THE EFFICACY OF *MONSONIA BURKEANA, MORINGA OLEIFERA* AND *TRICHODERMA HARZIANUM* ON TOMATO SOIL-BORNE FUNGAL PATHOGENS *FUSARIUM OXYSPORUM* AND *RHIZOCTONIA SOLANI* UNDER *IN VITRO* AND *IN VIVO* CONDITIONS

by

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TABLE OF CONTENTS

DECLARATION

I, Hlokwe Mapula Tshepo Pertunia, declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree Master of Science in Agriculture (Horticulture) has not been submitted previously by me or anybody for a degree at this or any other University. Also, this is my work in design and in execution, while related materials contained herein had been duly acknowledged.

Candidate: Ms Hlokwe Mapula

Signature	Date

DEDICATION

To my late Mom (Maleho Makoma Maria Mashela) and my grandmother (Elizabeth Koranta Ramosebudi).

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LIST OF TABLES

LIST OF FIGURES

APPENDICES

ABSTRACT

Tomato is second most cultivated crop globally and in South Africa it is planted by both commercial and smallholder farmers. However, the crop is susceptible to a number of diseases including those caused by fungal pathogens. Fusarium wilt caused by *Fusarium oxysporum f. sp. lycopersici* and seedling damping-off caused by *Rhizoctonia solani*, are known to cause serious yield loss in tomato production. Their management is mainly based on the application of synthetic fungicides and cultural practices. However, both methods have limitations which result in their inefficiency. Synthetic fungicides also have negative impact on the environment and human health. The ability of fungal pathogens to develop resistance to fungicides has also resulted in their reduced application. These challenges have led to a need to identify novel methods using plant extracts and biological control agents which can be used to manage these diseases. The objectives of this study were therefore to, firstly determine the efficacy of both plant extracts on mycelial growth of *F. oxysporum f. sp. lycopersici* and *R. solani* under laboratory conditions and secondly, to evaluate the effectiveness of both plant extracts as well as antagonistic fungi *Trichoderma harzianum* against Fusarium wilt and damping-off of tomato under greenhouse conditions. Food poisoning assay was used to investigate the efficacy of *M. burkeana* and *M. oleifera* extracts *in vitro*. Six (0, 2, 4, 6, 8, 10 g/ml) treatments were arranged in a completely randomised design and replicated four times. After 7 days of incubation at 25 °C, radial growth colony was measured. For the greenhouse experiment, Fusarium wilt was tested on cv. ‘HTX14’ as the most susceptible cultivar whilst seedling damping-off was tested on cv. ‘Money-maker’. Aqueous extracts were prepared by decocting different concentrations of *M. burkeana* (4, 6, 8 g/ml)

and *M. oleifera* (2, 4 and 6 g/ml) in 100 ml of distilled water at 100 °C for 15 minutes then left to cool before filtering and applying as a treatment. *Trichoderma harzianum* as a treatment was applied 7 days after inoculating the soil-borne pathogens. In-*vitro M. burkeana* treatments concentrations had the highest mycelia growth suppression against both *F. oxysporum f. sp. lycopersici* at 10 g/ml (76 %) whilst suppression on *R. solani* was at 8 g/ml (71 %) relative to control. *Moringa oleifera* extracts' highest pathogen suppression for both *F. oxysporum f. sp. lycopersici* and *R. solani* were respectively 35 % and 60 % relative to control at concentration 6 g/ml. Under greenhouse conditions shoot disease severity had highest suppression at 0.6 g/ml of *M. burkeana* and 0.4 g/ml of *M. oleifera* treatment concentrations resulting to 32 and 49 % relative to control. Whereas, treatment 0.8 g/ml of *M. burkeana* and 0.4 g/ml of *M. oleifera* suppressed stem and root discoloration by 39 and 54 % respectively. *Trichoderma harzianum* significantly ($P \leq 0.05$) reduced shoot severity and root and stem discolouration contributing the highest suppression of 49 % relative to control. In damping-off treatments, both plant extracts and *T. harzianum* also significantly reduced ($P \leq 0.05$) pre- and post-emergence damping-off incidence with *M. burkeana* recording the highest suppression at 78 % followed by *M. oleifera* at 64 %. *Trichoderma harzianum* reduced incidence of damping-off by 60 % relative to untreated control on both *M. burkeana* and *M. oleifera* experiments. The results of this study showed that *M. burkeana, M. oleifera* extracts and *T. harzianum* can be highly suppressive to both tested plant diseases. However, further studies should be conducted to determine their mode of action, application method and their effect on other soil microorganisms.

