings were inoculated with *Ralstonia solanacearum* using root dip method for thirty minutes (Mutuku *et al*., 2021). These were planted in four kilogram pots of forest soil with no history of bacterial wilt with two seedlings per pot. Non inoculated seedlings were also planted in the same soils serving as control. The four pots were replicated three times for each treatment bacterial wilt incidence and severity observed for three weeks.

3.2.5. Screening of *Trichoderma* isolates for antagonism against *Ralstonia solanacearum*

Using dual plate technique five days old cultures were used to determine antagonistic activity of the *Trichoderma* isolates on *Ralstonia solanacearum* (Abhiram & Masih, 2018; Veljović *et al*., 2017). The plates were replicated three times in RCBD while incubated at 18°C - 23°C with control plates having the *Trichoderma* isolates and *Ralstonia solanacearum*.

The fungal growth diameter of the *Trichoderma* species was measured in the control plates and used to determine the percentage growth inhibition. The distance of growth of the *Trichoderma* species from original point of inoculation in the treated plates towards the *Ralstonia solanacearum* on days three, six and nine was measured. The *Trichoderma* species growth over *Ralstonia solanacearum* then calculated by Equation 3.3 (Bunbury et al., 2019).

$$\%\ Trichoderma\ growth\ inhibition\ = \frac{R - R1}{R} \times 100 \qquad (3.3)$$

Where, R the distance from point of inoculation to colony margin in the control plate, $R1$ the distance of fungal growth from point of inoculation to colony margin in the treated plate in the direction of antagonist.

3.2.6. Data analysis

The data collected on percentage *Trichoderma* frequency and percentage *Trichoderma* growth inhibition were analyzed by both descriptive and inference statistical analysis (ANOVA) using GenStat® 15th edition owned by The Numerical Algorithms Group and Rothamsted Research. This was to determine whether the variations among the treatments were significant. Separation of means was done using Fisher's protected LSD at 5% significance level.

3.3. Results

3.3.1. The *Trichoderma* species isolated from the different habitats

The differences in colours of the species were the major characteristic and the first identification feature of the *Trichoderma* species (Andriani *et al*., 2021; Yadav *et al*., 2020). Conidia and phialides identification in terms of shape (Asis *et al*., 2021; Mistry & Bariya, 2022) enabled grouping into types shown in Table 3.1. The identified *Trichoderma* species were *Trichoderma harzianum* with ten isolates from compost soil, eight isolates from manure soil, four isolates from tomato soil, 15 isolates from forest soil, and five isolates from coffee soil and *Trichoderma asperellum* three isolates from compost, two manure and four forest soils with none from tomato and coffee soils.

The presence of *Trichoderma harzianum* was significantly high in the undisturbed environments of the forest, compost, and manure. *Trichoderma asperellum* had significantly

lower number of isolates with its highest populations originating from undisturbed habitats. The colony growths of *Trichoderma asperellum* and *T harzianum* observed in Figure 3.2 and are further differentiated in Table 3.1 in terms of the colony colour and colony reverse colour while the microscopic characteristics shown in Figure 3.3.

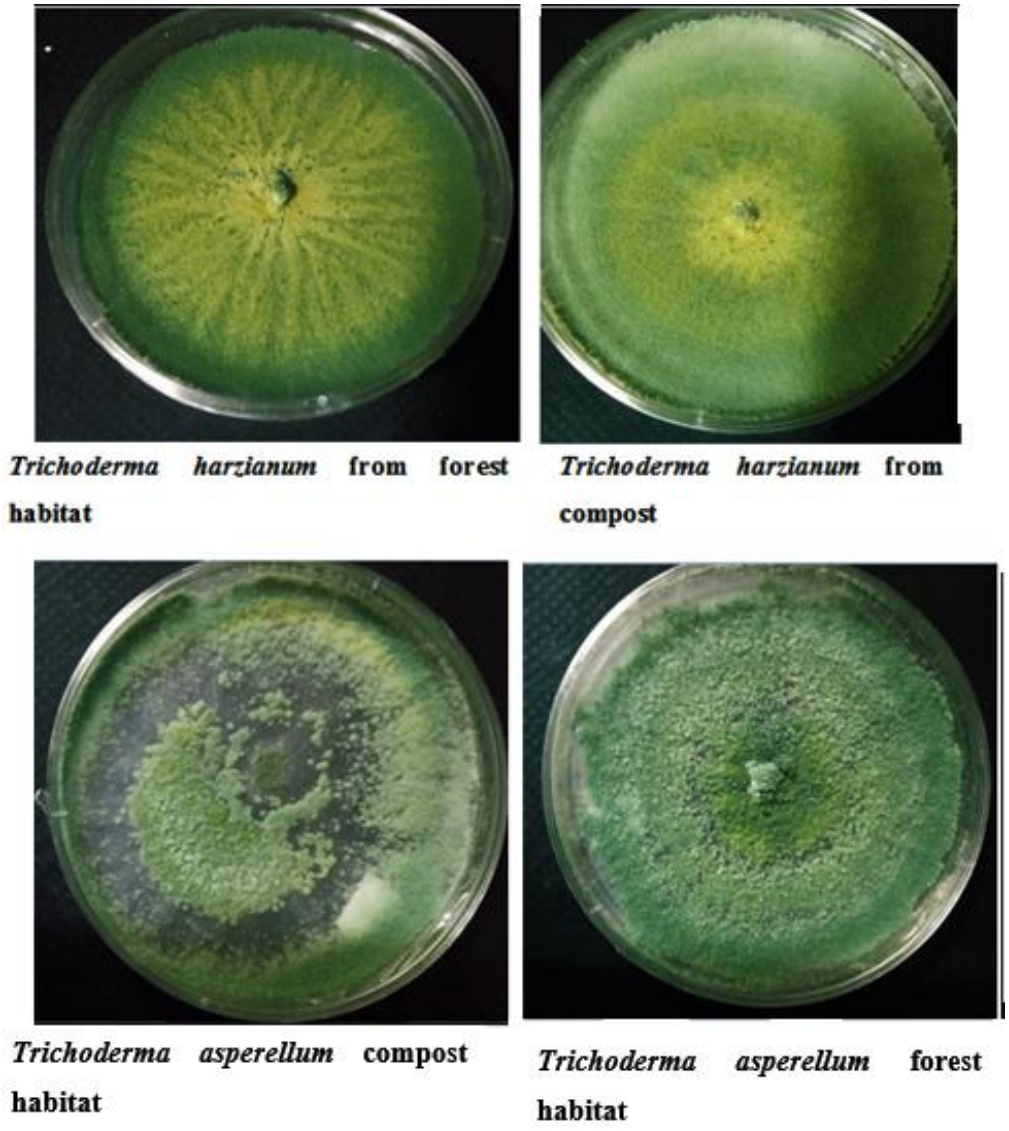

Figure 3.1: Colony growth of *Trichoderma spp* from different habitats at day four

Table 3.1: Morphological characteristics of identified *Trichoderma* species

Isolate type	Colony colour	Colony reverse	Phialides character	Conidia shape
T harzianum	Light green with yellow	Green	Cylindrical	Sub globose
T asperellum	Bluish-green	Colourless	Sub cylindrical	Globose

3.3.2. The isolated *Ralstonia solanacearum*

Colonies with pink to red-coloured centres were observed in Figure 3.1, identifying *Ralstonia solanacearum* on TZC media (Korayem *et al*., 2015; Mutimawurugo *et al*., 2019). Wilt symptoms were observed after seven days on the pot transplants for pathogenicity and causal agent confirmed through bacterial streaming test of the inoculated tomato plants and culture isolations of the pathogen colonies on TZC media were white with pink centers.

