DECLARATION

I Hope Takudzwa Mazungunye do hereby declare that this thesis is a result of my original research work except where clearly and specifically acknowledged. This thesis, "EVALUATION OF *Trichoderma* STRAINS AS BIOCONTROL OF *Fusarium oxysporum f.sp lycorpesici* IN TOMATO has not been submitted in any form before for any degree or examination in any other University.

Sign…… Date……..

Hope T Mazungunye

This is being submitted for the partial fulfilment of the requirements for Master of Science Honours degree in Crop Science (Agronomy) with the approval of the supervisors.

Sign ……. Date……

Dr Elizabeth Ngadze (Main Supervisor)

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ABSTRACT

Tomato (*Solanum lycopersicum L.)* is an important crop which provides people with essential nutrients e.g. potassium and phosphorus as well as anti-oxidants important in fighting cancers. Its production is threatened by the wilts caused by a fungus called *Fusarium oxysporum f.sp Lycopersici* which can cause yield losses of 50- 100 %. The disease is mainly controlled by use of synthetic chemicals which can pose a threat to human health and the environment. This study evaluated the use of *Trichoderma* strains as potential biocontrol of the *Fusarium oxysporum f.sp Lycopersici*. *In-vitro* experiment was a dual culture experiment with six treatments arranged in a Completely Randomized Design. The results showed *Trichoderma* strains (*T. harzianum, T. asperellum* – CA, C9, NY) reduced mycelial growth of *Fusarium* significantly ($p \leq 0.05$). The *Trichoderma* strains also showed a significantly high percentage inhibition of the pathogen ($p \leq 0.05$). The *in- vivo* experiment of tomato variety Tengeru evaluating two factors (2 methods of application and 3 biocontrol spp) and was arranged in completely randomized block design with six treatment combinations and three blocks. The experiment showed that *Trichoderma harzianum* and *Trichoderma asperellum* CA significantly ($p<0.05$) reduced the effects of the pathogen when compared with the control and improved the growth parameters of the tomato plants as well as chlorophyll content. The *Trichoderma strains* did not influence chlorophyll florescence ($p \leq 0.05$). Disease severity was significantly lower in *Trichoderma* inoculated treatments at ($p<0.05$) and lower in the control. Soil drenching proved to be a more effective method of application than seed treatment as shown in this study on many parameters. *Trichoderma* strains had significantly higher germination percentages ($p \leq 0.05$). The Trichoderma asperellum strains (CA and C9) significantly increased vigor Appendix ($p<0.05$) and significantly reduced the disease incidence percentage ($p<0.05$). *Trichoderma spp* was effective in suppressing *Fusarium oxysporum L*.

KEYWORDS: *Trichoderma harzianum, Trichoderma asperellum,* biocontrol, *Fusarium oxysporum*

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DEDICATIONS

I dedicate this to my mother who believed in me and pushed me to pursue a Master’s degree.

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LIST OF ABBREVIATIONS

TCA- *Trichoderma asperellum* (CA)

TC9 – *Trichoderma asperellum* (C9)

TNY- *Trichoderma asperellum* (NY)

T77- *Trichoderma harzianum*

M1- Seed Treatment

M2- Soil drenching

F- *Fusarium oxysporum L*

Spp- species

ST- Seed Treatment

SD- Soil drenching

FOL- *Fusarium oxysporum f.sp Lycopersici*

1 CHAPTER ONE

1.1 Background

Tomato (*Solanum lycopersicum L.)* is an important vegetable crop grown in Zimbabwe. Its popularity is due to its high nutritive value. Tomatoes are rich in many vital nutritional minerals (especially, phosphorus and potassium) and vitamins (B and C) (Barari, 2016a). Also, it helps in reducing the risk of common cancers like breast and prostate cancer. The crop provides an opportunity to generate cash income through sale of the fruit, offering many a livelihood. However, soil-borne pathogens cause a lot of economic yield losses through diseases (Barari, 2016a).

Approximately 800 million people in the developing world face food insecurity and at least 10% of food is lost due to plant diseases (Mrema *et al.*, 2014). Fungi has the biggest impact with regard to diseases and crop production losses compared to other plant parasites (Nisha *et al.*, 2011). Tomato and other solanaceous crops like potatoes are threatened by various fungal diseases which include vascular wilts. Tomato yield are significantly reduced by *Fusarium oxysporum f. sp. lycopersici* which destroys roots and also blocks the xylem of tomato plants at various growth stages, causing vascular wilt (Alwathnani and Perveen, 2012).

The use of synthetic chemicals remains the most widely used disease control method in the ongoing struggle against plant pathogens, even though there is risk of plant pathogens becoming resistant (Mecteau *et al.*, 2018). There is growing need for safe and sustainable disease control methods, and when coupled with an increasing export market potential for organically produced agricultural crops, it creates a need to assess other alternatives to reduce the use of environmentally hazardous synthetic chemicals (Mecteau *et al.*, 2018).

Several studies have shown the potential of biological agents in the control of Fusarium dry rots on solanaceae crops (Mejdoub-trabelsi et al., 2015). *Trichoderma* has been reported to show

potential antagonistic effects on *Fusarium* in other continents or regions on the African continent (Zaker, 2012). The control of the disease by *Trichoderma* may be achieved by competition, production of antibiotics or by mycoparasitism. However, biological efficacy of the *Trichoderma* spp may differ due to differences in ecosystems (Zaker, 2012). There is need to evaluate the effects of *Fusarium* spp which have been causing losses in the Zimbabwean tomato production sector and also apply the possible biocontrol agent (*Trichordema* spp) isolated from Zimbabwean soils.

1.2 **Problem statement**

Tomato *Fusarium* wilt has been on the rise in tomato production in Zimbabwe, resulting in yield and economic losses for farmers in Zimbabwe. *Fusarium oxysporum f.sp Lycopesici* is known to be one of the major *Fusarium* spp that cause wilt disease (Amini and Sidovich, 2010). The main method of control has been the use of synthetic chemicals, which are known to have detrimental effects to the environment when overused (Amini and Sidovich, 2010). Fusarium wilt is soil-borne in nature therefore, the application of fungicides to control this disease is significantly difficult (Alwathnani and Perveen, 2012). Fungicides are known to cause health hazards to the user and subsequent consumers, as chemical residues remain on the fruit (Barari, 2016a). Fungicides may also kill other various beneficial organisms in the soil and their toxic form persists, contaminating the whole environment. For example, it can cause underground water contamination which may affect non targeted fauna as well as drinking water for the human population (Sinha *et al*., 2018). There is risk of fungicidal resistance development through continued use of fungicides which will aid the whole fungal complex including *Fusarium* spp, *Pythium* spp and *Phytophlora* (Daami-Remadi *et al.*, 2006). Furthermore, new and improved pathogen spp have overcome host resistance creating a need to discover new resistant varieties which is expensive and difficult when the dominant gene is unknown (Amini and Sidovich, 2010).

The use of biocontrol (*Trichordema*) has been shown to reduce disease severity of *Ralstonia solanaceae,* isolated from infected potatoes from the seed potato producing area of Nyanga in Zimbabwe (Muhera, 2017). There is a growing problem of *Fusarium* wilts on tomato production in the country, as there is a knowledge gap in terms of the potential use of *Trichoderma* spp isolated from Zimbabwean soil for the control of *Fusarium oxysporum f.sp Lycopesici*.

1.3 **Justification**

Several studies, (Khalifa *et al.*, 2013; Schisler *et al.*, 2000 ; Ommati *et al*., 2013) have shown the potential of biological control of different *Fusarium* spp using *Trichoderma asperellum* in their regions. However in Zimbabwe, little information has been published so far concerning its potential as a biocontrol method. Therefore, there is a need to assess the potential of *Trichoderma* spp isolated from the Zimbabwean soils to control this pathogen.

The continued use of synthetic chemicals as the sole control method adopted by many farmers is of concern due to the risk of fungicide resistance development (Fravel *et al*., 2003). Thus, there is need for a more sustainable integrated control method of the disease which may substitute or compliment the use of synthetic chemicals in the control of *Fusarium* in tomato production (Lu *et al.*, 2004).

The reduced use of fungicides may be a lucrative option for produce destined for the European market which has increased its demand for organically produced vegetables (Ghazalibiglar *et al., 2016).* The risk of rejection of export tomato will be lowered due to the reduced risk of fungicide residues on the fruits (Alwathnani and Perveen, 2012).

If the *Trichoderma* isolates are successful in reducing the disease in the study, subsequently there will be a lesser reliance on fungicides or synthetic chemicals to control *Fusarium* and

other pathogens. As a result, the risk for environmental pollution is also reduced. (Muhera, 2017).

1.4 **Hypotheses**

1. The locally isolated soil-borne antagonists evaluated under *in-vitro* conditions are effective in suppressing *Fusarium oxysporum f.sp lycopesici* in tomatoes.
2. The locally isolated soil-borne antagonists evaluated under *in-vivo* conditions are effective in suppressing *Fusarium oxysporum f.sp lycopesici.*
3. Seedling treatment with Trichoderma is more effective in *Fusarium oxysporum f.sp lycopersici* suppression as compared to soil drenching.
4. *Fusarium oxysporum f.sp lycopersici* reduces tomato seed germination, plant vigour and increases disease incidence in tomato seedling production.

1.5 **Aims**

To evaluate *Trichoderma* strains as a biocontrol method against *Fusarium oxysporum f.sp lycopersici*.

1.6 **Specific Objectives**

1. To evaluate the use of *Trichoderma* spp in suppressing *Fusarium oxysporum f.sp Lycopersici in-vitro.*
2. To evaluate the use of *Trichoderma* spp in suppressing *Fusarium oxysporum f.sp Lycopersici* in-*vivo.*
3. To compare the most effective method of application of the antagonist between soil drenching and seedling treatment *in-vivo*.
4. To determine the effect of *Trichoderma spp* and *Fusarium oxysporum f.sp Lycopersici* on germination percentage, vigour Appendix and percentage disease incidence of tomato seedlings *in-vivo.*

2 CHAPTER 2

2.1 LITERATURE REVIEW

Tomato (*Solanum lycopersicum L*) is one of the most important commercial vegetable crops grown in Zimbabwe and around the world. Tomato belongs to the family Solanaceae and is a native crop of Peru and México (Verma *et al.*, 2017). Tomatoes are a nursery-based vegetable cultivated for the fleshy fruits which can be eaten raw or cooked (Verma *et al.*, 2017). They are also recommended by dieticians and nutritionists as they are known for controlling cholesterol and weight reduction (Mj *et al.*, 2017). It is also rich in medicinal value (Chavan *et al.*, 2011; Verma *et al.*, 2017) and is well reported to have antiseptic properties against intestinal infections. Being a rich source of lycopene, tomato is used in the treatment of cancer; especially the prostate cancer (Giovannucci, 1999; Verma *et al.*, 2017). Tomato is primarily used to produce soups, juices, ketchups, purees, pastes and powders.