Keywords: Damping-off, Fusarium wilt, Plant extracts, *T. harzianum,* Tomato plant

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

Tomato (*Solanum lycopersicum L*.) plants are the second most economically important vegetable crop worldwide. Global tomato production was estimated 178 million tonnes per hector in 2014 (FAOSTAT, 2017) with Asia (101 million tonnes) showing the strongest regional growth (FAOSTAT, 2017). South Africa remains a major regional tomato producer in Sub-Saharan Africa growing 54 million tonnes in 2014 on 11 % of the total cultivation area (DAFF, 2015). In South Africa, the crop is produced in all nine provinces under both tunnel and field conditions (DAFF, 2011). However, the highest production area is in the Limpopo Province, comprising 75 % of total field production (DAFF, 2011).

Tomato production is negatively affected by a number of constraints caused by both biotic and abiotic factors. Abiotic factors include crop management practices such as tillage, fertiliser application and climatic conditions (Agrios, 2005). However, biotic factors such as insect pest and diseases cause significant yield loss, especially under field conditions (Afroz *et al.,* 2010; Agrios, 2005). Moreover, tomato is susceptible to a number of soil-borne fungal pathogens affecting above- and below ground organs such as *Verticillium spp.* and *Fusarium spp.* both causal agents of tomato wilt and *Rhizoctonia solani* which causes seedling damping-off (Abu-Taleb *et al*., 2011; Chohan *et al.,* 2016). Crop damage due to soil-borne diseases occurs at all plant growth stages from seedling to maturity. According to FAOSTATS (2011), Fusarium wilt caused by *F. oxysporum f. sp. lycopersici* and seedling damping-off caused by *R. solani* are some of the major soil-borne diseases affecting tomato production globally.

Management of both Fusarium wilt and *Rhizoctonia* damping-off remains a very challenging task to most tomato farmers (Harari, 2016). Both diseases are normally managed by application of soil fumigants (Cerkauskas, 2001 and McGovern *et al*., 2001). However, due to the negative impact of synthetic chemicals on other soil microorganisms and human health, there has been a reduction in their application. Planting of resistant tomato cultivars is also used in the management of Fusarium wilt and seedling damping-off (Abu-Taleb *et al*., 2011). However, high genetic variability among pathogen isolates and loss of cultivar resistance are highly common in many tomato varieties leading to extreme loss of yield. Cultural practices such as crop rotation, intercropping, and organic manuring (Haggag and Nadia, 2012) can also be used in the management of both diseases. However, their effectiveness is normally affected by the saprophytic nature of the two pathogens and their ability to survive in the absence of the host (El-Mohamedy and Abdalla, 2014).

The effectiveness of plant extracts in suppressing fungal pathogens under both laboratory and field conditions has previously been reported (Dan *et al.,* 2013; El-Mohamedy and Abdalla, 2014; Shazia *et al.,* 2015). For example, extracts of *Artemisia afra*, *Rhamnus prinoides* and *Leucosidea serisea* were shown to suppress amaranthus and cabbage seedling damping-off caused by *R. solani* (Kena and Swart, 2011). The ability of plants to suppress fungal growth and disease occurrence has been attributed to the presence of secondary metabolites in plants with antifungal properties (Dan *et al*., 2013; Shazia *et al*., 2015; Singh, 2012).