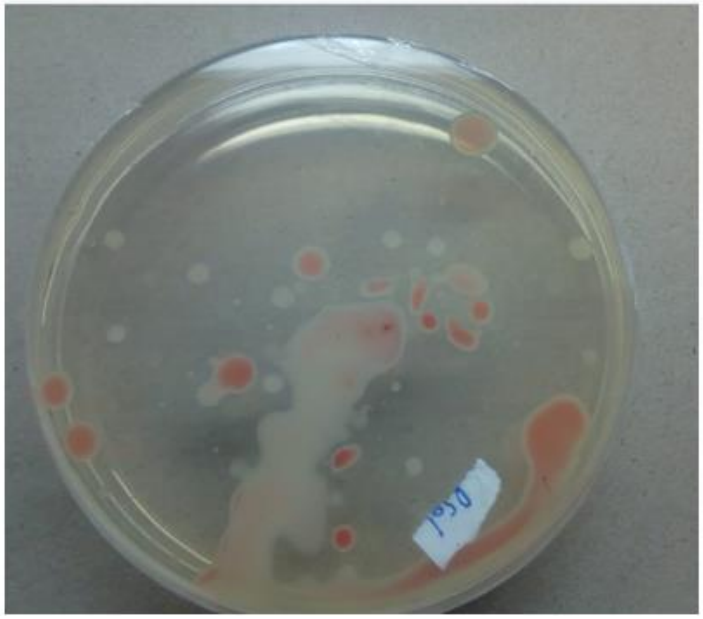

Figure 3.2: Isolated plant pathogen *Ralstonia solanacearum* with pink colored colonies on TZC media

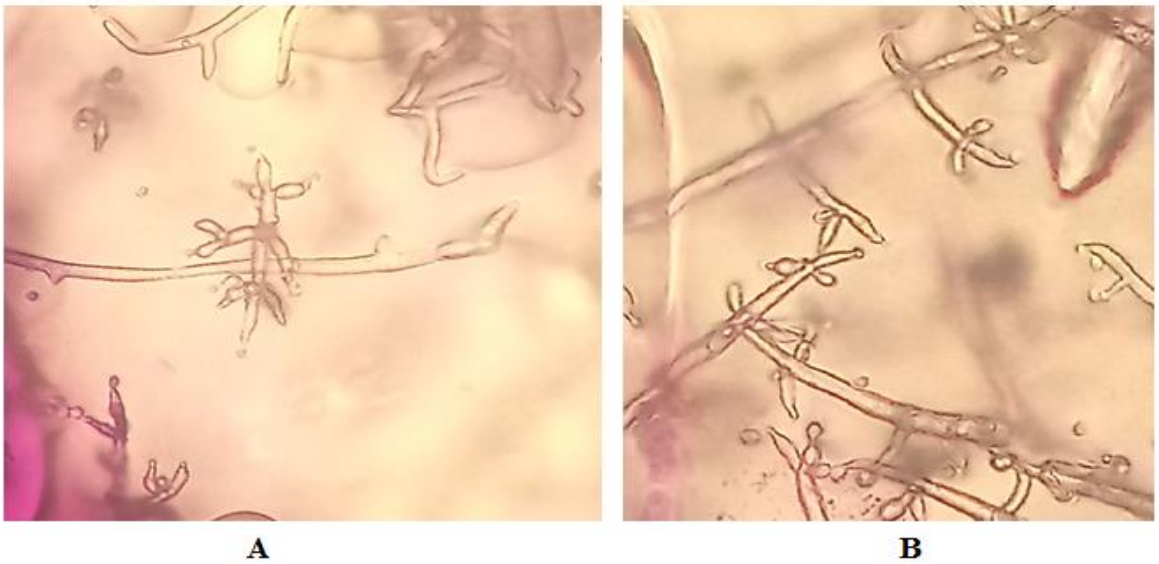

Figure 3.3: *Trichoderma harzianum* labelled A and *T asperellum* as B with different conidiophores shapes viewed at ×100

The conidiophores were observed to be pyramidal in shape in *Trichoderma harzianum* compared to *Trichoderma asperellum* form a whorl arrangement. The conidiophores' branches were observed to have their phialides arising from the main axis cylindrical in shape at the tip with lateral side branches similar in *Trichoderma asperellum* where the conidiophores branches had phialides also arising from the main axis. The phialides were enlarged in the middle sub-cylindrical; nearly sub globose (Figure 3.3) in *T harzianum* different as compared to the *T asperellum* where the phialides were more swollen in the middle and ovoid in shape. It is observed from Table 3.2 that the *Trichoderma spp* frequency was highest at incubation day three across the different habitats as compared to days six and nine, where there was no change in the percentage frequency therefore the *Trichoderma* populations were constant over incubation days six and nine. Compost and forest habitats had the highest *Trichoderma* frequency from the isolations done in the laboratory over days three, six, and nine of incubation. Although the compost environment had the highest *Trichoderma* frequency across the days of the incubation period, there was no significant difference at $P \leq 0.05$ between compost and forest habitats in *Trichoderma* frequency.

Table 3.2: *Trichoderma* frequency from the different habitats

Days of Incubation	Tomato	Forest	Coffee	Manure	Compost
DAY 3	20.5a	32.5 bc	25.8 ab	26.0 ab	36.4c
DAY 6	12.1a	24.2 bc	18.7 ab	20.2 b	28.6 c
DAY 9	12.1a	24.2 bc	18.7 ab	20.2 b	28.6 c
Mean	14.9 a	26.9 c	21.1ab	22.1 b	31.2 c
LSD-treatment	0.5	0.7	0.5	0.6	0.8
LSD-site	1.2	1.8	1.3	1.7	1.9
p-value	0.1	0.1	0.1	0.2	0.1
CV%	5.7	8.1	6.8	7.4	8.5

Means followed by the same letter(s) in each row are not significantly different at ($P \leq 0.05$); CV% = Coefficient of variation; LSD = Least Significant Difference at ($P \leq 0.05$)

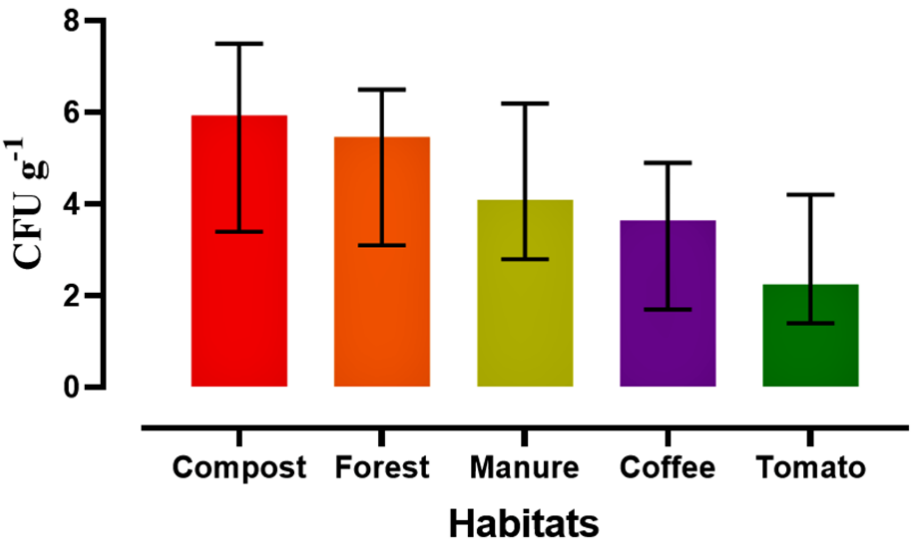

Figure 3.4: Populations of *Trichoderma* species from different soil habitats

The cultivated soils from tomato & coffee habitats were observed to have lower *Trichoderma* frequency than the undisturbed non-cultivated soils from the forest, manure, and compost habitats. There was no significant difference between forest and compost, but a statistically significant at $P \leq 0.05$ different from coffee, tomato, and manure across days three, six and nine. Tomato field being a continuously cultivated ecosystem had significant at $P \leq 0.05$ different mean from the forest, compost, coffee, and manure being the lowest across all the days of incubation.