The tomato plant is attacked by various diseases that include *Rhizoctonia solani*, *Phytopthora*, *Pithium* and *Fusarium* which lead to significant yield losses (Ghazalibiglar *et al*., 2016). *Fusarium* wilt is one of the most serious diseases affecting tomato yield. This disease is caused by *Fusarium oxysporum f. sp. Lycopersici* and the yield loss due to this disease is 25 - 50 % in Tunisia (Enespa, 2014; Mj *et al.*, 2017). *Fusarium* spp are well established soil borne pathogens in all soil types throughout the world. *Fusarium* spp. are saprophytes and are able to grow on soil organic matter for a prolonged period (Ghazalibiglar *et al*., 2016).

The most commonly used methods of control of tomato wilts are the use of fungicides, as well as planting of resistant or tolerant varieties (Fravel, 2003; Ghazalibiglar *et al*., 2016). However, fungicide application is mostly ineffective and difficult as the chemical may not reach the fungal spores which are widely distributed in the soil (Ghazalibiglar *et al*.,, 2016). It is difficult to control *Fusarium* wilt in tomatoes due to the pathogen progression within the vascular tissue, limiting the efficacy of fungicides (Verma *et al.*, 2017). Chemical application is also reported to

be an environmental hazard as well as health hazard to consumers who eat the fruit; resulting in a move towards more sustainable methods of controlling the pathogen (Theradimani *et al.*, 2018).

In the soil, there are organisms with antagonistic capabilities and one of the most popular are *Trichoderma* spp (Arenas1 *et al.*, 2018). The use of *Trichoderma* as a bio-control agent has been widely reported and interest has been ever growing as it is seen as a potential sustainable way of controlling *Fusarium oxysporum f.sp Lycopersici* (Ghazalibiglar *et al,* 2016; Mj *et al.*, 2017; Barari, 2016b). This study explores this option in Zimbabwean tomato production using local *Trichoderma* isolates and evaluates its use as a bio-control agent.

2.2 **Tomato Production in Zimbabwe**

Tomato production has been rising annually in Zimbabwe (Mrema *et al.*, 2014). Tomatoes are a foreign currency earner when grown for export. However, export markets have strict quality standards for the produce. Chemical residues on fruits may result in rejection for export and thus use of synthetic fungicides should be heavily regulated in disease and pests management (Sinha *et al*., 2018). This gives biological control an edge, making it an exciting prospect for use in Zimbabwean tomato production. In addition, with the ever growing population the demand for the crop increases as well, creating a need to reduce losses caused by *Fusarium* wilts (Sinha *et al*., 2018).

The production in Zimbabwe has increased from approximately ten thousand tons in 1980 to just below the 30 000 ton mark in 2017 (Figure 1. 1) (FAO, 2017). The main producers of the crop are China and India. In Africa, Egypt is ranked toped in terms of production. In comparison, Zimbabwe has a world share of 0.0% and is ranked 107th (Figure 1. 2)**.** One of the major constraints in the production of tomato in Zimbabwe is the effect of diseases particularly *Fusarium* wilts (FAO, 2017).

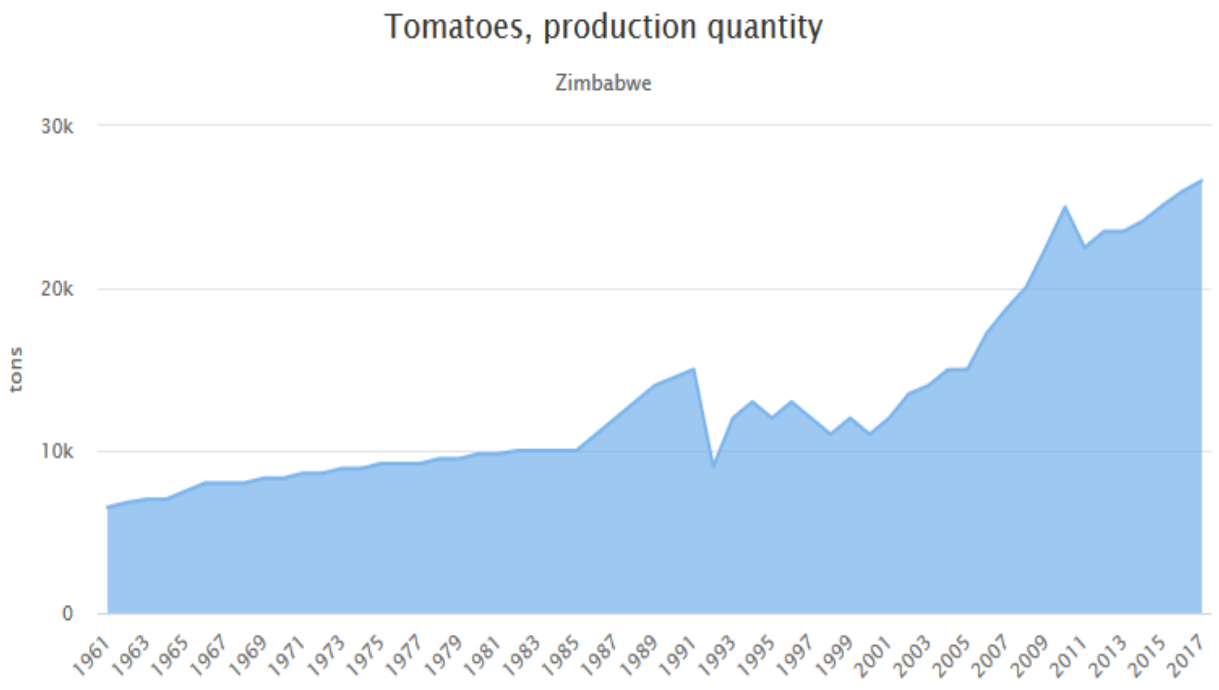

Figure 1. 1 Production quantity trend of tomatoes in Zimbabwe from 1961-2017 (FAO, 2017)

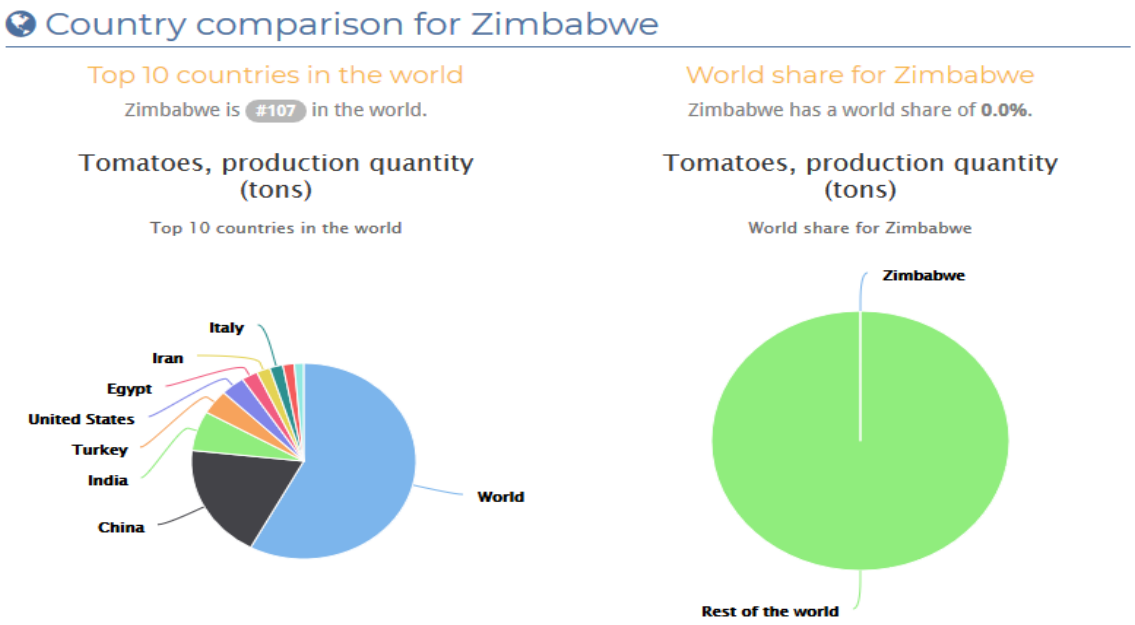

Figure 1. 2: World share statistics for tomato production quantity (tons) (FAO, 2017).

2.3 Tomato *Fusarium* wilt Pathogens

Fusarium is a wide genus classed to the Ascomycota phylum and includes more than hundred species that are distributed in soils or are linked with plants (Xu *et al.*, 2014). Fusarium has a global distribution and is responsible for severe vascular wilts, rots of various plant structures such as roots, cobs, stalks, tubers, bulbs, seedlings, and corms of a wide range of plants. In addition, *Fusarium* species also cause post-harvest dry rots and stem-end rots throughout the growing season in potatoes (Du *et al.*, 2012).

Fusarium oxysporum f.sp Lycopersici fungal colonies consist of whitish aerial mycelia that later produce dark violet pigments with time which are characteristic of the pathogen (Xu *et al.*, 2014). The pathogen has three types of conidia which are macroconidia, microconidia, and chlamydospores (Joshi and Chaturvedi ., 2013; Hristov, 2007). Its macro and micro-conidia characteristics are thin walled 3-5 septate, fusoid falcate macro conidia with somewhat hooked apex and pedicillate base respectively (Hristov, 2007). *Fusarium* spp. is a slow growing species

and is characterized as creamish white to creamy, light pink and light purple to violet (Elad and Chet, 2011).

2.4 **Symptoms of *Fusarium* wilts**

The most noticeable symptoms produced by *F. oxysporum fsp Lycopersici* occur in the transplantation of tomato seedlings and at the beginning of flowering (Arenas1 *et al.*, 2018). An infected tomato plant will begin yellowing on the bottom leaves. The yellowing will begin on one side of the leaf, shoot, or branch and then slowly spread out and up the vine. The vines will brown along the veins and eventually wilt permanently, resulting in a stunted plant. If the plant does not die, it will be weak and produces low quality tomatoes. The infected plants and their root systems become stunted and the degree of stunting is influenced by time of root infection. Plants infected early will be more severely stunted than those infected at a later stage (Cerkauskas, 2005).

2.5 **Conditions promoting disease development of *Fusarium oxysporum f.sp Lycopersici***

The infections mostly originate from the fungus from infected tomato debris. Other diseases like root knot nematode infections make wilt-resistant varieties more susceptible to the fungus because of physiological changes in the root as it is a wound pathogen (Verma *et al.*, 2017).

Disease development is ideal in warm temperatures (from 27/28 °C), acidic soil (pH 5–5.6) and dry weather conditions. Rapidly growing, tender tomato plants fertilized with ammonium nitrate are mainly susceptible to the disease. The fungus can be disseminated by infected seed or by transplants grown in infested soil. The fungus can be introduced into a field on contaminated equipment, training stakes, packing crates or shoes. Soil particles from infested fields may be blown into disease-free fields (Theradimani *et al*., 2018).

2.6 Control measures

2.6.1 Chemical control

Ammonium based nitrogen fertilizer makes tomato more susceptible to oxysporum L infection. Therefore, use of other nitrogen based fertilizers for example calcium nitrate would reduce the incidence of the pathogen (Cerkauskas, 2005). Several fungicides can also be used to control the fungus for example methyl bromide, which unfortunately is not environmentally friendly (Fravel *et al*., 2003). However, control of *Fusarium* wilt of tomato is very difficult because pathogen affects the vascular tissues which limits the effectiveness of fungicides (Verma *et al.*, 2017). The raising of soil pH to 6.5–7.0 can be another chemical control method (Cerkauskas, 2005).