Application of different biological agents and their mode of action in suppressing soil-borne diseases have been widely acknowledged (Ashwani *et al*., 2004; Harman, 2006; Shalini and Dohroo, 2005). Saravanakumar *et al*. (2007) reported that *Talaromyces flavus* reduced incidence of Verticillium wilt caused by *Verticillium dahliae* and increased yield of eggplant. *Talaromyces flavus* occupy rhizospheres of tomato, cotton, and eggplant decreasing germination of microsclerotia of *V. dahliae* (Saravanakumar *et al*., 2007). Unlike *T. flavus*, *Trichoderma spp.* use different mechanisms for the control of phytopathogens which include mycoparasitism, competition for space and nutrients, secretion of antibiotics and a fungal cell wall degrading enzymes (Benítez *et al*., 2004; Harman *et al*., 2004; Kubicek *et al*., 2001). In addition, *Trichoderma spp.* has a stimulatory effect on plant growth (Naseby *et al*., 2000) as a result of modification of soil conditions. The current study focused on the efficacy of plant extracts (*Monsonia burkeana*, *Moringa oleifera*) and biological control *Trichoderma harzianum* on tomato soil-borne diseases, namely Fusarium wilt and seedling damping-off.

1.2 Problem statement

Tomato is susceptible to different types of soil-borne diseases including Fusarium wilt caused by *F. oxysporum f. sp. lycopersici* and seedling damping-off caused by *R. solani* (Martinez, 2007; Strange and Scott, 2005). Though infected soil by both diseases can be managed by soil fumigants, their negative effect on the environment and human health has prompted for the development of alternative strategies (Harari, 2016). These alternative strategies include the use of plant extracts as botanical fungicides and biological control agents as they are effortlessly accessible, biodegradable and are not a threat to the environment as well as the ecosystem at

large. This study, therefore, tested the efficacy of plant extracts and biological control agent against fungal pathogens, *F. oxysporum f. sp. lycopersici* and *R. solani* under both laboratory and greenhouse conditions.

1.3 Rationale of the study

Plant extracts and biological control agents are being investigated as alternative measures in the management of crop pests and diseases. Plants have been shown to contain secondary metabolites such as alkaloids, tannins, quinones, phytoalexins, coumarins and phenolic compounds, which are known for antifungal activity (Kagale *et al*., 2005). Also, Hermosa *et al*. (2012) reviewed developments in the biological control of plant pathogens and found their repertoire of extracellular lytic enzymes that cause necrotrophic action through lysis of fungal cell walls as well as the role they play in plants. *Trichoderma* species affects disease development through promotion of plant growth, directly and indirectly, tolerance to abiotic stresses and induced disease resistance on affected plants (Joshi *et al*., 2012). This study provides information necessary for the development of management strategies using plant extracts and biological agents.

1.4 Aim and objectives

1.4.1 Aim

The aim of this study was to develop an effective management strategy using *M. burkeana*, *M. oleifera* plant extract and *T. harzianum* against Fusarium wilt and seedling damping-off in tomato production.

1.4.2 Objectives

The objectives of this study were to determine whether:

1. *Monsonia burkeana* and *M. oleifera* would suppress the growth of *F. oxysporum f. sp. lycopersici* and *R. solani* in *vitro*.

2. *Monsonia burkeana*, *M. oleifera* and *T. harzianum* would suppress *F. oxysporum f. sp. lycopersici* and *R. solani* disease severity *in vivo*.

1.4.3 Hypotheses

1. *Monsonia burkeana* and *M. oleifera* would suppress the growth of *F. oxysporum f. sp. lycopersici* and *R. solani in vitro*.

2. *Monsonia burkeana*, *M. oleifera* and *T. harzianum* would suppress *F. oxysporum f. sp. lycopersici* and *R. solani* disease severity and incidence under greenhouse conditions.

1.5 Reliability, validity and objectivity

In this study, reliability of data was based on statistical analysis of data at the probability level of 5%. The validity was achieved by repeating the experiments at least twice and finally, the objective was achieved by ensuring that the findings are discussed based on empirical evidence, thereby eliminating all forms of subjectivity.

1.6 Bias

Bias was minimised by ensuring that the experiment error was reduced through adequate replications. Also, treatments were assigned randomly within the selected research designs (Leedy and Ormrod, 2005).

1.7 Structure of mini-dissertation

Following the description and detailed outline of the research problem (Chapter 1), the work done and not yet done on the problem statement was reviewed (Chapter 2). Then the subsequent chapters (Chapter 3 and 4) addressed objective one and two of this report highlighting results to provide the significance of the findings. Summary, conclusion and recommendations with respect to future research (Chapter 5) tied together the entire study. Citations and references were used following the Harvard style as prescribed by Senate of the University of Limpopo.