The population of *Trichoderma* species varied in the five different soil habitats from Figure 3.4 above. Compost and forest soil habitats had the highest CFU/g of *Trichoderma* species compared to the rest of the soil habitats. It is observed that the most cultivated soils had a slightly lower CFU/g of *Trichoderma* species, notably in coffee and tomato fields' soil samples. Manure soil habitat also had a relatively high *Trichoderma* CFU/g compared to the cultivated soil habitats in Figure 3.4. The *Trichoderma* colony forming units were highest in samples from the sites with low disturbance as compared to the disturbed habitats.

3.3.3. Antagonism of *Trichoderma* species against *Ralstonia solanacearum*

Trichoderma harzianum and *T asperellum* were checked for antagonism against *Ralstonia solanacearum*. The two *Trichoderma* species were observed to have high antagonistic activity over the plant pathogen.

Table 3.3: *Trichoderma harzianum* from different habitats antagonism on *Ralstonia solanacearum* at day 3, 6 and 9

Days of incubation	Tomato	Forest	Coffee	Manure	Compost	Control
DAY 3	20.47a	32.48 bc	25.84 ab	26.04 ab	36.43c	0.00d
DAY 6	12.06a	24.20 bc	18.69 ab	20.15 b	28.57 c	0.00d
DAY 9	12.06a	24.20 bc	18.69 ab	20.15 b	28.57 c	0.00d
Mean	44.08 d	12.52 a	32.61 c	29.67 b	29.02 b	0.00e
LSD Treatment	0.03	0.12	0.5	0.31	0.33	0
LSD Site	2.1	1.7	1.9	1.8	1.8	0
CV%	7.1	4.5	6.9	6.85	6.8	0

Means followed by the same letter(s) in each row are not significantly different at($P \leq 0.05$); CV% = Coefficient of variation; LSD = Least Significant Difference at ($P \leq 0.05$)

They both showed high growth activity in the presence of *Ralstonia solanacearum* where isolated *Trichoderma harzianum* from undisturbed habitat of forest had significantly at $P \leq 0.05$ high antagonistic effect over the pathogen at 87.5% and 71% respectively. Isolated *Trichoderma asperellum* from undisturbed habitats antagonistic effects were 85.1%, 83.3%, and 80.4% respectively. The *T harzianum* in the disturbed environments of coffee and tomato fields had lower growth activity of 67.4% and 55.9% respectively *in vitro* in the pathogens presence. The antagonistic effect of *Trichoderma harzianum* from disturbed environment was significantly at $P \leq 0.05$ lower compared to the undisturbed environment. *T asperellum* from the forest habitat was more active compared to those from compost and manure at 81.2%, 80.8%, and 80.2% respectively, and can be seen in Table 3.3, 3.4 and 3.5.

Table 3.4: *Trichoderma asperellum* from different habitats antagonism on *Ralstonia solanacearum*

Days of incubation	Forest	Manure	Compost	Control
DAY 3	31.33 b	26.12 ab	34.42c	0.00d
DAY 6	22.10 ac	21.10 b	27.54 bc	0.00d
DAY 9	22.10 ac	21.10 b	27.57 c	0.00d
Mean	14.91 a	28.07 b	28.13c	0.00d
LSD Treatment	0.13	0.33	0.31	0.0
CV%	7.0	6.7	6.8	0.0

Means followed by the same letter(s) in each row are not significantly different at ($P \leq 0.05$); CV%=Coefficient of variation; LSD=Least Significant Difference at ($P \leq 0.05$)

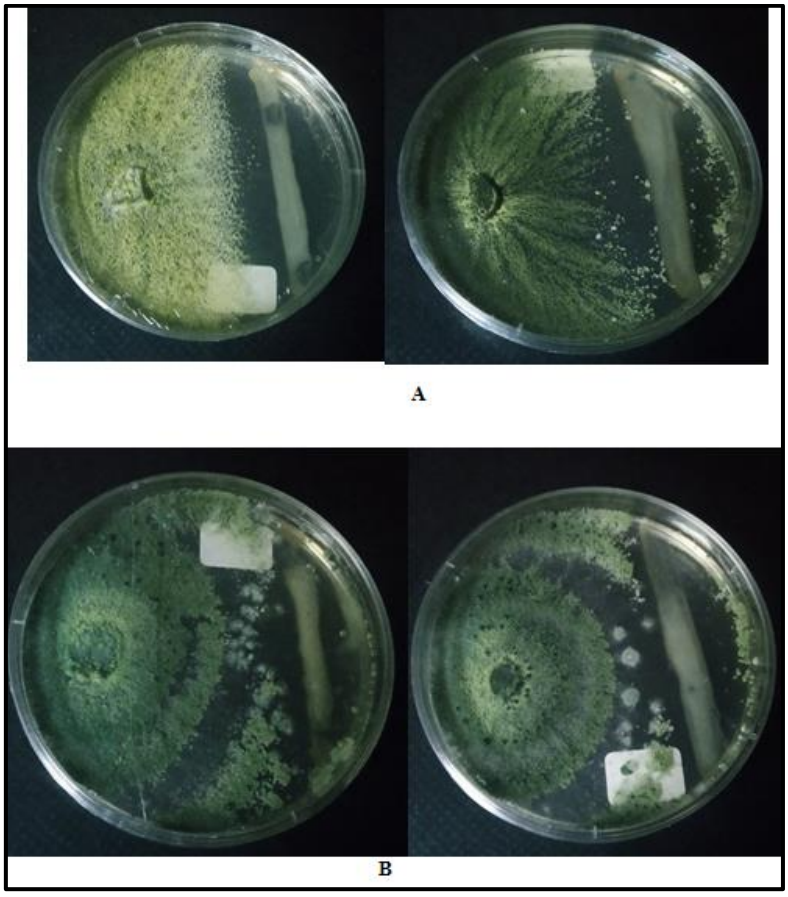

Figure 3.5: Growth activity of A - *Trichoderma harzianum* and B -*Trichoderma asperellum* antagonism against *Ralstonia solanacearum* by dual plate technique on PDA

3.4. Discussion

In this study, the populations, identification, and screening of *Trichoderma species* from different habitats against *Ralstonia solanacearum* were done to evaluate these biological control agents' capabilities in managing the pathogen which is similar to studies in other research works involving *Trichoderma* species managing plant diseases (Konappa *et al*., 2020; Yan & Khan 2021). High *Trichoderma* species populations were observed in undisturbed habitats of forest, compost, and manure which agreed with studies by Vinale *et al*. (2008). Reporting that *Trichoderma spp,* were more frequent in undisturbed high organic matter ecosystems. Similarly, Hyder *et al*. (2017) and Maina *et al*. (2016) established that the abundance of *Trichoderma* species depends on the history of ecosystems disturbance.

The habitats influenced the *Trichoderma* populations as few *Trichoderma* species were isolated from the cultivated habitats of tomato similar to findings by Bhale *et al*., (2012) Bourguignon *et al,* (2008) and Prabowo *et al*., (2021) show that cultivated soil habitats have

low microbial life due to disturbance. Similarly, Bolo *et al*. (2021) reported that populations of *Trichoderma* species in ecosystems with reduced tillage practices were very high compared to cultivated habitats that required the addition of inorganic fertilizers to enhance soil microbial life. Therefore concurs with the results of the study with higher *Trichoderma* species populations in undisturbed habitats.

Different research works have shown that *Trichoderma* species have a very high antagonistic ability. Use of *Trichoderma* spp on *Fusarium oxysporum* causing fusarium wilt of tomatoes (Hassan & Barde 2020; Moin *et al*., 2021) especially *Trichoderma viride* and on *Pythium* causing damping off in tomatoes (Silva *et al*., 2017; Kashyap *et al*., 2020 ; Shashikumar *et al*., 2019; Verma *et al*., 2017). Management of *Fusarium solani* resulting in fruit and root rot in tomatoes by *Trichoderma harzianum* (Salim *et al*., 2017) with several other studies evaluating *Trichoderma spp* antagonistic potential against tomato plant pathogens (Jamil, Musheer & Kumar 2021; Jogaiah *et al*., 2018; Salim *et al*., 2017).