2.6.2 Cultural control

Good fertilizer management, use of clean resistant varieties, weeding are some of the good cultural practises that reduce the pathogen incidence in tomatoes (Cerkauskas, 2005). The crops should only be weeded when extremely necessary since it's a wound pathogen and the fungus gains entry into plant tissue through injured tomato roots. Crop rotation for example the use of paddy rice (*Oryza sativa*) in rotation with tomato will reduce inoculum in the soil. These however do not result in the complete eradication of the pathogen but rather can help reduce the inoculum in the soil (Cerkauskas, 2005).

2.6.3 Phytosanitary control

The use of contaminated tools, shoes, clothing and hands of workers poses a high risk of infestation. Therefore, the use of clean equipment to avoid infesting new fields is a control measure. There is also need to use clean non-infested water and not use pond or ditch water located near infested fields to irrigate. Disinfecting areas where transplants are grown. Sanitization of greenhouse structures, crates, benches, tools and flats is also a vital way of reducing pathogen inoculum and infection (Cerkauskas, 2005). On-field phytosanitary measures

also include use of footbath before entering greenhouses or tomato fields. On a national level, strict phytosanitary measures have been put in place and these include the strict regulation of plant material at borders.

2.6.4 Biological control

The examples of agents responsible for biocontrol activity include rhizosphere competent fungi and bacteria, which are also capable of inducing growth responses by controlling minor pathogens or producing growth stimulating factors (Alwathnani and Perveen, 2012). There are advantages reported to be associated with the use of the biological control agents such as self-sustaining spread on their own after initial establishment, reduced inputs of or renewable resources and offers long term disease suppression in an environmentally friendly manner (Feng, 2012).

The examples of biocontrol agents include cyanobacteria which has been studied for the control of plant pathogenic fungi, particularly soil borne (Alwathnani and Perveen, 2012). Biological control, therefore, holds promise as a strategy for disease management. Biocontrol agents (BCAs) including fluorescent Pseudomonas, a non-pathogenic *Fusarium* strain, *Trichoderma* spp , have been reported to provide control of *Fusarium* wilt (Ghazalibiglar *et al.,* 2016*).*

2.6.5 *Trichoderma* as biocontrol agent

Trichoderma spp are commonly known soil borne fungi that have become popular for their biocontrol of some diseases as well as enhancing plant growth (Lu *et al.*, 2004). *Trichoderma* spp. are found in almost all soil types including cultivated soil, garden soil, fallow and pasture land, forest soil etc. (Gary *et al.,* 2004; Verma *et al.*, 2017). The antagonistic activity of

biocontrol by *Trichoderma* strains is attributed complex mechanisms which include the nutrient competition, anti-biosis, action of cell wall-lytic enzymes, initiation of a systemic resistance, parasitism and improved plant nutrient availability (Theradimani *et al*., 2018).

A previous *in-vivo* study showed the occurrence of and sequence for the several stages and gene expressions of necrotrophic parasitic interaction between *Atroviride* and *P. ultimum* Lu *et al* (2004). The chemotactic growth of *Atroviride* to the host and the winding around the host hyphae were commonly observed in a study by (Lu *et al.*, 2004). The growth of the helix-shaped hyphae by the *T. atroviride* mycoparasite happened in both the presence and absence of the host (Verma *et al.*, 2017).

Trichoderma atroviride reduced disease incidence of *Fusarium* spp and increased plant yield in tomato in a study by (Verma *et al.*, 2017). Moreover, in that same study, many plant growth characteristics including fresh weight, dry weight, root length, plant height were increased. Also, the *Fusarium* severity was lowered in *Atroviride* treatments as compared to the *Fusarium* spp control (Verma *et al.*, 2017).

The search for *Trichoderma* with high antagonistic potential against various plant pathogens in several crops has gained more attention and momentum as it is a sustainable option for disease management (Muhera, 2017). In many other countries, the use of *Trichoderma* isolated from their local soils has been extensively studied with some even pointing out that the action of *Trichoderma* can be different due to different environmental conditions or microclimates (Daami-remadi *et al*., 2006). This has prompted this study to assess the biocontrol potential of *Trichoderma* isolates from Zimbabwean soils *in-vitro* and *in-vivo* and contribute to literature.

3 CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Experimental Study site

The *In-vitro* and *In-vivo* experiment were conducted at the Department of Crop Science, University of Zimbabwe (17.78^0S, 31.05^0E and altitude of 1523 meters). This experiment was conducted to account for the antagonistic effect of *Trichoderma* spp against *Fusarium oxysporum f.sp Lycopersici*.

3.2 In-vitro Experiment

3.2.1 Pathogen used in the study

Fusarium oxysporum f.sp Lycopersici was regenerated from the University of Zimbabwe Plant Pathology lab culture collection. The *Fusarium spp* was cultured on Potato Dextrose Agar (PDA) for one week before use in the dual cultures.

3.2.2 *Trichoderma* strains used in the study

Trichoderma harzianum strain T77, *T. asperellum* strain Nyehu, *T. asperellum* C9, *T.* CA was obtained from culture collection. They were cultured on PDA (Potato Dextrose Agar) at 27 ^oC for one week and then used in the dual cultures against *F. oxysporum f.sp Lycopersici*.

3.2.3 Experimental design

A completely randomized factorial design where treatments (*Trichoderma spp* and controls as well as *Fusarium* spp were the fixed factors. Means were separated using Fisher's projected LSD at ($P < 0.05$).

3.3 In-vitro antagonistic activity of *Trichoderma* spp against *Fusarium oxysporum f.sp lycopersici*

The dual culture technique was used in the evaluation of biocontrol potential of the *Trichoderma* spp against *Fusarium oxysporum f.sp L* on PDA. The 90 mm diameter Petri dish was divided into two segments and the pathogen and antagonist were placed 20 mm equidistant from the line. A cork borer was used to obtain discs of 6 mm from the sub cultured pathogen

and *Trichoderma* plates and placed on 20 mm equidistant points on opposite sides of the Petri dishes. Control Petri dishes had the same microorganism on either side so as to compare with pathogen antagonist treatments. A total of six treatments were used and the treatments were replicated three times.

Table 3. 1 Treatment structure of the in-vitro experiment

Treatment	Pathogen	*Trichoderma* spp
1	*Fusarium oxysporum* L	*Trichoderma asperellum*. (NY)
2	*Fusarium oxysporum* L	*Trichoderma harzianum* (T77)
3	*Fusarium oxysporum* L	*Trichoderma asperellum* (TCA)
4	*Fusarium oxysporum* L	*Trichoderma asperellum* (TC9)
5	*Fusarium oxysporum* L	*Fusarium oxysporum* L
6	*Trichoderma asperellum(*NY*)*	*Trichoderma asperellum (*NY*)*

3.4 Data collection

Radial growths as well as inhibition zones were measured at one, four, seven and ten days after culturing. Radial growth was measured using a ruler.

Pathogen growth inhibition was calculated using the following formula:

$$\text{Growth inhibition } \% = ((C1 - C2) / C1) \times 100$$

Where, C1: Mean diameter of pathogen colony in control plates and C2: Mean diameter of pathogen colony in presence of antagonist. Statistical analysis was performed using Genstat 14.

3.5 Experiment 2: *In-vivo*

3.5.1 Experimental site

In-vivo experiment was carried out at the Department of Crop Science greenhouses, University of Zimbabwe (17.78^0S, 31.05^0E and altitude of 1523 meters).

3.5.2 Choice of Tomato variety

The tomato seedling variety used is the local variety Tengeru. This is a determinate tomato variety with fruits that are firm with very good red color both internal and external color.

A mature fruit weighs between 90-120g and fruits mature at the same time and thus can be harvested at once. The variety has intermediate resistance to pests like nematodes and also intermediate resistance to bacterial wilt, tomato yellow leaf curl virus disease, tomato mosaic virus and fusarium wilt.

3.5.3 *In-vivo* Antagonistic activity of *Trichordema spp* against *Fusarium spp* in tomato

Black plastic bags were dipped for 24 hours in 1% sodium hypochlorite solution at the crop science field laboratory and then rinsed with distilled water before planting commenced. The plastic pots were filled with red fersialitic soil. The soil was oven dried for 24 hours at 100^{o}C temperature to sterilize it. The *Trichoderma* species trial had one commercial strain *t, harzianum* (T77) and four *Trichoderma* isolates from culture collection namely, *T. asperellum* (NY), *T. asperellum* (C9) and *T. asperellum* (CA) which were tested for their antagonism in this experiment. The tomato variety which was used is Tengeru which is one of the popular local varieties on the market.

3.5.4 Experimental design

The experimental design was a 2×3 factorial in Randomized Complete Block Design replicated 3 times i.e. (2 methods by 3 Trichoderma treatments. The two methods were (method 1- soil drenching and method 2 - seedling treatment). The control was with pathogen and without antagonist.

Table 3. 2 Treatment combinations of the in-vivo experiment

Treatment no	Treatment description 1	Treatment description 2
1	T77 + method 1	T77 + method 2
2	T CA + method 1	TCA + method 2
3	Control with pathogen + method 1	Control with pathogen + method 2

Key: T77 – *Trichoderma harzianum*, TCA - *Trichoderma asperellum*, **method 1**- seedling dressing, **method 2** soil drenching.

3.5.5 Application of antagonists

The Trichoderma spp *T. harzianum* (T77), *T. asperellum* (TCA and TC9) were sub cultured on PDA for 14 days and then the spores were scrapped off into 100 ml of sterile distilled water and shaken for 30 minutes in the magnetic stirrer. The number of spores was determined shortly after by the hemocytometer and the concentration adjusted to 10^4 spores ml^{-1} by using the following equation:

$$\text{Concentration} = \frac{\text{Required Concentration}}{\text{Inital Concentration}} \times \text{Volume To Be Made}$$

To apply the seedling treatment, the antagonists were applied before transplanting. The seedlings were soaked for 30 minutes in the antagonists' suspension mixed adjusted to 10^4 spores ml^{-1} for *Trichoderma*. The treated seedlings were then transplanted.

Seedlings that were not treated were immersed in sterile distilled water for 30 minutes. These seedlings were later drenched with the antagonist which was the second method of application of the antagonists (soil drenching) by drenching with 100ml of the 10^4 spores ml^{-1} concentrated solutions of the antagonists (T77, TCA). This method was conducted a week after the inoculation with the pathogen.

3.5.6 Inoculation with *Fusarium oxysporum f.sp lycopersici*

Fusarium oxysporum f.sp lycopersici was cultured in PDA for 14 days and then scrapped off and mixed with sterile water and put on a magnetic shaker for 30 minutes. The final

concentration was determined by the hemocytometer and the concentration was adjusted to 10^4 spores ml^{-1}. The tomato plants in all treatments for the experiments were inoculated with the pathogen at the third and fourth leaf stage by punching each plant with sterilized needles at the base of the stem above the upper secondary roots (Muhera, 2017). A volume of 100 ml of the suspension were drenched in blocks 1, 2 and 3, respectively in all pots over the wounded area. After the inoculation process, all the pots were covered by polythene bags for 24 hours to maintain high humidity (Maji and Chakrabartty, 2014; Muhera, 2017).