CHAPTER 2
LITERATURE REVIEW

2.1 Fusarium wilt and seedling damping-off of Tomato: Causal organisms, symptoms and conditions favouring disease development

2.1.1 Fusarium wilt: Causal pathogen biology

Fusarium wilt of tomato caused by *F. oxysporum f. sp. lycopersici* was first described by G.E. Massee in England in 1895 (Bawa, 2016). *Fusarium oxysporum f. sp. lycopersici* is classified under the order Hyphales and the genus *Fusarium*. It produces three types of asexual spores; microconidia, macroconidia and chlamydospores (Bawa, 2016) and all play an important role in plant infection. The pathogen has three physiological races (1, 2 and 3) and is distinguished by their specific pathogenicity on tester plants carrying dominant race-specific resistance genes (Cai *et al.,* 2013). Physiological strains are important in the development of resistant cultivars. Some strains of *F. oxysporum* are not pathogenic and may even antagonise the growth of pathogenic strains hence used as biological agents (Fravel *et al*., 2003). In solid culture medium such as potato dextrose agar (PDA), different isolates of *F. oxysporum* have varying appearances. In general, the aerial mycelium first appears white and then changes to a variety of colours ranging from violet to dark purple depending on the strain. The fungus also forms sporodochia which appear cream or orange in colour depending on the growth media used for culturing (Bawa, 2016). The fungus is present in all important tomato growing regions of the world (Agrios, 2005).

2.1.2 Disease symptoms

Symptoms of *F. oxysporum* in infected tomato plants first appear as slight vein clearing on the outer portion of the young leaves followed by epinasty of the older

leaves (Sally *et al.,* 2006). This symptom occurs on one side of the plant or on one shoot. Successive leaves become yellowish, wilt and die before the plant reaches maturity. As the disease progresses, growth is typically reduced, and little or no fruit develops. If the main stem is cut, dark brown streaks may be seen longitudinally through the stem. The browning of the vascular system is characteristic of the disease and generally can be used for its identification (Mui-Yun, 2003). On the outside of affected stems, white, pink or orange fungal growth can be seen especially in wet conditions (Ajigbola and Babalola, 2013).

2.1.3 Conditions favouring Fusarium wilt development

Fusarium oxysporum occurs, survives and grows in soils of all types, however, sandy soils provide conditions that are most favourable for its growth and development (Lowell, 2001). Soil and air temperature of 28 ºC is optimum for disease development. When soil temperatures are optimum and air temperatures are below optimum, the pathogen will extend into the lower parts of the stem although the plants will not exhibit external symptoms (Mui-Yun, 2003). The pathogen is primarily spread over short distances by irrigation water and contaminated farm equipment (Stephen and Ndre, 2003). It can also be spread over long distances either in infected transplant or soil (Stephen and Ndre, 2003).

Fusarium oxysporum enters the plant through root tips (Akrami and Yousefi, 2015). The mycelium grows in the xylem vessels where water supply is cut-off resulting to wilting (Stephen and Ndre, 2003). There is often an association of Fusarium wilt and nematode colonisation where the nematodes provide an entry route for the fungus (Bawa, 2016). Enzymes such as pectinolytic and cellulolytic may also facilitate

Fusarium penetration into plant host (Babalola, 2010). Infection and disease development in Fusarium wilt is favoured by warm soil temperature and low soil moisture (Lewis, 2003).

2.1.4 Seedling damping-off: Causal pathogen biology

Seedling damping-off of tomatoes is caused by a soil-borne saprophytic fungus *R. solani* (Moussa, 2002). The pathogen belongs to the phylum Basidiomycota, class Hymenomycetes, order Ceratobasidiales and family Ceratobasidiaceae (Tsukiboshi, 2002). For proper identification and classification*, R. solani* has common morphological characterization used which include high cellular nuclear numbers wider main runner hyphae (Abu-Taleb *et al*., 2011). It also forms buff-coloured to dark brown mycelium, sclerotia which are shaped in an irregular form and light to dark brown colour without variation (Abu-Taleb *et al*., 2011). This fungus can be further characterised by the presence of multinucleate cells with dolipores, production of sclerotia, and lack of conidia (Laroche *et al*., 1992). The source of infection is mainly mycelium which grows into plant roots through chemotaxis (Ceresini, 1999). After connection to the external surface of the host, *Rhizoctonia* penetrates the plant roots and causes damping-off.