Similarly Alka and Prajapati (2017) and Sallam *et al*. (2019), this study established that total and partial inhibition on the pathogen occurred using *Trichoderma spp.* This is attributed to high mycoparasitism as reported by Bhat (2017) and Salim *et al*. (2017) while Benlamoudi *et al*. (2021) and Jagraj *et al*. (2018) presented a high *Trichoderma spp* sporulation invaded *Fusarium oxysporum* colonies. The different *in vitro* assessments of antagonistic activities involving *Trichoderma spp* have also shown that antibiotic secretion of dermadin, trichodermin, sesquiterpene, trichovirdin are among the contributing factors to the growth inhibition of pathogens (Misra & Ansari, 2021; Yan & Khan, 2021). Studies by Hernandez *et al*. (2011) leading to the classification of *Trichoderma spp* was confirmed where the *Trichoderma* species grew completely towards the pathogen. The in vitro antagonism check justifies that this research work concurs with previous classifications and behavior of isolates of *Trichoderma* on plant pathogens.

3.5. Conclusion

The study showed that *Trichoderma* species were abundant in habitats with high organic matter and especially undisturbed of forest, compost, and manure ecosystems. The populations of the *Trichoderma* species in these undisturbed habitats were higher compared to the tomato and coffee habitats. *Trichoderma harzianum* and *asperellum* were the most common species during the isolations from both the undisturbed and disturbed soil habitats.

The active *Trichoderma asperellum* and *harzianum* isolates were from the forest and compost soils, with a significant difference from those of coffee, tomato, and manure soils. In this study, the results show that *Trichoderma* species have a biocontrol capacity *in vitro* against bacterial wilt of tomatoes agreeing with studies done by Guzmán-Guzmán *et al*., (2019). The isolation of native *Trichoderma* spp from native soils indicates that *Trichoderma* species isolation is possible with mass multiplication in simple formulations. Biological control agents (BCA), in this case, are important alternative in the management of plant pathogens, which is an environment-friendly approach to managing plant pathogens with food safety in mind. The application their application can be adopted for management of bacterial wilt of tomatoes when applied after establishment of the crop.

CHAPTER 4:

EFFICACY OF *TRICHODERMA* SPECIES IN MANAGING *RALSTONIA SOLANACEARUM* IN TOMATOES

Abstract

Ralstonia solanacearum causes bacterial wilt in tomatoes resulting in significant crop losses. The different management measures available for bacterial wilt of tomato include crop rotation, sanitation, grafted planting materials, soil solarization, fumigation, chemicals among others. Biological control agents have been used in managing various diseases hence the need to check the efficacy of native *Trichoderma* isolates and those commercially available in managing bacterial wilt. Greenhouse experiments were conducted in three sites already infested with bacterial wilt in Mirera, Naivasha. *Ralstonia solanacearum* concentration in the infested soil was determined by serial dilution. The most antagonist *Trichoderma* isolates were mass multiplied in sterile sorghum grains. Transplanting of Anna F1 tomato seedlings in infested potted soils was done with three treatment applications. Light green and yellow masses of growth on sterile sorghum grains indicated *Trichoderma* species multiplication. *Trichoderma harzianum* had a concentration of 1.94 $\times 10^6$ spores g-1 while *T asperellum* was at 2.06 $\times 10^6$ spores g^{-1}. Bacterial wilt incidence and severity in the isolated *T harzianum* treated plots was 11.8%, *T asperellum* 12.7%, commercial *Trichoderma harzianum* 11.81% and commercial *Trichoderma asperellum* 11.9%. The control had high bacterial wilt incidence and severity at 27.7%. There was 100% stem browning in the control treatment with 60% observation on Trichoderma species treated plots. The commercial *Trichoderma harzianum* had high plant growth at 14.7% while the isolated *Trichoderma harzianum* 14.6%, commercial *Trichoderma asperellum* 14.5% and *Trichoderma asperellum* isolate at 14.3%. The control treatments had the low plant growth at 13.1%. The combined *Trichoderma* species treatments had high yield in kilograms with the commercial combined *Trichoderma spp* at 17% and the isolated *Trichoderma spp* at 16.4%. The single applications of commercial *Trichoderma harzianum* and *Trichoderma asperellum* both had 16.3% yields. The isolated *Trichoderma harzianum* yielded 15.5% and *Trichoderma asperellum* 15.4% significantly high compared to the control. The application of *Trichoderma* species reduces the levels of bacterial wilt incidence and severity on the tomato crops additionally improving the yield. The application of *Trichoderma* species as a method of managing bacterial wilt of tomato should be adopted.

Keywords: Bacterial wilt, Incidence, *Trichoderma*, *Ralstonia solanacearum*, Severity, Yield

4.1. Introduction

Bacterial wilt in Kenya has affected tomato production and experienced by a large number of farmers (Shitiavai *et al*., 2021). This leads to up to100% crop loss or yield loss (Kamuyu, 2017; Mbaka *et al*., 2013). The pathogen has proven to persist for a long time in the soils with a wide geographical distribution (Mihovilovich *et al*., 2017). Methods of managing bacterial wilt that have been adopted include the incorporation of bio fertilizer (Dong *et al*., 2020) enabling amendment of the soil nutrient content suppressing the disease (Zheng *et al*., 2020). The use of crop rotation has also been adopted as a bacterial wilt management strategy. This involves the establishments of crops from different families in the same land after a tomato cropping season (Li *et al*., 2019). Proper farm hygiene minimizes the pathogens inoculum from spreading. The disinfection of farm implements should be adhered to reducing the pathogens introduction during cultural activities (Haile *et al*., 2020). This additionally achieved by removal of crops showing disease symptoms, burying or burning of plant residues in one place (Belete *et al*., 2021). The solarization and fumigation of bacterial wilt infested soil, improves the structure of the soil hence plant growth promotion (Jibat & Alo, 2020; Panth *et al*., 2020). The use of chemicals that are less lethal like silver nanoparticles, magnesium oxide nanoparticles indicated management of bacterial wilt in tomatoes (Santiago *et al*., 2019). There are drawbacks of these methods solarisation solely depends on the sun thus time consuming for farmers results (Iraboneye *et al*., 2021; Mamphogoro *et al*., 2020; Manda *et al*., 2020). Fumigants have negative impacts on the environment from the products used these include methyl bromide and chloropicrin (Shen *et al*., 2021). The available resistant rootstocks are expensive to farmers although good management method (Maurya *et al*., 2019). The larger category of available chemical solutions are not environmentally friendly and are harmful to human health but are able to manage the disease (Ali *et al*., 2021; Li *et al*., 2021; Ravikumar *et al*., 2021).

There is need for alternative safe, sustainable, and effective methods in controlling bacterial wilt of tomatoes (Morais *et al*., 2019). BCAs exhibit characteristics that involve self-sustaining ability, reduced input of non-replenishable resources, scattered across after the first establishment, and provision of continuous disease suppression (Whipps, 2007). According to Kumar (2017), (BCAs) have been used as antagonistic agents of plant pathogens. This ensures the farmers' safety during handling and application of the biological control agents in regards to their health (Abd-Elgawad, 2020), management of disease and

safety to environmental microbial life (Kumari *et al*., 2020) hence this study. This involved the use of natively isolated *Trichoderma* spp from different soil habitats and evaluating their efficacy in managing bacterial wilt of tomato. Additionally was determining the hypothesis that native *Trichoderma* species had no effects on bacterial wilt incidence and severity in field conditions.