3.5.7 Data Collection

The tomato plants were monitored for the development of wilt symptoms at 14, 21 and 35 days after transplanting (DAT). The disease Appendix data were recorded using the scale developed by Mandal *et al.* (2017)

Table 3. 3 Disease severity scores used in the in-vivo experiment (Adapted from **Mandal *et al.* (2017)**

Scale	Description
0	No wilting
1	1-25 %
2	26-50%
3	51-75%
4	76-100%

3.5.8 Plant growth assessments

Fourteen days after the application of soil antagonist isolates, the effect of tested antagonists on plant growth were measured in terms of plant height, number of nodes per plant, number of flowers per plant 40 days after inoculation.

3.5.9 Chlorophyll content and fluorescence

Chlorophyll content was measured at two weeks after inoculation at the same hour by a portable chlorophyll meter. The readings were done on two fully expended leaves (two readings per leaf). The chlorophyll fluorescence was measured using a chlorophyll fluorescence meter.

3.5.10 Statistical analysis

Disease severity, plant height, number of nodes per plant, number of flowers per plant and chlorophyll data were subjected to the repeated measures ANOVA using Genstat. Fishers LSD was used to separate the means at 5% significance level.

3.6 Experiment 3: *In-vivo* germination test

3.6.1 Choice of Tomato Variety

The tomato variety used was the local variety Tengeru from prime seeds.

3.6.2 Experimental design

Randomized complete block design (RCBD) was used with three blocks

3.6.3 *In-vivo* germination test to assess antagonism of *Trichoderma* spp against *F Oxysporum f.sp lycopersici*

Oven sterilized fersialitic soil was inoculated with the *F. oxysporum f.sp lycopersici* spores (1.0×10^4 spores/g of soil mixture) before the seeds were sown. Surface sterilized (0.1% sodium hypochlorite) tomato seeds were planted in each compartment. The inoculums of *T. asperellum* (TCA and TC9) were prepared in the form of a conidial suspension (10^4 spores / ml) as described above. Tomato seedlings were raised in seedling trays. The control was seedlings with pathogen without antagonist and the plants were watered regularly.

3.6.4 Data collection

The percentage seed germination was determined 14 days after sowing by using the following equation:

$$\text{Germination Percentage} = \frac{\text{Number of seedlings germinated}}{\text{Total number of seeds sown}} \times 100$$

Disease assessment for incidence of wilt was determined after three weeks of sowing and Percent Disease Incidence was recorded at 14 days after sowing. Percent disease incidence was determined using the following equation:

$$\text{Percent Disease Incident} = \frac{\text{Number of seedlings affected}}{\text{Number of seedlings germinated}} \times 100$$

Seedling vigour Appendix was calculated by using the formula as described by Abdul-Baki and James (1973) and Sinha (*et al*. 2018). Data on root length and shoot length was recorded 14 days after sowing. The data was used in the calculation of vigour Appendix.

$$\text{Vigour Appendix} = (\text{Mean root length} + \text{Mean shoot length}) \times \text{Germination percentage.}$$

3.6.5 Data analysis

ANOVA was done using Genstat 14 package. The means were separated using the Fischer's protected LSD at $P < 0.05$.

4 CHAPTER 4

RESULTS

4.1 Experiment 1 (*in-vitro*)

4.1.1 Radial Growth

Trichoderma spp. significantly reduced ($P < 0.05$) radial growth of *Fusarium oxysporum f.sp Lycopersici*. The lowest radial growth of 23 mm was recorded in *T. asperellum* (NY), while the highest 37.75 mm was recorded in *T. asperellum* (NY) control. The second highest radial growth of 32.42 mm was recorded in the pathogen control (Figure 4. 1).

Table 4. 1: A radial growth of *Fusarium oxysporum f.sp Lycopersici* in dual cultures with Trichoderma isolates *in-vitro* assays.

Biocontrol	Radial Growth
T. asperellum NY	23 a
T. harzianum	27.67 b
T. asperellum CA	26.58 ab
T. asperellum C9	25.08 ab
Fusarium(control)	32.42 c
T, asperellum(NY)(control)	37.75 d
F value	**<0.01**
s.e.d	**1.981**
LSD	**3.948**
CV%	**15.7**

Means followed by the same superscript do not differ significantly at p < 0.05.

4.1.2 Percentage Inhibition

Trichoderma harzianum and Trichoderma asperellum (CA) showed the lowest inhibition % of *F. oxysporum f.sp Lycopersici* ($P < 0.05$). These two were not significantly different ($p \leq 0.05$) from each other. *Trichoderma asperellum* (NY) had significantly ($P < 0.05$) higher inhibition % of the pathogen at day four and day seven. *Trichoderma asperellum* (C9) had a significantly ($P < 0.05$) higher percentage inhibition of the pathogen at day one and day ten (Figure 4. 1).

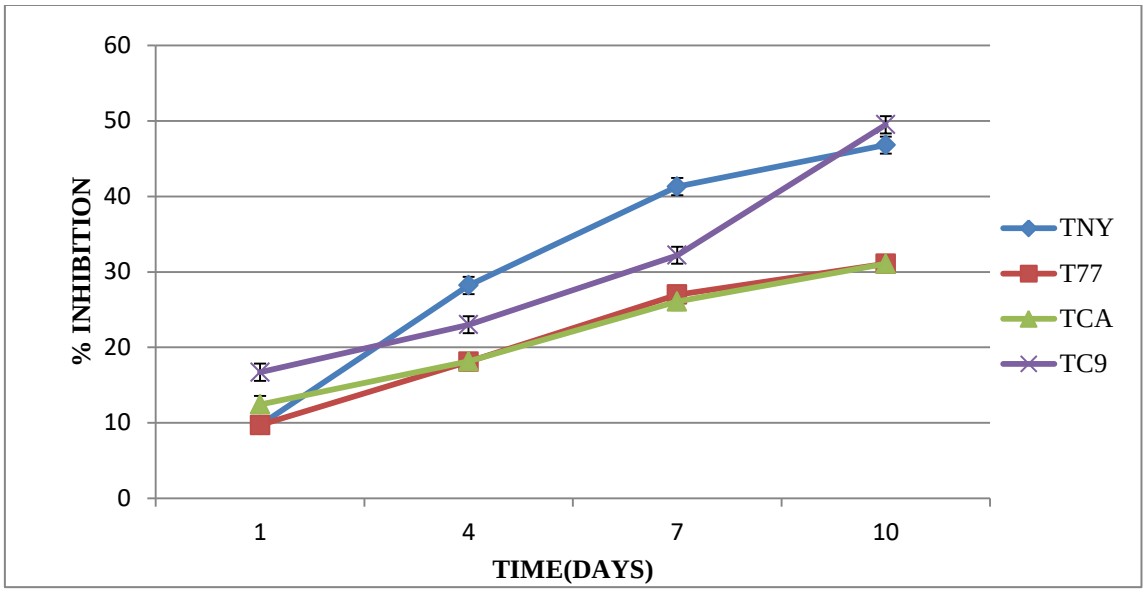

Figure 4. 1: Effect of *Trichoderma* isolates on % growth inhibition of *Fusarium oxysporum spp L* over time (dual culture method). Means with error bars that overlap do not differ significantly p (<0.05). Error bars represent standard error differences of the means.

4.2 Experiment 2 (*in-vivo*)

4.2.1 Plant height

There was a significant ($p<0.05$) interaction between application method and biocontrol agent used. *Trichoderma harzianum* applied using the soil drenching methods recorded significantly ($P < 0.05$) taller plants with an average height of 20.93 cm at 14 Days after application. There was no significant difference between the *Trichoderma* treatments applied using the drenching method (Figure 4. 2). *Fusarium oxysporum f.sp Lycopersici* applied using the soil drenching method recorded significantly ($P < 0.05$) shorter plants with an average height of 9.43 cm at 14 days after application of biocontrol. *Trichoderma asperellum* applied using the drenching method recorded significantly ($P <0.05$) taller plants at 21 days after application. There was no significant difference between the *Trichoderma* treatments applied using the drenching methods (Figure 4. 2).

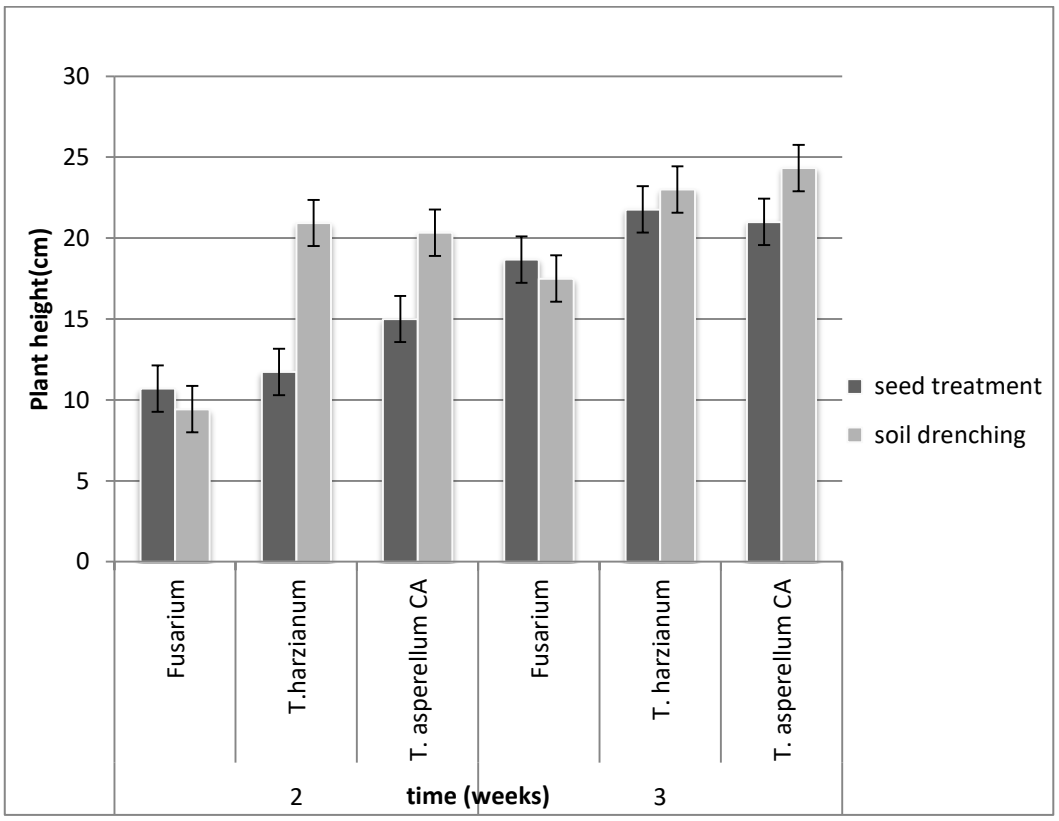

Figure 4. 2: Showing the interaction of biocontrol and method of application on tomato plant height at weeks 2 and 3 after inoculation. Bars with error bars that overlap do not differ significantly at (P < 0.05). Error bars represent the standard error of differences of means.