2.1.5 Pre- and post-emergence damping-off symptoms

Pre-emergence damping-off occurs immediately after planting, when a pathogen infected seed, result in decay and death of the seed before germination (Agrios, 2005). Post-emergence damping-off occurs when the stem starts decaying at soil level, causing it to collapse due to lack of supporting tissues within the plant (Agrios, 1997). Post-emergence damping-off, appears as reddish-brown lesions on the stem

at soil level and girdle the stem when conditions are favourable (Dorrance *et al*., 2001). The stem may appear water soaked and with a soft crown, causing the plant to collapse.

2.1.6 Conditions favouring damping-off development

Rhizoctonia solani can be found in cool and warm soils with disease development being more established when temperatures are adverse to the host (24-32 °C) (Jones *et al*., 1997). The mildly virulent strain causes most disease at 24 °C, while the moderately virulent strain causes disease at 32 °C and the highly virulent strain causes disease at 24, 27, and 32 °C (Harikrishnan and Yang, 2004). Rainfall followed by cool weather in a subnormal rainfall season also favours the development of *R. solani* diseases (Dorrance *et al*., 2003). The sources of infection are natural soil, contaminated weed or rotation crops, plant debris and infected seeds (Dorrance *et al*., 2003). Survival and inoculum potential are influenced by soil factors such as temperature, moisture, pH, and competitive activity with associated organisms (Jones *et al*., 1997). According to Ceresini (1999), *R. solani* can survive for a long time by producing small, irregular shaped, brown to black structures (sclerotia) in soil and on plant tissue. It can also survive as mycelium by colonising soil organic matter as a saprophyte (Olsen and Young, 1998).

2.2 Work done on the research problem

2.2.1 Current trends in the management of Fusarium wilt and seedling damping-off

Several approaches have been established for the management of Fusarium wilt and seedling damping in the tomato industry and they include cultural practices, chemical treatments, the use of resistant cultivars, grafting, soil solarisation and

biological control. However, severe losses still occur due to ineffectiveness of these approaches as determined by pathogen resistance (Hibar *et al*., 2007; Jabnoun-Khiareddine *et al*., 2010).

2.2.2 Potential uses of plant extracts as a management strategy

Natural plant products have been reported as important sources of agricultural bio-fungicides due to their ability to suppress fungal growth and disease development (Ijato *et al.,* 2010). Most plants found to have medicinal properties have also been shown to be effective against plant pathogens (Dissanayake, 2014). Examples of such reports include antifungal activity of *Oxalis corniculata L*., *Ocimum gratissimum L., Tithonia diversifolia, A. Gray*, *Azadirachta indica, A. Juss*, *Kaempferia galangal* L. and *Zingiber officinale* against fungal strains of *F. oxysporum* and *R. solani* to name a few (Dissanayake, 2014; Dwivedi and Shukla, 2000). Ginger from the Zingibereaceae family, for instance, has aromatic compounds that affect the morphology of the hyphae and mycelia structure of the fungal pathogens, viz., *Pythium spp.* and *F. oxysporum* (Jahromi *et al*., 2012; Vidyasagar and Tabassum, 2013). Since a number of medicinal plants have been tested and found to be effective in controlling fungal pathogens, the current study also aimed to test *M. burkeana* and *M. oleifera* plant extracts against two soil-borne pathogens, namely, *F. oxysporum f. sp. lycopersici* and *R. solani*.