4.2. Materials and Methods

4.2.1. Description of the experimental site

Greenhouse experiments were conducted between months of March to September 2021 in Mirera, Naivasha located at -0.782°, 36.45° S. Mirera in Naivasha is at an elevation range of 900-2086 m above sea level with bimodal rainfall range of 500-1800 mm. Temperature ranges between 15°C to 28°C were a representative of zone IV (Charles *et al*., 2019; Kariuki *et al*., 2019) where Laikipia, Machakos and Naivasha areas are found. The vegetation were sparse and majority xerophytic shrubs, trees and ephemeral grasses (Manzi & Gweyi-Onyango, 2020). The common vegetation around this locality are shrubs comprising of camphor bushes (*Tarchonanthus camphoratus*) , grasses, euphorbia trees and acacia trees of different species that include *Acasia senegal*, *Acacia seyal* and *Acacia brevispica*. The grasses found were *Pennisetum mezianum*, *Themeda triandra, Pennisetum straminium*, *Pennisetum massaiense*, *Eragrostis* spp, *Hyperenia* spp, *Seteria* spp and *Digiteria* spp. The soils are Calcisols mostly developed from colluvial, alluvial and aeolian deposited from of the base weathering material. The greenhouse were made of polyethene which is anti-drip, controls U.V rays with air spaces for aeration retaining the absorbed heat, creating a stable temperature within the greenhouse. The cover materials were on steel providing stability and durability. The greenhouse sizes were 10 m by 15 m housing about 350 plants, which were planted directly on the soil under a drip irrigation system. The crop history of the sites includes tomatoes, capsicum, brinjals and rotation with cow peas.

4.2.2. Culture and multiplication of *Trichoderma spp* for field application

The best-performing *Trichoderma* isolates against *Ralstonia solanacearum* were maintained by sub culturing every ten days on PDA. Mass multiplication for application was done using a sterilized sorghum carrier (Kumar, 2017). Two separate 250g of sorghum was weighed into conical flasks supplemented with 5% anhydrous dextrose. The flasks were

corked using aluminum foil and cotton wool and autoclaved for sterilization (Boblina *et al*., 2019). This was done for one hour at 1.5 bars in 121°C (Dreger *et al*., 2019). Five milimetre disk of each of the active *Trichoderma* species were introduced into the flasks using sterile borer. The flasks were properly shaken after every three days for uniform inoculation of the sorghum grains at 18°C - 23°C. The sorghum grains were air dried and then using a grinding machine ground and packed in sterile bags and kept in 18°C - 23°C , dry place for application (Williams *et al*., 2022).

The already colonized sorghum grains were carefully removed using a sterile glass rod onto clean aluminum from the conical flasks and each of the species was ground into powder using a grinding machine. Sterile conical flasks were used to suspend five grams of the already colonized carrier into 100ml of sterile distilled water. This was shaken in a rotary shaker for 20 minutes. Serial dilution to six-fold was carried out to determine the inoculum levels of each species and plating on Kelman's TZC media. The number of colonies were counted over the days 3, 6, and 9 and colony-forming units calculated by Equation 4.1 (Mondal *et al*., 2020).

$$\text{CFU/g} = \frac{(\text{Average number of colonies} \times \text{dilution factor})}{\text{concentration per plate}} \qquad (4.1)$$

4.2.3. Isolation and multiplication of *Ralstonia solanacearum*

Homogenous mix of six soil samples from the three greenhouses was done. Serial dilution to eight-fold was conduct from one gram of the homogenious soil sample suspension in sterile distilled water. Plating on Kelman's TZC media was done and the *Ralstonia solanacearum* inoculum in the soil calculated by Equation 3.1. Infestation levels in the three greenhouses on the previous crop calculated by Equation 4.2 (Razia *et al*., 2021).

$$\frac{\text{Total number of plants with symptoms}}{\text{Total number of plants assessed}} \times 100 \qquad (4.2)$$

Tomato plants collected from infected greenhouses in Mirera, Naivasha were used for *Ralstonia solanacearum* inoculum preparation. The stems were chopped into two centimetre pieces and surface sterilized with 2 % sodium hypochlorite for two minutes then rinsed with sterile distilled water. The stems were mashed using a sterile glass rod inside a universal bottle with sterile distilled water, (Aley & Elphinstone, 1995; Jeong *et al*., 2007). Sterile wire loop was inserted into the obtained suspension and streaked onto plates with TZC agar

(Kelman, 1954). This was then incubated at 18°C - 23°C (Mallory *et al*., 2020; Yadav *et al*., 2020). Using sterile glass rod the bacterial colonies were flooded with sterile distilled water and scrapped off into a sterile conical flask containing 1000 ml sterile distilled water. These were mixed using a centrifuge for 20 minutes to attain uniformity (Morel *et al*., 2018).Serial dilution was carried out to seven fold and concentration of inoculum calculated (Uwamahoro *et al*., 2020).Potting was done with bacterial wilt infested soil from the greenhouses whose infestation levels had been determined by Equation 4.1. The pots were watered for two hours and 35 ml of the prepared bacterial wilt inoculum added to the center of each pot (Marquès *et al*., 2020).

4.2.4. Experimental design and layout

In the greenhouse there were seven blocks that were13m long and 2m wide, each with seven plots. In each plot there were six pots of 6’’by 9” separated by a path of one metre by one metre with one plant each, arranged in RCBD. This was replicated in the two remaining greenhouses. The treatments applied included each five grams of isolated *Trichoderma asperellum*, isolated *Trichoderma harzianum* and combination of the isolated *T harzianum* and *T asperellum*. The plots with no treatment application were also included, five gram each of standard check were applied of commercial *Trichoderma harzianum*, commercial *Trichoderma asperellum*, a combination of commercial *Trichoderma asperellum* and *T harzianum*. The Anna F1 seedlings were raised at commercial plant nursery. The seeds were sown in trays containing sterile coco peat and peat moss premixed with fertilizer high in Nitrogen, Potassium and Phosphorous. Irrigation was carried out daily, weeding and scouting for any pest and diseases.

On establishment of the experimental crop, 150 kg Ha-1 of Diammonium phosphate (47% P_20_5) was used. Two weeks after transplanting, 200Kg ha-1 CAN (27% N) was applied, followed by second application in the fourth week. In weeks seven, nine, and twelve, NPK compound fertilizer (N=17%, P_20_5=17%, K=17%) was applied at 150Kg ha-1. The crop was manually weeded and kept clean. Crop support (trellising) was done as per farmers' practice. Pruning was done to remove side shoots, laterals, old leaves, diseased leaves, and branches to reduce fungal diseases and increase air circulation within the crop canopy. The standard pest and disease management program was followed.

4.2.5. Assessment of bacterial wilt incidence and severity

The disease incidence determination was done by counting the number of wilted plants per pot on day five of every week up to the end of the experiment. Disease incidence was calculated as a percentage of wilted plants in each of the treatments (Ayana *et al*., 2011) as given in Equation 4.3.

$$\mathrm{I} = \frac{NPSWS}{NPPT} \times 100 \qquad (4.3)$$

Where I the Wilt incidence, $NPPT$ Number of plants per treatment, and $NPSWS$ Number of plants with wilt symptoms.

Disease severity was checked every fifth day of the week up to the end of the experiment. This was conducted by checking the levels of wilt on a scale of 1-4 (Wei *et al*., 2013) where at one no symptoms were visible, two half of the foliage to one leaf wilting, three with 60% of the foliage wilting and at four the entire plant had wilted and died. Additionally stem browning was also scored for at a scale of 0-3 (Elphinstone *et al*., 1998) from the fifth day of the first week of transplanting to the last week of the experiment. Where at zero no browning, one there was slight brown color 2 cm from the stem base, two there was light brown color more than 2 cm from the base, at three there was very dark brown color widespread browning of vascular tissue. Finally counting the number of wilted tomatoes per plot on the fifth day weekly was confirmed by checking for bacterial ooze. Ooze rate score range at 0-3 (Pradhanang *et al*., 2005) with zero indicating no ooze, one had thin strands of bacteria oozing that stops in three minutes, two very continuous flow that stops in five minutes and three with heavy ooze turning the water turbid.