4.2.2 Disease severity

There was a significant interaction ($p<0.05$) between biocontrol and method of application of the antagonist. The control plants which were soil drenched with *Fusarium* but not treated with the biocontrol recorded a significantly ($P < 0.05$) high disease severity (1.498). *Trichoderma harzianum* soil drenched recorded a significantly ($P <0.05$) low disease severity. There was no significant difference between the Trichoderma treatments (Figure 4. 3).

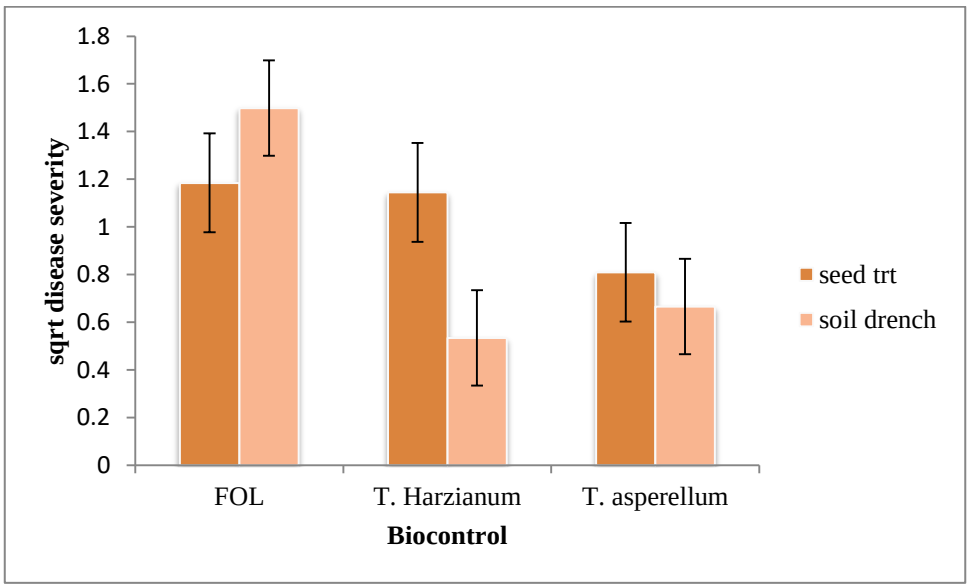

Figure 4. 3: Showing effect interaction of method of application and biocontrol. Bars with error bars that overlap do no differ significantly at ($p \leq 0.05$). Error bars represent the standard error of difference of the means.

4.2.3 Nodes per plant

There was a significant interaction ($p< 0.05$) between method of application and biocontrol for the number of nodes count per plant. *Trichoderma harzianum* soil drenched treatments recorded significant ($P < 0.05$) high numbers of nodes per plants (16.5). The *Trichoderma* treatments were not significantly different from each other (Figure 4. 4). The soil drenched control plants recorded a significantly ($P < 0.05$) low number of nodes 7.5 (Figure 4. 4).

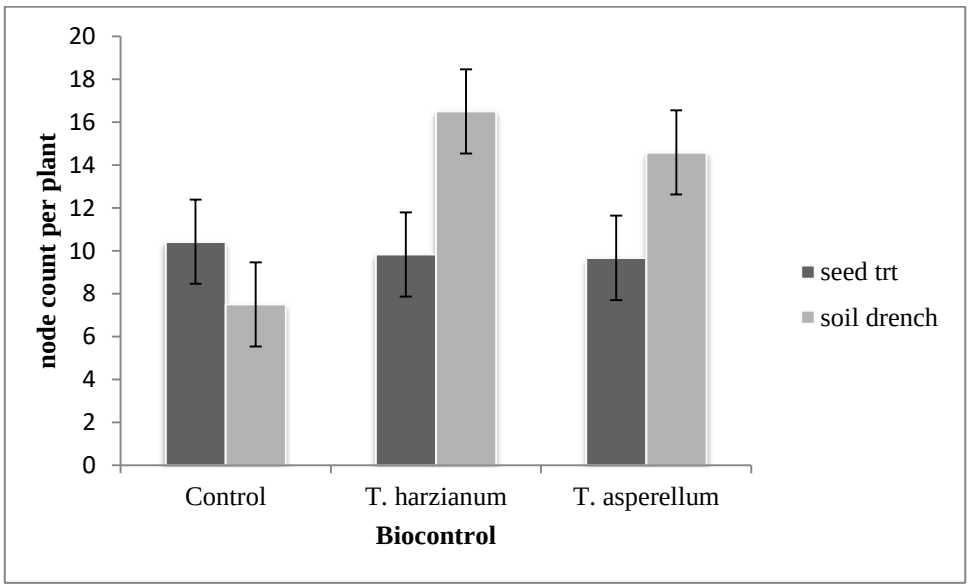

Figure 4. 4: Interaction of biocontrol and method of application on number of nodes count. Bars with error bars that overlap do not differ significantly (P < 0.05). Error bars represent the standard error of differences of means.

4.2.4 Chlorophyll content and fluorescence

The control and biocontrol significantly ($P \leq 0.05$) recorded different levels of chlorophyll (Figure 4. 5). There was no significant difference ($p<0.05$) between the biocontrol treatments and *T. harzianum* (46.76) recorded the highest chlorophyll content at week 3 whilst the control with pathogen without antagonist recorded the least (Figure 4. 5). There was an interaction between time and method of application.

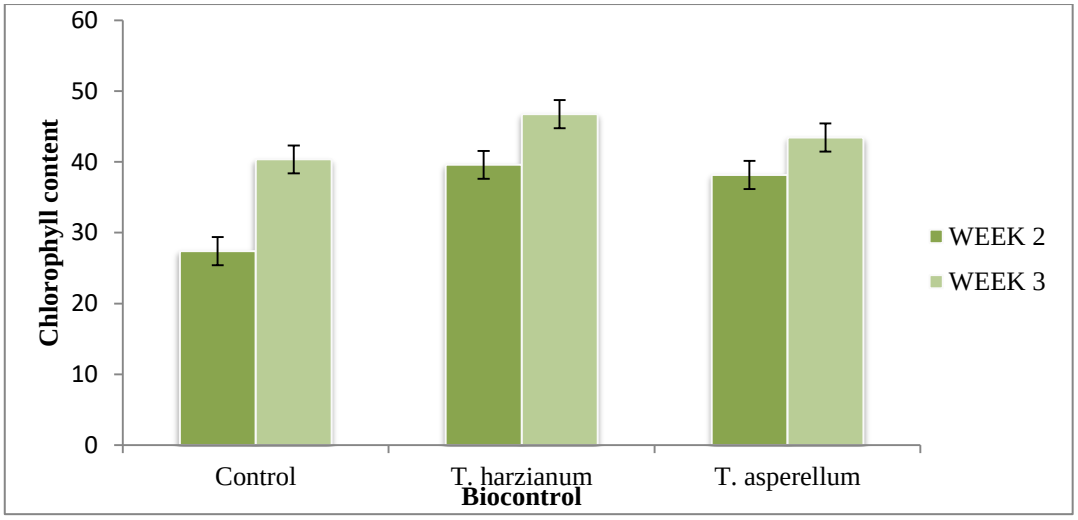

Figure 4. 5 Effect of biocontrol on Chlorophyll content at week 2 and 3. Bars with error bars that overlap do not differ significantly (P < 0.05). Error bars represent standard error of differences of the means.

Soil drenched plants at week three had a significantly (P<0.05) high chlorophyll content 46.39 (Figure 4. 6). Chlorophyll content of soil drenched treatments was significantly (P < 0.05) high at week two than seed treated treatments at both week two and three (Figure 4. 6).

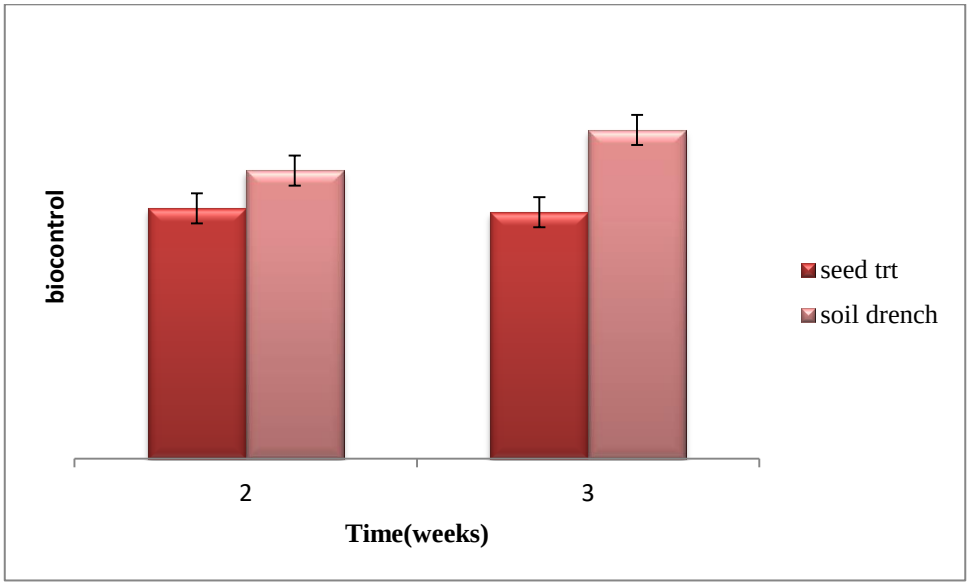

Figure 4. 6: Interaction between method of application and time on chlorophyll content. Bars with error bars that overlap do not differ significantly (P < 0.05). Error bars represent standard error of differences of the means.

Chlorophyll fluorescence showed no significant difference in terms of biocontrol, method of application as well as the interaction of biocontrol and method of application and time.

4.2.5 Flower count

The Trichoderma strain, *Trichoderma harzianum* recorded a significantly (P < 0.05) higher number of flowers per plant 8.5 flowers (Figure 4. 7). The control treatment with pathogen without antagonist recorded the least number of flowers per plant (1.5 flowers) (Figure 4. 7). *Trichoderma asperellum* recorded the second highest number of flowers (5 flowers) and was

significantly (P < 0.05) higher than the control treated plants and significantly lower than *Trichoderma harzianum* (Figure 4. 7)

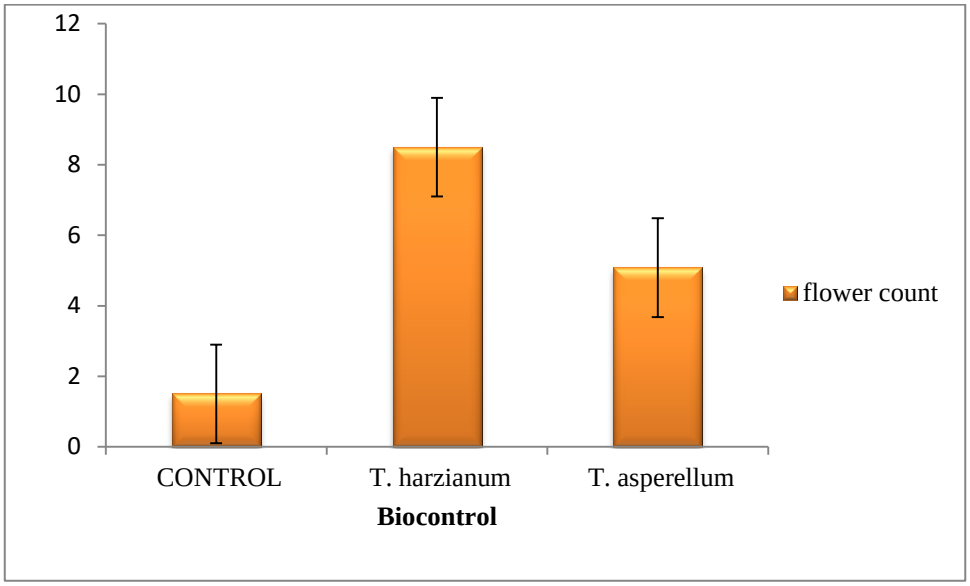

Figure 4. 7 Effect of biocontrol on the number of flowers per plan. Bars with error bars that overlap do not differ significantly (P < 0.05). Error bars represent standard error of the difference of means.