Monsonia burkeana is widely used by local people in South Africa as special tea, with various ethno-medicinal claims. The plant has been reported to have high quantities of phenolics, essential nutrient elements and antioxidants with the highest concentrations being on the leaves (Mamphiswana *et al*., 2010; Mamphiswana *et al*.,

2011). Additionally, extracts from *M. burkeana* have potent antifungal properties, with the potential of being used in product development for managing phytosanitary risks in greenhouse production systems (Tshivhandekano *et al*., 2014). Particularly those that involve tomato plants, which are highly susceptible to bacterial and fungal wilt diseases as well as damping-off (Agrios, 2005). Mamphiswana *et al*. (2010) reported the efficacy of *M. burkeana* to suppress bacteria *Ralstonia solanacearum* Biovar 2 race 3 growth *in vitro,* the causal agent of wilt in potatoes and tomatoes. Though there is little information on the use of *M. burkeana* in managing fungal diseases, a recent study by Kena (2016) demonstrated the antifungal effect of methanolic extract of this plant against *P. digitatum* in *vitro* and subsequent reduction of citrus green mould severity in *vivo.*

Moringa oleifera Lam. (Family: Moringaceae) has gained much importance in the recent days due to its multiple uses as a medicinal plant and increased benefits in agricultural industry (Ashfaq *et al*., 2012). Roots, flowers, bark, stem, leaves and seeds of Moringa possess antimicrobial properties (Anjorin *et al.,* 2010; Dwivedi and Enespa, 2012). The fungicidal effect of Moringa extracts on some soil-borne fungi such as *Rhizoctonia spp., Pythium spp.* and *Fusarium spp. in vitro* was reported in a number of studies (Dwivedi and Enespa, 2012; El-Mohamedy and Abdalla, 2014; Moyo *et al*., 2012). Leaves of *M. oleifera* are rich in zeatin, a cytokinin in addition to other growth enhancing compounds like ascorbates, phenolic and minerals like Ca, K, and Fe that makes it an excellent crop growth enhancer (Phiri and Mbewe, 2010). *Moringa oleifera* provides a rich and rare combination of zeatin, quercetin, b-sitsterol, caffeoylquinic acid and kaempferol which have antifungal and antibacterial activities (Anjorin *et al.,* 2010; El-Mohamedy and Abdalla, 2014).

2.2.3 The use of biocontrol agents for suppression of soil-borne pathogens

Several antagonistic organisms have been successfully used as biocontrol agents for controlling soil-borne pathogens (Afzal *et al.,* 2013; Kowsari *et al.,* 2014). Soil application of biocontrol agents, *viz*. *Chaetomium spp.*, *Penicillium spp., Trichoderma viride, T. harzianum,* fluorescent *Pseudomonas spp.* and *Bacillus subtilis* have effectively reduced root rot caused by soil-borne pathogens in several crops (Loganathan *et al.,* 2010; Shafique *et al.,* 2015). Naraghi *et al.* (2010) reported successful biological control of tomato Verticillium disease by antagonistic effects of *Talaromyces flavus*.

Increasingly, *Trichoderma* species are being reported to control soil-borne diseases on a range of crops (Hafiza *et al.,* 2016). Afzal *et al.* (2013) found that endophytic *T. viride* was effective in suppressing *F. solani, F. oxysporum, R. solani* and root-knot nematode induced diseases on okra when used alone or in combination with *Pseudomonas aeruginosa.* The inhibitory effect of *Trichoderma spp.* might be due to direct mycoparasitism in addition to competition for nutrients and production of toxic metabolites (Afzal *et al.,* 2013; Hafiza *et al.,* 2016; Kowsari *et al.,* 2014). Since *T. harzianum* is viewed as a strong competitor for development as biocontrol agents, the current study then tested its potential against Fusarium wilt and seedling damping-off under laboratory and greenhouse trials.

2.3 Focus of the current study

In an effort to meet the demands of tomato, farmers resort to using of synthetic fungicides. However, target markets have set strict quality requirements that require limited use of chemicals (FAOSTAT, 2016). Some soil-borne pathogens that affect

tomatoes have a sophisticated morphology which makes them complicated to manage especially where fungicides are applied (Mizubuti *et al*., 2007). This complexity therefore, calls for a multi-faced approach in managing the diseases they induce in crops. Amongst the integrated strategies introduced, use of plant extracts, especially medicinal plants and biocontrol agents have received much attention. Hence the current study intended to test the antifungal activity of *M. burkeana* plant and *M. oleifera* leaves as well as the biocontrol agent *T. harzianum* against the troublesome soil-borne pathogens of tomato, *F. oxysporum f. sp. lycopersici* and *R. solani* since the information has not been documented.