4.2.6. Assessment of growth and yield

The plant height from the tip to the plant base per treatment was observed and measured using a tape measure. This was recorded from transplanting to the first fruit set after every two weeks. Physiologically mature fruits were harvested per treatment. These were sorted and marketable fruits put aside according to size using a vernier caliper (Behera *et al*., 2019). They were weighed using a weighing scale, and these were recorded throughout harvest twice a week.

4.2.7. Data analysis

The data collected on on bacterial wilt incidence, severity and tomato yield percentage were analyzed by both descriptive and inference statistical analysis (ANOVA) using GenStat® 15th edition owned by The Numerical Algorithms Group and Rothamsted Research. This was to determine whether the variations among the treatments were significant.

4.3. Results

4.3.1. Multiplied *Trichoderma* species and *Ralstonia solanacearum*

The growth of *Trichoderma* species was quantified by green and yellow mass on the sorghum grains after 3 days in Figure 4.1. The inoculum concentration of *T asperellum* was found to be 1.94 $\times 10^6$ spores g^{-1} and *T harzianum* was 2.06 $\times 10^6$ spores g^{-1} once ground into powder forms and applied in the experiment. In the three greenhouses, the bacterial wilt infestation levels were at 42.85%, 51.42%, and 57.14% respectively. The *Ralstonia solanacearum* levels in the infested collected soil samples was at 2.18 $\times$ 10^5 spores g^{-1} with inoculations into the pots with inoculum of 1.69 $\times$ 10^5 CFU ml^{-1} of *Ralstonia solanacearum* in sterile distilled water for uniformity.

Figure 4.1: Pure cultures of *Ralstonia solanacearum* isolated from the infested greenhouse

4.3.2. Bacterial wilt incidence and severity

The incidence of bacterial wilt was significantly higher in control with no treatments at 27.7% cross compared to the other treatments. The combination of the commercially available *Trichoderma harzianum* and *Trichoderma asperellum* treatment had significantly lower incidence of bacterial wilt of 11.2% than the other treatments. The commercial *Trichoderma asperellum* bacterial wilt incidence was 11.84% while the commercial

Trichoderma harzianum observed 11.81%. The isolated *Trichoderma asperellum* had bacterial wilt incidence levels of 12.7%, while *T harzianum* 11.88%. Combination of the isolate *T harzianum* and *T asperellum* had bacterial wilt incidence of 11.7%. There was no significant difference at ($P \leq 0.05$) among the *Trichoderma* treatments as they had reduced incidence and severity of bacterial wilt which was significantly different from control treatment with high disease incidence as seen in Table 4.1 and Figure 4.3.

Figure 4.2: Mass multiplication of *Trichoderma spp* on sorghum grains

The severity of bacterial wilt across the three greenhouses was highest in the control at 22.1%, *Trichoderma asperellum* isolate recorded lower bacterial wilt severity compared to the control in the tomatoes at 13.1% while the commercial *Trichoderma asperellum* was at 12.9%. The *Trichoderma harzianum* isolate bacterial wilt severity was significantly lower compared to the control at 12.8% but higher compared to the commercial *Trichoderma harzianum* at 12.7%. The treatments with the combined *Trichoderma* species had reduced bacterial wilt severity where the combination of the commercial *Trichoderma* species recorded 12.2% while the isolated combinations of *Trichoderma* species at 12.4%. The tomatoes in the control treatment were 100% affected by stem browning as compared to the other treatments on *Trichoderma* species where 80% of browning on the stems was only observed on the crops that had been adversely affected by bacterial wilt and at the later stages seen in Table 4.3. There was no significant difference between the treatments on *Trichoderma* species as compared to the control

Table 4.1: Bacterial wilt incidence across the treatments

Treatments	Replicate 1	Replicate 2	Replicate 3
Trichoderma asperellum	2.9c	2.1a	1.1a
Trichoderma harzianum	2.6bc	2.3a	1.5ab
Trichoderma (*harzianum* + *asperellum*)	2.5ab	2.1a	1.3a
Trichoderma harzianum commercial	2.6b	2.1a	1.3a
Trichoderma asperellum commercial	2.5ab	2.1a	1.8b
Trichoderma (*harzianum* + *asperellum*) commercial	2.1a	1.8a	1.5ab
Control - (no application)	4.6d	4.5b	4.1c
LSD	0.4	0.4	0.4
CV%	9.3	9.7	10.1

Means followed by the same letter(s) in each row are not significantly different at ($P \leq 0.05$); CV% = Coefficient of variation; LSD = Least Significant Difference at ($P \leq 0.05$).

Ralstonia solanacearum presence was checked and scored from the ooze rate and found to be significantly higher in the control treatments compared to the other treatments. Higher bacterial ooze observed from the wilted and dead plants. There was no significant difference among the *Trichoderma* treatments as they showed reduced pathogen presence in the tomato plants checked for bacterial ooze scoring as compared to the control Table 4.4. The combination of the commercially available *Trichoderma harzianum* and *Trichoderma asperellum* treatment had no significant difference with the isolated *Trichoderma* species. Bacterial streaming test indicated that the control treatment had the highest levels of the pathogen *Ralstonia solanacearum* at 98% while the *Trichoderma* species treated plots had no significant difference from each other at 65%, significantly at $P \leq 0.05$ lower than the control.

Table 4.2: Bacterial wilt severity across the treatments

Treatments	Replicate 1	Replicate 2	Replicate 3
Trichoderma asperellum	2.9c	2.1a	1.1a
Trichoderma harzianum	2.6bc	2.3a	1.5ab
Trichoderma (*harzianum* + *asperellum*)	2.5ab	2.1a	1.3a
Trichoderma harzianum commercial	2.6b	2.1a	1.3a
Trichoderma asperellum commercial	2.5ab	2.1a	1.8b
Trichoderma (*harzianum* + *asperellum*) commercial	2.1a	1.8a	1.5ab
Control - (no application)	4.6d	4.5b	4.1c
LSD	0.4	0.4	0.4
cv%	9.3	9.7	10.1

Means followed by the same letter(s) in each row are not significantly different at ($P \leq 0.05$); CV% = Coefficient of variation; LSD = Least Significant Difference at ($P \leq 0.05$).

Table 4.3: Stem browning across the treatments

Treatments	Replicate 1	Replicate 2	Replicate 3
Trichoderma asperellum	0a	0a	0a
Trichoderma harzianum	0a	0a	0a
Trichoderma (*harzianum* + *asperellum*)	0a	0a	0a
Trichoderma harzianum commercial	0a	0a	0a
Trichoderma asperellum commercial	0a	0a	0a
Trichoderma (*harzianum* + *asperellum*) commercial	0a	0a	0a
Control - (no application)	0.8a	0.8a	0.9b
LSD	0.4	0.4	0.4
CV%	38.9	15.5	19.2

Means followed by the same letter(s) in each row are not significantly different at ($P \leq 0.05$); CV% = Coefficient of variation; LSD = Least Significant Difference at ($P \leq 0.05$).

Figure 4.3: Bacterial streaming test using wilted tomato plant stem section suspended in plain water

Table 4.4*:* *Ralstonia solanacearum* ooze from the wilted tomatoes in the treatments

Treatments	Replicate 1	Replicate 2	Replicate 3
Trichoderma asperellum	0a	0a	0a
Trichoderma harzianum	0a	0a	0a
Trichoderma (*harzianum* + *asperellum*)	0a	0a	0a
Trichoderma harzianum commercial	0a	0a	0a
Trichoderma asperellum commercial	0a	0a	0a
Trichoderma (*harzianum* + *asperellum*) commercial	0a	0a	0a
Control - (no application)	0.3b	0.2b	0.9b
LSD	0.1	0.2	0.1
CV%	18.9	19.3	19.2

Means followed by the same letter(s) in each row are not significantly different at ($P \leq 0.05$); CV% = Coefficient of variation; LSD = Least Significant Difference at ($P \leq 0.05$).