4.3 Experiment 3: Effect of Biocontrol on Germination %, Vigor Appendix and disease Incidence %

4.3.1 Germination percentage

The results show that biocontrol has an effect on germination percentage (Table 4. 2). The combination between *T. asperellum* CA and *T. asperellum* C9 (48.45c) had the highest germination percentage followed by *T. asperellum* CA (37.33b). There was a significant difference on germination percentage from the control (Table 4. 2). TCA (37.33b) and TC9 (35.78b) did not differ significantly (Table 4. 2).

Table 4. 2: Biocontrol effects on germination, vigor and disease incidence of tomato seedling *in-vivo*.

Biocontrol	Germination %	Vigor Appendix	Disease incidence %
T CA	37.33b	293.0c	31.07b
T CA+ TC9	48.45c	431d	16.42a
TC9	35.78b	228b	17.57a
Control F	25.28a	182a	38.27c
F value	**<0.01**	**<0.01**	**<0.01**
SED	**2.607**	**0.0986**	**2.662**
LSD	**5.526**	**0.2231**	**6.021**
CV%	**12.3**	**4.9**	**14.6**

Means following the same superscript are not significantly different from each other at (p<0.05).* means of 3 randomly sampled seedlings.

4.3.2 % Disease incidence

The results showed that the control had the highest percentage disease incidence and the *Trichoderma* spp had a significantly ($p<0.05$) lower disease incidence as compared with the control (Table 4. 2). The combination of TC9+TCA is showing to have the lowest percentage disease incidence. However, (TC9+TCA) and TCA are not significantly different from each other in terms of disease incidence (Table 4. 2**).**

4.3.3 Vigor Appendix

All four biocontrol treatments are significantly different ($P<0.05$) from each other when it comes to vigor Appendix of the tomato seedlings. The combination of TC9+TCA showed the highest vigor Appendix and the control with pathogen showed the least vigor Appendix. *T. asperellum* CA showed a significantly ($p<0.05$) higher vigor Appendix than *T. asperellum* C9 (Table 4. 2).

5 CHAPTER 5

DISCUSSION

5.1 In-vitro bioassay of *Trichoderma* spp

The *Trichoderma* isolates *T. asperellum* (NY, C9, CA and Nyehu) and *T. harzianum* (77) which were tested showed a higher mycelial radial growth and inhibitory effects on the growth of *Fusarium oxysporum f.sp Lycopersici.* The findings of this study findings are in agreement with Verma *et al.* (2017) study on *T. viridae.* The dual culture in this study displayed different levels of mycelium growth inhibition. *Trichoderma asperellum* (NY) showed a high radial growth rate over time and also the highest % inhibition. This isolate outperformed the commercialized *T. harzianum* in the *in-vitro* experiment. Isolate C9 also had a higher percentage of growth inhibition than T77. The advancing hyphae of isolates NY, CA, T77 and C9 covered the entire Petri dish suppressing the growth of *F. oxysporum.* The findings from the current study showed that *Trichoderma* strains have inhibitory effects on the growth of *Fusarium oxysporum* strain in all the treatments in the dual culture. The results also revealed that *Trichoderma* strains competed with *F. oxysporum* for nutrients and space, as well as mycoparasitism over the pathogen and probably secretion of antibiotics. This supports the findings from a study conducted by Theradimani *et al*. (2018) where activities of *Trichoderma viridae* and *T. harzianum* showed convincing results in terms of % inhibition zone shown against *Fusarium* species. The general mechanisms of antagonistic activity of *Trichoderma* spp include antibiosis, lysis, competition and mycoparitism. Ramesh and Pandey (2018) showed that *T. Viridae* produced diffusible substances toxic to the pathogens and these toxins could effectively act as ectoparasites by overcrowding the other organisms. Nisha (*et al.*, 2011 and B.Hunchoth (*et al.*, 2015) stated that *Trichoderma* spp. secrete different compounds that have the potential to effectively suppress *F. oxysporum f.sp Lycopersici* although antibiotic substances from *Trichoderma* strains were not extracted and determined in this study. However, some antibiotics

such as tubercidin, candicidin, phosphlactomycin, phenasin and 4-diacetylphloroglucinol, which have been produced by some antagonists, like Pseudomonas flourescens, Streptomyces spp. and Trichoderma spp., have been reported (Barari, 2016b). Another mechanism of mycoparasitism is described by Lu *et al.* (2004) and showed the branching of *T. atroviride* hyphae as an active, chemotactic response to the presence of the host. Papilla-like erections at the *T. atroviride* hyphal tips were observed and these occurred in the presence and in the absence of direct contact with the pathogen (Lu *et al.*, 2004). In that same study, there was an observation of adherence *of T. atroviride* spores to the hyphae of *P. ultimum* where the germination happened and parasitized the host pathogen. All these are possible reasons which can explain the inhibitory effect of *Trichoderma* on the radial growth of *Fusarium oxysporum f.sp Lycopersici* shown in this study.

5.2 *In-vivo* bioassay of biocontrol

The use of *Trichoderma* spp. as biocontrol agents is promising in tomato production as it improves plant physiological and growth factors and thus improving crop yield. The general plant health is also improved and shows no adverse effect on the environment and consumers.

5.2.1 Plant height and number of nodes per plant

Trichoderma isolates employs several mechanisms in influencing plant growth. Plant height and number of nodes are indicators of plant growth. This study showed that plant height and number of nodes were significantly affected by treatment of *Trichoderma* isolates. *T. harzianum* recorded significantly higher plant heights and number of nodes. The results are in affirmation with the study of Liu *et al.* (2012), where an increase in plant height and biomass was observed when plants were treated with *Trichoderma viridae*. Soil drenching application of the *Trichoderma* spp recorded the highest plant heights as well as number of nodes. This supports the study conducted by Velmurugu *et al.* (2009) who stated that seeds inoculated with *Trichoderma* had no significant effect in seedling height of tomato plants. Further supporting

results by Ramesh and Pandey (2018) showed that in the *Trichoderma* seed inoculation treatments there was no significant effect observed in plant height. The plant heights in seed treated treatments were not significantly different from the control at week 3. Muhera (2017) stated that method of *Trichoderma* introduction is important in the success of *Trichoderm*a action in seedling growth improvements. This is due to *Trichoderma* action, depending on the ability of *Trichoderma* spp to survive and develop in the rhizosphere. Therefore seedling treatment may not offer effective inoculation compared to soil drenching into the root zone. The increase of plant growth due to *Trichoderma* inoculation is also associated with secretion of auxins, gibberellins and cytokinins which are important hormones that boost the development of roots and shoots. The results from the current study are in line with those reported by Verma *et al.*, (2017) which showed the increase in plant growth factors related to yield by *Trichoderma* strains.

5.2.2 Disease severity

The application of *Trichoderma* spp on tomato plants under greenhouse conditions reduced disease severity of *Fusarium* wilts as evidenced by the significantly lower wilt severity on plants inoculated with the pathogen. *T. harzianum* (77) was the most effective agent in suppressing disease development followed by *T. asperellum* (CA). Disease severity was the highest in the control treatments were the plants were inoculated with *Fusarium* only. There was a significant reduction on disease severity by addition of the *Trichoderma* antagonists. Interestingly, Verma *et al.* (2017) stated that one of the mechanisms for disease reduction caused by *Trichoderma* spp. is their ability to induce a potentiated state in the plant enabling it to become resistant to subsequent pathogen (*Fusarium*) infection. The same study also reported other mechanisms which include the induction of systemic resistance (Madden and Dorrance, 2006), the production of antibiotics to restrict the growth of the pathogen and competition with pathogens for nutrients or ecological niches (Tian *et al.*, 2018). *T. harzianum* showed the best

efficacy compared to *T. asperellum.* This effect was also consistent as shown by results recorded at two, three and five weeks after inoculation with the *Trichoderma* strains.

5.2.3 Chlorophyll content and fluorescence

Chlorophyll content was higher in the leaves of *Trichoderma* treated plants with *Trichoderma harzianum* 77 inoculated plants showing the highest significant ($P < 0.05$) chlorophyll content compared to the controls. There was no difference in biocontrol between soil drenched treatments and seed treated treatments for chlorophyll content. In this study, the control recorded lower chlorophyll content as compared to treated plants and this can be attributed to the wilting caused by the pathogen in control plants. The results are in agreement with the findings by Madden and Dorrance (2006), that indicated the occurrence of patchy stomatal closure in dehydrating leaves limiting the photosynthetic activity of these leaves. The control plants were stunted as well and this therefore reduced the leaf size, leaf area Appendix and subsequently the chlorophyll content (Verma *et al.*, 2017). *Trichoderma* treatments were superior in terms of growth parameters like plant height, number of nodes, and also vigour Appendix and all these are precursors to the amount of chlorophyll produced. This explains why the *Trichoderma* treatments produced more chlorophyll than the control treatment with pathogen only (Enespa, 2014).

Trichoderma did not affect the chlorophyll florescence in this study as there was no significant difference between the control and the *Trichoderma* treatments. This could be attributed to the timings of the reading, and could potentially have an effect when readings are taken closer to physiological maturity. A study by Bora *et al*. (2016), observed that as the disease progressed, the maximum quantum efficiency of PS11 photochemistry decreased in the leaves of tomato plants with *Fusarium* wilt. The *Fusarium* wilt disease decreased the leaf area – there is a reduction in the ability of the tomato plants to capture photosynthetically active radiation, therefore determining the lower levels of chlorophyll content which is in agreement with this

study. The experiment also expected to show lower chlorophyll fluorescence in pathogen inoculated treatments. However, this study showed no difference between the biocontrol treatments and the pathogen inoculated treatments.

5.2.4 Flower count per plant

The number of flowers produced per plant in this study was higher in *T. harzianum* followed by T. *asperellum* and the least number of flowers by the plants inoculated with the pathogen only. Clearly, the pathogen is affecting the number of flower produced or at least delaying the flowering process. Flowers produced are a proxy to the number of fruits to be produced and thus the yield of the tomato plants (Ramesh and Pandey, 2018). Therefore, *Trichoderma* is showing positive signs of biocontrol of the pathogen in terms of number of flowers produced.