CHAPTER 3
THE EFFICACY OF *MONSONIA BURKEANA* AND *MORINGA OLEIFERA* ON MYCELIA GROWTH OF *FUSARIUM OXYSPORUM f. sp. LYCOPERSICI* AND *RHIZOCTONIA SOLANI IN VITRO*

3.1 Introduction

Fungal diseases cause significant loss in many economic crops including tomato plants. Crop losses due to these diseases in tomato production is estimated to be more than 14 % worldwide (Agrios, 2005; FAOSTAT, 2011). Fusarium wilt of tomato caused by *F. oxysporum f. sp. lycopersici*, as well as seedling damping-off caused by *R. solani* are some of the most widely spread and economically important fungal diseases in tomato production (Hanaa *et al*., 2011). Although both diseases can be managed with the application of synthetic fungicides, the negative effects of such fungicides on the environment, human health and their inaccessibility to most emerging farmers have led to a need to develop alternative control measures. Most plants and their derivatives have expressed fungitoxicity against spore germination and mycelia growth of many fungal pathogens (El-Mohamedy *et al*., 2013; Talreja, 2010).

Monsonia burkeana and *M. oleifera* are indigenous plants found in various parts of Southern Africa including South Africa. Both plants are known for their medicinal properties and are used to treat different human and animal ailments (Mamphiswana *et al*., 2011; Moyo *et al.,* 2012). Extracts from the two plants have been reported to possess secondary metabolites which have potential for plant disease suppression (Ashfaq *et al*., 2012; Kena, 2016, Mamphiswana *et al*., 2011). However, information on their application in the management of Fusarium wilt and damping-off of tomatoes

is lacking. Hence focus of the current study was to test *M. burkeana* and *M. oleifera* efficacy on *F. oxysporum f. sp. lycopersici* and *R. solani* as major soil-borne pathogens of tomato *in vitro.*

3.2 Materials and methods

3.2.1 Study site and collection of materials

The study was conducted in the Plant Pathology laboratory at the University of Limpopo, South Africa. Healthy whole plant of *M. burkeana* (including roots, leaves, flowers, stems) and *M. oleifera* leaves were collected from the University of Limpopo experimental farm, Limpopo Province, South Africa (23°53'10" S, 29°44'15" E). A representative specimen for each plant was taken to the University of Limpopo herbarium for confirmation.

3.2.2 Preparation of methanol extracts

Monsonia burkeana plant and *M. oleifera* leaves were gently washed under slow running tap water, patted with a paper towel to remove excess water and shade dried for approximately 21 days. Dry plant materials were then ground to powder using a laboratory grinder (Model FZ-102). One hundred grams *M. burkeana* and *M. oleifera* powder were added separately to 700 ml methanol and placed on a rotary shaker for 24 hours. Methanol was then evaporated on a rotary evaporator under reduced pressure at 64 °C. The obtained extract was oven-dried for 21 days at 35 °C to constant weight, yielding green solid suspension. Prepared plant extracts were kept at 4 °C until further use.

3.2.3 Fungal cultures

Pure cultures of *F. oxysporum f. sp. lycopersici* (PPRI 5457), and *R. solani* (PPRI 13845), were acquired from the Mycology unit at the Bio-systematic division of Agricultural Research Council-Plant Protection Research Institute (ARC-PPRI), Pretoria, South Africa. *Fusarium oxysporum f. sp. lycopersici* was isolated from infected tomato plants whilst *R. solani* was isolated from maize roots showing root rot symptoms. The two fungal cultures were kept at 4 °C until further use.

3.2.4 Analytical procedure and data collection

An amount of 0, 2, 4, 6, 8 and 10 g of the resultant suspension of each plant was separately dissolved in 10 ml sterile distilled water and thoroughly mixed before being added to 200 ml bottles containing PDA. Bottles were then autoclaved at 121 °C. Six concentrations (0, 2, 4, 6, 8 and 10 ml) for each plant extract were used as treatments. Extract treatments were prepared by adding different concentrations in separate 200 ml bottles of sterilized Potato Dextrose Agar (PDA) medium and transferred equally into 48 petri plates. The extract amended PDA was then allowed to solidify overnight.