4.3.3. Growth of the tomato plants in the treatments

The growth of the plants in regards to the height from the stem base of the plant to the first fruit set was observed to be significantly ($P \leq 0.05$) lower in the control treatments than the other treatments. The combination of the *Trichoderma* treatments had a significant ($P \leq 0.05$) difference in height with the plants being taller compared to the other *Trichoderma* treatments done singly. *Trichoderma* treated plants' growth was significantly ($P \leq 0.05$) higher as compared to the control where no treatment application was being done Table 4.5 and Figure 4.5. Plant growth was observed to be greatly influenced by the *Trichoderma* species in all the treatments containing *Trichoderma* application. The tomato plants' growth in regards to height was better in the *Trichoderma* species treated plots at 99% compared to the control at 52%. The *Trichoderma* species treated plots had no significant difference from each other on the height of the tomato plants although the commercial *Trichoderma harzianum* had the highest growth at 14.7% while the isolated *Trichoderma harzianum* had 14.6%. The commercial *Trichoderma asperellum* had a plant growth of 14.5% and the isolated *Trichoderma asperellum* at 14.3%. The combination treatments of the isolated *Trichoderma asperellum* and *T harzianum* and the commercial *Trichoderma harzianum* and *asperellum* both were not significantly ($P \leq 0.05$) different from each other at 14.3% each. The control treatments had the lowest plant growth at 13.1%.

4.3.4. Yield of the tomato plants in the treatments

The treatments with *Trichoderma* species recorded the highest yield in kilograms significantly ($P \leq 0.05$) different from the control with lowest. The treatments with combination of *Trichoderma* species had highest yield in kilogram significantly ($P \leq 0.05$) different from the single *Trichoderma* species. The single *Trichoderma* treatments had better yield which were significantly ($P \leq 0.05$) different from the control, which was lower. The control treatment had a significantly low yield in kilograms Table 4.5, Figure 4.5.

The *Trichoderma* combined treatments had the highest yield in kilograms with the commercial *Trichoderma* combinations at 17% and the isolated *Trichoderma harzianum* and *Trichoderma asperellum* at 16.4%. The commercial *Trichoderma harzianum* and *Trichoderma asperellum* had significant ($P \leq 0.05$) yield in kilograms at 16.3% and 16.1% respectively. There was a significant difference between the isolated *Trichoderma harzianum* and *T asperellum* in the yield at 15.5% and 15.4% respectively significantly ($P \leq 0.05$) higher to the control 13.2%.

Table 4.5: Tomato height up to 1st fruit set in the treatments

Treatments	Replicate 1	Replicate 2	Replicate 3
Trichoderma asperellum	26.6e	26.1a	28.9c
Trichoderma harzianum	26.6cd	25d	28.9c
Trichoderma (*harzianum* + *asperellum*)	26.5e	24b	28.9c
Trichoderma harzianum commercial	26.4de	24.8c	29.1c
Trichoderma asperellum commercial	26.1c	24.9cd	28.9c
Trichoderma (*harzianum* + *asperellum*) commercial	25.4b	23.7a	24.1a
Control - (no application)	24.1a	23.6a	24.1a
LSD	0.3	0.2	0.8
CV%	1	0.3	0.8

Means followed by the same letter (s) in each row are not significantly different at ($P \leq 0.05$); CV% = Coefficient of variation; LSD = Least Significant Difference at ($P \leq 0.05$).

Table 4.6: Tomato yield in kilograms per treatment

Treatments	Replicate 1	Replicate 2	Replicate 3
Trichoderma asperellum	0.9a	2.6b	2.6b
Trichoderma harzianum	3.0b	2.6b	2.7b
Trichoderma (*harzianum* + *asperellum*)	3.2c	2.8c	2.8c
Trichoderma harzianum commercial	3.2c	2.7c	2.8c
Trichoderma asperellum commercial	3.2c	2.8c	2.8c
Trichoderma (*harzianum* + *asperellum*) commercial	3.3c	2.9d	2.9d
Control - (no application)	0.5a	0.4a	0.3a
LSD	0.2	0.2	0.1
CV%	5.2	2.8	2.1

Means followed by the same letter (s) in each row are not significantly different at ($P \leq 0.05$); CV% = Coefficient of variation; LSD = Least Significant Difference at ($P \leq 0.05$).

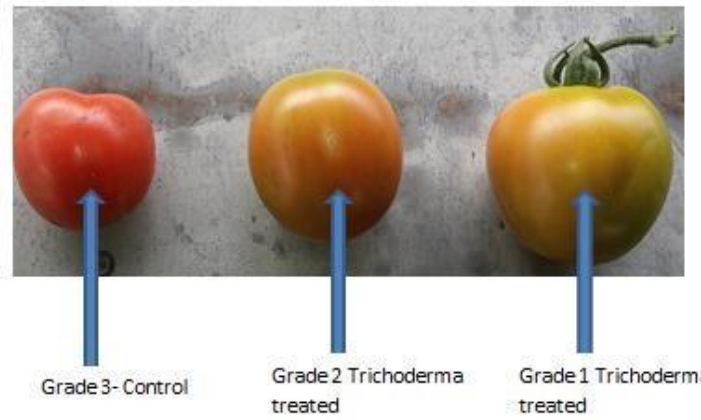

Figure 4.4: The harvested tomato grades: grade 1, 2 and 3

4.4. Discussion

The multiplication of *Trichoderma* species have been done on various substrates Sabalpara *et al*., (2014). According to Bhagat *et al*. (2010), sorghum and wheat were the commonly used solid substrates for the mass production of *Trichoderma viride* and reported to be more effective. In this study *Trichoderma* species were multiplied on sorghum grains according to (Dreger *et al*., 2019; Williams *et al*., 2022). The species growth was quantified by bluish green and yellow growth on the sorghum as observed in work done by (Iqbal *et al*., 2020). The linear growth and spore production of *Trichoderma* species are higher on sorghum grains compared to the other substrates (Patel & Singh, 2021). *Ralstonia solanacearum* preparation by flooding of the cultures with sterile distilled water and scrapping off the bacterial colonies allows for higher bacterial concentrations (Konappa *et al*., 2018). The quantity of inoculum inoculated ranges in various research work in this study 35 ml was drenched into the potted soils according to (Choudhary *et al*., 2018; Oussou *et al*., 2020). *Ralstonia solanacearum* inoculation of soil by drenching increases infestation levels of the pathogen due to direct contact with the soil particles (Morel *et al*., 2018). The *Trichoderma* species antagonistic activities are influenced by its source habitats (Al-Ani, 2018; Islam *et al*., 2021).

Trichoderma species isolated from marshy ecosystems when inoculated to plants have been observed to enhance the plant survival in water deficit areas (Singh *et al*., 2020). The plants in this case were able to withstand the pathogen a stress factor in the soil to the tomato plants due to the *Trichoderma* species concurring with findings by (Cornejo-Ríos *et al*., 2021) where the Trichoderma induced stress tolerance to tomato plants. The habitats with high organic matter enhance antagonistic, competitive and mycoparasitic ability of *Trichoderma* species (Ferreira & Musumeci, 2021; Mukherjee *et al*., 2022). *Trichoderma* spp increases the uptake of micronutrients and also helps in solubilisation of phosphates (Kamala, 2018).*Trichoderma* species are associated with plant roots growth impacting on plant vegetative growth and development (Zhang *et al*., 2019).By inducing plant growth promotion through colonizing roots the rooting system improves as they release secondary metabolites acting as auxins produce (Sofo *et al*., 2011).The *Trichoderma* species enhance uptake of ammonia nitrogen and minerals by the plant roots (Harman and Bjorkman, 2005). In this study, the *Trichoderma* species are observed to enhance the tomato plant growth concurring with studies showing them as plant growth promoters and soil borne disease suppressors (Adnan *et al*., 2019; Al-Askar *et al*., 2021). Their ability to stimulate plant growth when

applied as a treatment in this research work agrees with the work done by (Álvarez Romero *et al*., 2021; Bader *et al*., 2020; Ferreira and Musumeci 2021). The growth tomato exhibited in *Trichoderma* treatments implied better uptake of nutrients and water required by the plants for growth agreeing with the findings by (Abdullah *et al*., 2021; AL-surhanee 2022). Additionally (Al-Askar *et al*., 2021; Yu *et al*., 2021) found that the interaction between *Trichoderma asperellum* and *T harzianum* on tomato plants improved growth of root hairs mass on the roots which enhances nutrient uptake hence better growth. This further validated by (Rakibuzzaman *et al*., 2021) in the ability of *Trichoderma* species to enhance vegetative growth of plants. *Trichoderma* species have beneficial effects on crops, presented by (Elshahawy *et al*., 2017; Vinale & Sivasithamparam 2020) concurring with results from this work where plant height in the treated sections were better (Rostaminia *et al*., 2021).