5.3 Assessment of biocontrol on germination % disease incidence and vigour of seedlings

5.3.1 Germination %

Germination percentage was clearly higher in *Trichoderma* inoculated seeds than in control seeds. This was due to competition parasitism and biosis of the pathogen when the plants were germinating. A combination of *T. asperellum* CA and C9 even further improved the germination %. This was supported by the research of Sinha *et al.* (2018) who indicated that treated seeds showed protective effect for seed germination against the invasion by soil borne pathogens. In this study, the seed treated with Trichoderma spp isolates proved to be effective in reducing the effects of fusarium pathogen on germination (Sinha *et al*., 2018). However, it must be noted that the germination percentages were quite low when compared with other studies for biocontrol (Prasad *et al.*, 2002)

5.3.2 Percent disease incidence

This study shows that disease incidence was higher in the control and was lower in the *Trichoderma* treated pots. The affected growing seedlings showed wilting and yellowing

symptoms and some even died despite growing conditions being suitable, suggesting that the pathogen may be the cause. However, due to production of anti-biotics, mycoparasitism by *Trichoderma* and competition for nutrients, % disease incidence was lowered. This was in agreement with other studies (Prasad *et al.*, 2002 and Sinha *et al*., 2018).

5.3.3 Vigor Appendix

This basically gives an indication of seedling vigor and high seedling vigor was observed in *Trichoderma* treatments. This is due to the general improvement of growth parameters by *Trichoderma* spp (Sinha *et al*., 2018). Root lengths seemed to have been increased by the strains which are a component of the vigor Appendix and enhanced root health will result in a shoot with high vigor Appendix. The study highlighted a significantly lower vigor Appendix in pathogen control seedlings in comparison to the Trichoderma treated seeds. It is in agreement with studies by Prasad *et al.*, (2002) and Sinha *et al*., (2018) which showed that biocontrol improves vigour Appendix of seedlings and reduces effect of *Fusarium oxysporum f.sp Lycopersici.*

6 CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

Trichoderma is effective in suppressing *Fusarium oxysporum f.sp Lycopersici in-vitro* as shown by the study. *T. asperellum* (NY) had the highest inhibition percentage of *Fusarium.* The other strains (*T. harzianum*, *T.asperellum* CA, C9) also significantly reduced the pathogen radial growth and had high percentage inhibition of the pathogen. These locally isolated *Trichoderma* strains are also effective in reducing the effects of *Fusarium oxysporum Lycopersici in-vivo.* Soil drenching in this study is a more effective method of application of the *Trichoderma* spp than seed treatment. *Fusarium* also reduces the germination percentage, vigor and increases the percentage disease incidence in tomato seedlings.

6.2 Recommendations

The bio-efficacy evaluations of *Trichoderma* strains produced promising results and since this study was only done in the lab and in the greenhouse, there is need to further the study and do field evaluations. The experiment should include all strains and assess the residual efficacy under field conditions. The *T. asperellum* strains showed great potential *in-vitro* and *in-vivo*. Farmers can easily adopt biocontrol methods as the fungicides proliferate in the soil and will offer environmentally and health hazard free method of *Fusarium* wilt control in tomato production.

7 REFERENCES

Abdul-Baki, A. A. and James, A. (1973) 'Vigor determination in Soybean by multiple criteria', *Aliiance of Crop, Soil and Enviromental Scince societies*, 13(6), pp. 630–633.

Alwathnani, H. A. and Perveen, K. (2012) 'Biological control of *Fusarium wilt* of tomato by antagonist fungi and cyanobacteria', *African Journal of Biotechnology*, 11(5), pp. 1100–1105.

Amini, J. and Sidovich, D. F. (2010) 'The effects of fungicides on *Fusarium oxysporum f.sp. lycopersici* associated with *Fusarium* wilt of Tomato', *Journal of Plant Protection Research*, 50(2).

Arenas1, O. R. (2018) Biological Control of *Fusarium oxysporum* in Tomato Seedling Production with Mexican Strains of *Trichoderma*. Puebla: Intech Open, pp. 165–166.

Barari, H. (2016a) 'biocontrol of tomato *Fusarium* wilt by *Trichoderma* species under in vitro and in vivo conditions', *Cercetări Agronomice în Moldova*, 1(165), pp. 91–98. doi: 10.1515/cerce-2016-0008.

Barari, H. (2016b) 'biocontrol of tomato *Fusarium* wilt by *Trichoderma* species under in vitro and in vivo conditions', *Cercetări Agronomice în Moldova*, 165(1), pp. 91–98.

BHunchoth, A. Phironrit, N. Leksomboon. C. Chatchawankanphanich, O. Kotera, S. Narulita, E. Kawasaki. T. (2015). 'Isolation of *Ralstonia solanacearum*-infecting bacteriophages from tomato fields in Chiang Mai, Thailand, and their experimental use as biocontrol agents', *Journal of Appiled microbiology*, 118(4), pp. 1023–1033.

Bora, P., Bora, L.C., Deka, P.C., Borkotoki, B., Sharma, A.K., Dutta, H.S., Buhagohain, D. 2016. Efficacy of *Pseudomonas fluorescens* and *Trichoderma viride* based bioformulation for management of bacterial wilt disease of ginger. Int. J. Plant Sci. 11, 180–186.

Cerkauskas, R. (2005) '*Fusarium Wilt* on Tomato', *Journal of plant protection*. Shanhua, 60(2), 2.

Chavan Chetan, TamboliAshpak, Patil Priyanka, C. A. (2011) 'pharmacognostical and pharmacological screening oftridax procumbensl.', *International research journal of pharmacy*, 2(7), pp. 154–159.

Daami-Remadi, M. Ayed. F. Khiareddine, J. Hibar, K. Mahjoub, M. E. (2006) 'Effects of some Bacillus sp. Isolates on *Fusarium spp* in-vitro and Potato Tuber Dry Rot Development in-vivo', *Plant pathology Journal*, 5(3), 283–296.

Daami-remadi, M., Agriculture, O. and Hibar, K. (2006) 'Effect of Two *Trichoderma* species on Severity of Potato Tuber Dry Rot Caused by Tunisian *Fusarium* Complex', Plant pathology Journal 10(2), 126- 122

Du, M. Xiangyu, R. Qinghua, S. Wang, Y (2012) 'Characterization of *Fusarium spp* . Causing Potato Dry Rot in China and Susceptibility Evaluation of Chinese Potato Germplasm to the Pathogen', 175–184.

Elad, Y. and Chet, I. (2011) 'Degradation of plant pathogenic fungi by Trichoderma harzianum', Canadian Journal of Microbiology, 28(September 2015), 719–725.

Enespa, D. S. (2014) ‘Effectiveness of some antagonistic fungi and botanicals against *Fusarium solani* and *Fusarium oxysporum f. sp. lycopersici* infecting brinjal and tomato plants.’, Asian Journal Plant Pathology, 8, 18–25.

FAO (2017) *Food and Agriculture Organization of the United Nations, Food and Agriculture Organization of the United Nations*. Rome. Available at: Food and Agriculture Organization of the United Nations (Accessed: 23 May 2019).

Feng, X. (2012) ‘Identification of a novel nuclear-localized adenylate kinase 6 from Arabidopsis thaliana as an essential stem growth factor’, Plant Physiol Biochem, 61, 180–186.

Fravel, D. Olivain, C. and Alabouvette, C. (2003) ‘*Fusarium oxysporum* and its biocontrol’, New Phytologist Trust, 157(3), 493-502

Gary, H,.Viterbo, C. Howell, I. Chet. (2004) ‘*Trichoderma* species-Oppotunistic, avirulent plant symbionts’, Nature reviews Microbiology, 2(1), 43–56.

Ghazalibiglar, H., Kandula, D. R. W. and Hampton, J. G. (2016) ‘Biological control of *Fusarium* wilt of tomato by *Trichoderma* isolates’, New Zealand Plant Protection, 63(69), 57–63.

Giovannucci, E. (1999). Tomatoes, Tomato-Based Products, Lycopene, and Cancer: Review of the Epidemiologic Literature.’, Journal of the National Cancer Institute, 91(4), 317–331.

Hristov, A. C. (2007). The Johns Hopkins microbiology newsletter. 05. Baltimore.

Joshi K and Chaturvedi P. (2013) ‘Efficient in vitro propagation protocol of *Centella asiatica*

(L.) Urban: an endemic and underutilized nutraceutical herb', African J. Biotech, 12(33), 5164–5172.

Khalifa, E. Z. Ammar, M. M. Mousa, E. M. Hafez, S. L. (2013) 'Biological control of the disease complex on potato caused by root-knot nematode and *Fusarium* wilt fungus', Nematol. medit, 40, 169–172.

Lu, Z. Woo, T, R. Zeilinger, S. Lorito, S. Jansson, M. Janet, K. (2004). *In-vivo* study of *Trichoderma* -Pathogen-Plant Interactions , using constitutive and inducible green fluorescent protein reporter systems', 70(5), 3073–3081.

Madden, L. V and Dorrance, A. (2006) 'Systemic Resistance Induced by *Trichoderma spp* .: Interactions Between the Host , the Pathogen , the biocontrol Agent , and soil organic matter quality', *Phytopathology*, 96, 186–189.

Mandal, H. Chakraborty, P. D., , Arnab, S.S. (2017) 'Biocontrol of Virulent Ralstonia solanacearum isolates by an Indigenous Bacillus cereus', *Jornal of Agricultural Technology*, 13(1), 19–30.

Mecteau, M. R. Joseph, A. Mecteau, M. R. Tweddell, R. J. (2018) 'Effect of different salts on the development of *Fusarium solani var* . coeruleum , a causal agent of potato dry rot, Phytoprotection, 89(1), 1–6.

Mejdoub-trabelsi, B., Jabnoun-khiareddine, H. and Daami-remadi, M. (2015) 'Plant Pathology & Microbiology Interactions between Four Fusarium Species in Potato Tubers and Consequences for Fungal Development and Susceptibility Assessment of Five Potato Cultivars under Different Storage Temperature', Microbiol, J Pathol, Plant, 6(8), 1–25.

Mj, B., Keswani, K., Singh, C.H.B. (2017). Biological management of *Fusarium* wilt of tomato using biofortified vermicompost', Mycosphere, 8, 467–483.

Muhera, S. (2017) Evaluation of *Trichoderma* in the biocontrol of *Ralstonia solanaceum in Potatoes*. University of Zimbabwe.

Nisha, A. T. Garg, S. G. Nandini, K, Rajesh, M. (2011) 'In Vitro Antifungal Potency of Plant Extracts Against Five Phytopathogens', Brazilian Archives of Biology and Biotechonology, 54(6). 1093–1098.

Ommati, F., Zaker, M. and Mohammadi, A. (2013) 'Biological control of *Fusarium* wilt of potato (*Fusarium oxysporum f . sp . tuberosi*) by *Trichoderma* isolates under field condition and their effect on yield', 2(4), 435–442.

Prasad, R Prasad, R K Srivastaval R D Rangeshwaran, R Wasnikar, A R Singh, S P Rao, N S (2002) 'A rapid in vivo bioassay method for testing and selection of fungal antagonists of plant pathogens', J.Biol.Control, 16(2), 173–176.

Ramesh, G. C. and Pandey, B. R. (2018) 'Evaluation of *Trichoderma spp ., Pseudomonas fluorescens and Bacillus subtilis* for biological control of *Ralstonia wilt* of tomato', F1000Research, (0), 1–22.

Schisler,D.A. Slininger, P. Kleinkopf, G. Bothast, R.J. Ostrowski,R.C. (2000) 'Biological Control of *Fusarium* dry rot of potato tubers under commercial storage conditions', American journal of Potato Research, 77, pp. 29–40.

Sinha, A., Singh, R. and Verma, A. (2018) 'Bioefficacy of *Trichoderma harzianum* and

Trichoderma viride against *Fusarium oxysporum f . sp . capsici* causing wilt disease in chilli', Journal of Pharmacognosy and Phytochemistry, 7(5), 965–966.

Theradimani, M., Susitha, S. and Amudha, C. (2018) 'Biocontrol of *Fusarium* Wilt in Tomato caused by *Fusarium oxysporum f . sp . lycopersici*', International Journal of Current Microbiology and Applied Sciences, 7(09), 420–429.

Tian, Y. Tian, Y. T. Yanglan Y, Zheng, L. Yucai, C. Jie Boevre. M.D. (2018) 'Antagonistic and Detoxification Potentials of *Trichoderma* Isolates for Control of Zearalenone (ZEN) Producing *Fusarium graminearum*', Frontiers in Microbiology, 1–11.

Velmurugu, J. Wijeratnam, S. Wijesundera, W. R. L. (2009) '*Trichoderma* as a Seed Treatment to Control Helminthosporium Leaf Spot Disease of *Chrysalidocarpus lutescens*', World Journal of Agriculutural Sciences. 5(6). 720-728.

Verma, N. P. Sinha, A. and Singh, H and (2017) 'Efficacy of *Trichoderma* in controlling *Fusarium* wilt in tomato (*Solanum lycopersicum L* .) Efficacy of *Trichoderma* in controlling *Fusarium* wilt in tomato (*Solanum lycopersicum L* .)', Res.Environ.Life *Sci.*, 10(7), 636–639.

Xu, S., Joon-young W, Jae-hyoun H, Young-il, K. Byung-sup (2014) 'Mycobiology First Report of Potato Stem-End Rot Caused by *Fusarium oxysporum* in Korea', The Korean Society of Mycology. Gangneung, 206–209.

Zaker, M. (2012) 'Evaluation of some *Trichoderma* isolates for biological control of potato wilt disease (*Fusarium oxysporum*) under lab . and green house conditions', Journal of Plant Protection, 1(4), 279–286.

Appendix 1: Analysis of variance for radial growth of *Fusarium Oxysporum f.sp Lycopersici*.

Analysis of variance

Variate: RAD_GROWTH_1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	3	15095.36	5031.79	213.78	<.001
TRT	8	3038.50	379.81	16.14	<.001
Day.TRT	24	1013.72	42.24	1.79	0.030
Residual	72	1694.67	23.54		
Total	107	20842.25			

APPENDIX 2: Analysis of variance for percentage inhibition in dual culture method

Analysis of variance

Variate: %INHIBITION_1,%INHIBITION_4,%INHIBITION_7,%INHIBITION_10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
REPS	2	8.93	4.46	0.58	0.588
biocontrol	3	1032.29	344.10	44.85	<.001
Residual	6	46.04	7.67	0.20	
Subject.Time stratum					
d.f. correction factor 0.6249					
Time	3	5112.31	1704.10	44.18	<.001
Time.REPS	6	226.22	37.70	0.98	0.453
Time.biocontrol	9	600.78	66.75	1.73	0.204
Residual	18	694.26	38.57		
Total	47	7720.83			

(d.f. are multiplied by the correction factors before calculating F probabilities)

APPENDIX 3: Analysis of variance of repeated measures of plant height (week 2, 3).

Analysis of variance

Variate: PH2,PH_3WKS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Block	2	16.602	8.301	1.68	0.235
Biocontrol	2	306.502	153.251	31.02	<.001
Method	1	106.778	106.778	21.61	<.001
Biocontrol.Method	2	110.237	55.119	11.16	0.003
Residual	10	49.402	4.940	0.91	
Subject.Time stratum					
d.f. correction factor 1.0000					
Time	1	443.804	443.804	81.70	<.001
Time.Block	2	26.301	13.150	2.42	0.139
Time.Biocontrol	2	18.404	9.202	1.69	0.233
Time.Method	1	8.604	8.604	1.58	0.237
Time.Biocontrol.Method	2	6.204	3.102	0.57	0.582
Residual	10	54.323	5.432		
Total	35	1147.160			

(d.f. are multiplied by the correction factors before calculating F probabilities)

APPENDIX 4: Analysis of variance of repeated measures for disease severity at week 2, 3, 5 and 7).

Analysis of variance

Variate: sqrt_disease_severity_wk2,sqrt disease_severity_wk3,sqrt_disease_severity_wk5,sqrt_disease_severity_7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr
Subject stratum					
Block	2	0.3113	0.1556	0.61	0.565
Biocontrol	2	5.0067	2.5033	9.73	0.005
Method	1	0.3899	0.3899	1.52	0.246
Biocontrol.Method	2	2.5581	1.2791	4.97	0.032
Residual	10	2.5724	0.2572	0.95	
Subject.Time stratum					
d.f. correction factor 0.7043					
Time	3	4.7420	1.5807	5.82	0.009
Time.Block	6	0.6184	0.1031	0.38	0.830
Time.Biocontrol	6	2.2346	0.3724	1.37	0.277
Time.Method	3	1.3557	0.4519	1.66	0.212
Time.Biocontrol.Method	6	1.2289	0.2048	0.75	0.573
Residual	30	8.1448	0.2715		
Total	71	29.1627			

(d.f. are multiplied by the correction factors before calculating F probabilities)

APPENDIX 5: Analysis of variance of repeated measure of number of nodes per plant for week 2, 3, 5 and 7.

Analysis of variance

Variate: NODES_PER_PLANT_WK_2,NODES_PER_PLANT_WK_3,NODES_PER_PLANT_WK_5,NODES_PER_PLANT_WK_7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Block	2	5.25	2.62	0.11	0.894
Biocontrol	2	230.58	115.29	4.99	0.031
Method	1	150.22	150.22	6.51	0.029
Biocontrol.Method	2	312.53	156.26	6.77	0.014
Residual	10	230.92	23.09	1.23	
Subject.Time stratum					
d.f. correction factor 0.3608					
Time	3	1115.28	371.76	19.85	<.001
Time.Block	6	20.64	3.44	0.18	0.850
Time.Biocontrol	6	54.97	9.16	0.49	0.640
Time.Method	3	64.44	21.48	1.15	0.313
Time.Biocontrol.Method	6	334.81	55.80	2.98	0.090
Residual	30	561.86	18.73		
Total	71	3081.50			

(d.f. are multiplied by the correction factors before calculating F probabilities)

APPENDIX 6: Analysis of variance of repeated measures for chlorophyll content (week 2 and 3).

Analysis of variance

Variate: CHLOROPHYLL_CONTENT_WK2,CHLOROPHYLL_CONTENT_WK3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Block	2	116.13	58.06	2.45	0.136
Biocontrol	2	559.96	279.98	11.82	0.002
Method	1	60.45	60.45	2.55	0.141
Biocontrol.Method	2	2.39	1.19	0.05	0.951
Residual	10	236.80	23.68	1.41	
Subject.Time stratum					
d.f. correction factor 1.0000					
Time	1	646.43	646.43	38.44	<.001
Time.Block	2	82.88	41.44	2.46	0.135
Time.Biocontrol	2	95.39	47.69	2.84	0.106
Time.Method	1	89.15	89.15	5.30	0.044
Time.Biocontrol.Method	2	16.73	8.37	0.50	0.622
Residual	10	168.17	16.82		
Total	35	2074.47			

(d.f. are multiplied by the correction factors before calculating F probabilities)

APPENDIX 7: Analysis of variance for repeated measures of chlorophyll florescence (week 2 and 3).

Analysis of variance

Variate: CHLOROPHYLL_FLORESCENCE_WK2, CHLOROPHYLL_FLORESCENCE_WK3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Block	2	0.001730	0.000865	0.57	0.581
Biocontrol	2	0.002019	0.001010	0.67	0.534
Method	1	0.001028	0.001028	0.68	0.428
Biocontrol.Method	2	0.005275	0.002638	1.75	0.223
Residual	10	0.015082	0.001508	0.81	
Subject.Time stratum					
d.f. correction factor 1.0000					
Time	1	0.023784	0.023784	12.75	0.005
Time.Block	2	0.003035	0.001517	0.81	0.471
Time.Biocontrol	2	0.000869	0.000434	0.23	0.796
Time.Method	1	0.000460	0.000460	0.25	0.630
Time.Biocontrol.Method	2	0.000984	0.000492	0.26	0.773
Residual	10	0.018660	0.001866		
Total	35	0.072926			

(d.f. are multiplied by the correction factors before calculating F probabilities)

APPENDIX 8 Analysis of variance for Flower count

Analysis of variance

Variate: FLOWER_COUNT_WK_5,FLOWER_COUNT_WK_6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Subject stratum					
Block	2	55.056	27.528	2.34	0.147
Biocontrol	2	294.056	147.028	12.50	0.002
Method	1	1.361	1.361	0.12	0.741
Biocontrol.Method	2	35.389	17.694	1.50	0.268
Residual	10	117.611	11.761	2.52	
Subject.Time stratum					
d.f. correction factor 1.0000					
Time	1	46.694	46.694	10.02	0.010
Time.Block	2	12.056	6.028	1.29	0.317
Time.Biocontrol	2	19.389	9.694	2.08	0.176
Time.Method	1	0.694	0.694	0.15	0.708
Time.Biocontrol.Method	2	2.056	1.028	0.22	0.806
Residual	10	46.611	4.661		
Total	35	630.972			

(d.f. are multiplied by the correction factors before calculating F probabilities)

APPENDIX 9: Analysis of variance for germination percentage

Analysis of variance

Variate: Germination_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr
Method_of_Application	1	36.75	36.75	1.80	0.198
Biocontrol	3	1617.86	539.29	26.46	<.001
Method_of_Application.Biocontrol	3	78.96	26.32	1.29	0.311
Residual	16	326.11	20.38		
Total	23	2059.69			

APPENDIX 10: Analysis of variance for vigor Appendix

Analysis of variance

Variate: vigor_Appendix

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr
Block stratum	3	0.01035	0.00345	0.18	
Block.*Units* stratum					
biocontrol	3	14.34610	4.78203	245.86	<.001
Residual	9	0.17505	0.01945		
Total	15	14.53150			

APPENDIX 11: Analysis of variance for percentage disease incidence

Analysis of variance

Variate: %_disease incidence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	350.12	116.71	8.24	
Block.*Units* stratum					
biocontrol	3	1355.95	451.98	31.90	<.001
Residual	9	127.53	14.17		
Total	15	1833.60			