The tomato plants were observed to grow with reduced bacterial incidence and severity, especially in the treated plots due to the ability of the *Trichoderma* species, helping the plants build resistance to the pathogen as observed in reports by (Guzmán-Guzmán *et al*., 2019; Sallam *et al*., 2019; Tseng *et al*., 2020). The severity of the pathogen on the tomatoes in the treated plots was greatly reduced, concuring with (Khan *et al*., 2020; TopolovecPintarić 2019). Similarly, Sood *et al*., (2021) reported similar findings that *Trichoderma* influences rapid growth and vigor (Al-Ani, 2019), hence reducing pathogen severity on a crop. *Trichoderma harzianum* and *T asperellum* have managed *Botrytis cinerea* in strawberry (Kuzmanovska *et al*., 2018) and have effectively controlled grey mould (*Botrytis cinerea*) in tomatoes (Herrera-Téllez *et al*., 2019; Risoli *et al*., 2022; Zhao *et al*., 2021). Fusarium wilt in tomatoes caused by *Fusarium oxysporum* have been managed by application of *Trichoderma harzianum* and *T asperellum* (Aleaghaee *et al*., 2018; Sallam *et al*., 2019; Vargas-Inciarte *et al*., 2019). Early blight management in tomatoes have been managed using *Trichoderma asperellum*, *Trichoderma harzianum* and *Trichoderma viride* (Ayodeji *et al*., 2022; Ghazanfar *et al*., 2019; Khalil *et al*., 2021).

Trichoderma species, when applied singly, have shown significant antagonistic effects against pathogens (Andrade-Hoyos, Silva-Rojas, & Romero-Arenas 2020; Irawati *et al*., 2020; Lava & Babaeizad 2021). This resulted in a lot of research work in different crops with single applications agreeing with the results in this work. Research has indicated the ease in single *Trichoderma* applications (Sánchez-Montesinos *et al*., 2021). In tomatoes, various *Trichoderma* strains have been applied singly and proven management of soil-borne pathogens as observed in these studies (Alelign 2020; Hasan *et al*., 2021; Sudhasha 2020). *Trichoderma* species in the management of soil-borne diseases have been sorted after single

applications and combinations showed good results in both scenarios concurring with the results herein (Mazen, 2021).

Soil treatments with mixtures containing *Trichoderma* isolates were more effective than the individual treatments. This attributed to the synergistic effect between *Trichoderma* isolates (Singh & Singh 2014). Hence suppression was enhanced with the synergistic effect of the interaction of the *Trichoderma* species. *Trichoderma harzianum* combined with *Trichoderma viride* had better results in suppressing *Fusarium verticillioides* causing fungal infection in maize (Kumar *et al*., 2021). *Trichoderma harzianum* and *T asperellum* have been used to manage *Pythium* causing damping off in tomatoes (Elshahawy & El-Mohamedy, 2019). Yields of the *Trichoderma* treatments were seen to be higher in this work and similar to previous studies (Abd-El-Kareem *et al*., 2019), this also concurs with Kumar *et al*. (2021). Commercially some countries have *Trichoderma* species combined into one formulation, which has been tested and shown promising results against plant pathogens (Gilardi *et al*., 2020), concurring with the obtained results.

4.5. Conclusion

Sterile sorghum grains support the growth of *Trichoderma* species. *Trichoderma* species from different habitats and manage bacterial wilt of tomatoes by reducing bacterial wilt incidence and severity. *Trichoderma* species mode of actions allowed nutrient uptake hence plant growth indicated by the high plant height. Hence yield increase in the *Trichoderma* species treated. The single and combined *Trichoderma* species were able to manage bacterial wilt of tomatoes.

CHAPTER 5:

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1. General Discussion

Trichoderma species were more abundant in undisturbed habitats than the disturbed due to high organic matter content in such ecosystems. *Trichoderma* populations were influenced by the soil biota which is responsible for the levels of biological communities in the soil. The more disturbed the soil biota, the lower its functional diversity and the biological communities living in it. The dense root volumes in the undisturbed habitats like the forests, manure and compost act as food reservoirs for the survival and multiplication of the beneficial biological communities. *Trichoderma* species populations are influenced by high organic matter and plant exudates mainly existing in undisturbed ecosystems (Graham & Strauss, 2021; Pandey et al., 2022; Vannucchi et al., 2021).Natural undisturbed ecosystems have higher microbial phyla, *Trichoderma* species being among them (Kumar et al., 2021). This concurs with findings that the cultivation activities in the disturbed ecosystems, damage the soil biota reducing microbial life (Certini *et al*., 2021).

Trichoderma harzianum and *T asperellum* were the most common *Trichoderma* species. The undisturbed habitats had *Trichoderma* species with the highest antagonistic activity against *Ralstonia solanacearum*. These habitats being undisturbed, had microbial life with high antagonistic interactions (Sarria *et al*., 2021). The isolated *Trichoderma harzianum* was more dominant in antagonism compared to isolated *Trichoderma asperellum* which concurs with work done by (Konappa *et al*., 2022). The incidence and severity of bacterial wilt in the planted tomatoes were lower in the *Trichoderma* species treatments due to their ability to antagonize the pathogen. This according to (Misra & Ansari, 2021) *Trichoderma* species gives the plants protective effect against the pathogen additionally the work by (Hasan *et al*., 2021; Jamil 2021) concurs with this study. Higher plant height, less stem browning, and better yield were observed in the *Trichoderma* treatments. El-Komy *et al*., 2022 found that there were synergistic effects when *Trichoderma* species are combined in the soil as seen in this study. The yield of the tomato plants was higher with the application of *Trichoderma* species agreeing with studies by (Kumar *et al*., 2021; Sani *et al*., 2020) that

Trichoderma species enhance growth hence yield increment. The applications of locally isolated *Trichoderma* species, from undisturbed environments were more sustainable.

5.2. Conclusion

The populations of *Trichoderma* species in different local habitats were found to be higher in the undisturbed habitats as compared to the disturbed habitats with *Trichoderma harzianum* and *Trichoderma asperellum* being the dominant species. *Trichoderma harzianum* and *asperellum* from the undisturbed habitats had higher antagonistic effect on *Ralstonia solanacearum* as compared to the same species from the disturbed soil habitats out of the fifty-one isolates. The efficacy of the carrier material for the multiplication of isolated *Trichoderma* species needs more stable formulation. The efficacy of the *Trichoderma* species in managing bacterial wilt of tomato was confirmed. This study validated that *Trichoderma* species can be isolated from different soil habitats locally, screened for antagonism, multiplied for application at field levels and manage bacterial wilt of tomatoes.

5.3. Recommendations

This study can be put into practical use by:

1. Further exploitation of *Trichoderma* species occurring in local environments. Enabling isolation of *Trichoderma harzianum* and *T asperellum* for use in the management of bacterial wilt in tomatoes from the undisturbed habitats.
2. More studies on carrier materials formulations that can be used on natively isolated *Trichoderma* species as antagonists as a solution to the local farmers for bacterial wilt management in tomatoes.
3. Application of *Trichoderma* species at early stages of tomato crops for the management of bacterial wilt of tomatoes. More bio-prospecting for other antagonists for *Ralstonia solanacearum* should be pursed locally to increase the solution scope for managing bacterial wilt in tomatoes.

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