Seven-day old *F. oxysporum f. sp. lycopersici* and *R. solani* culture disk of 5 mm in diameter were cut from the edge of actively growing culture and was placed at the centre of extract amended petri plates. Inoculated petri plates were then incubated at 25 °C under aseptic condition for seven days. Non-amended Potato Dextrose Agar (PDA) medium plates served as control treatments. Pathogen colony growth was measured using a transparent ruler (Sallam and Kamal, 2012) after ±7 days.

Mycelia growth inhibition was calculated using the formula:

$$\text{Relative treatment effect (RTE)} = [(T/C) - 1] \times 100$$

Where C = average diameter of fungal colony in control plates and T = average diameter of fungal colony in extracts amended plates.

3.2.5 Experimental design and data analysis

The experiment was laid down on a completely randomized design with six treatments and 4 replicates. Data were subjected to SAS statistical program to generate partial ANOVA (Gomez and Gomez, 1984). Mean separation was achieved by using Fisher's least significant difference (LSD) at probability level of 5 %. Mean suppression level (y-axis) and *M. burkeana* or *M. oleifera* concentration level (x-axis) were subjected to the lines of the best fit using excel 2016. The responses of mean suppression to increasing *M. burkeana* or *M. oleifera* concentration level were modelled by the regression curve estimations resulting to a quadratic equation: $Y = b_2 x^2 + b_1 x + a$, where Y = Mean suppression levels; x = *M. burkeana* or *M. oleifera* concentration level using $x = -b_1 / 2b_2$ relation for the saturation point for each extract.

3.3. Results

Both *M. burkeana* and *M. oleifera* significantly suppressed mycelia growth of *F. oxysporum f. sp. lycopersici* and *R. solani* (Appendices 3.1-3.4). *Monsonia burkeana* had the highest suppression at treatment concentrations 10 and 8 g/ml for *F. oxysporum f. sp. lycopersici* and *R. solani,* respectively (Table 3.1; Figure 3.1). *Moringa oleifera* highest suppression was measured at treatment 6 g/ml for both *F. oxysporum f. sp. lycopersici* and *R. solani* (Table 3.2; Figure 3.2). Relative to untreated control, *M. burkeana* suppressed the growth of *F. oxysporum f. sp. lycopersici* and *R. solani* from 36 to 76 % and 20 to 71 %, respectively (Table 3.1).

Moringa oleifera suppressed *F. oxysporum f. sp. lycopersici* from 1 to 35 %, whereas *R. solani* was suppressed from 13 to 60 % (Table 3.2).

Table 3.1: Effect of *Monsonia burkeana* on mycelia growth suppression of *Fusarium oxysporum f. sp. lycopersici* and *Rhizoctonia solani in vitro.*

Treatment	*F. oxysporum*		*R. solani*	
(g/ml)	Mean[a]	RTE (%)[b]	Mean	RTE (%)
0	76[a]	–	85[a]	–
2	36[c]	–53	68[b]	–20
4	49[b]	–36	34[c]	–60
6	36[c]	–53	26[d]	–69
8	30[d]	–61	25[d]	–71
10	18[e]	–76	27d	–68

[a]Means in the same column followed by the same superscript were not different (P ≤ 0.05) according to Fisher's least significant difference (LSD) test.

[b]RTE = [(treatment / untreated control) –1] × 100.

Table 3.2 Effect of *Moringa oleifera* on mycelia growth suppression of *Fusarium oxysporum f. sp. lycopersici* and *Rhizoctonia solani in vitro.*

Treatment (g/ml)	*F. oxysporum f. sp. lycopersici*		*R. solani*	
	Mean[a]	RTE (%)	Mean	RTE (%)
0	68[a]	–	85[a]	–
2	59[bc]	–13	74[ab]	–13
4	54[c]	–21	52[cd]	–54
6	44[d]	–35	34[d]	–60
8	55[c]	–19	67[abc]	–21
10	67[ab]	–1	63[bc]	–26

[a]Means in the same column followed by the same superscript were not different (P ≤ 0.05) according to Fisher's least significant difference (LSD) test.

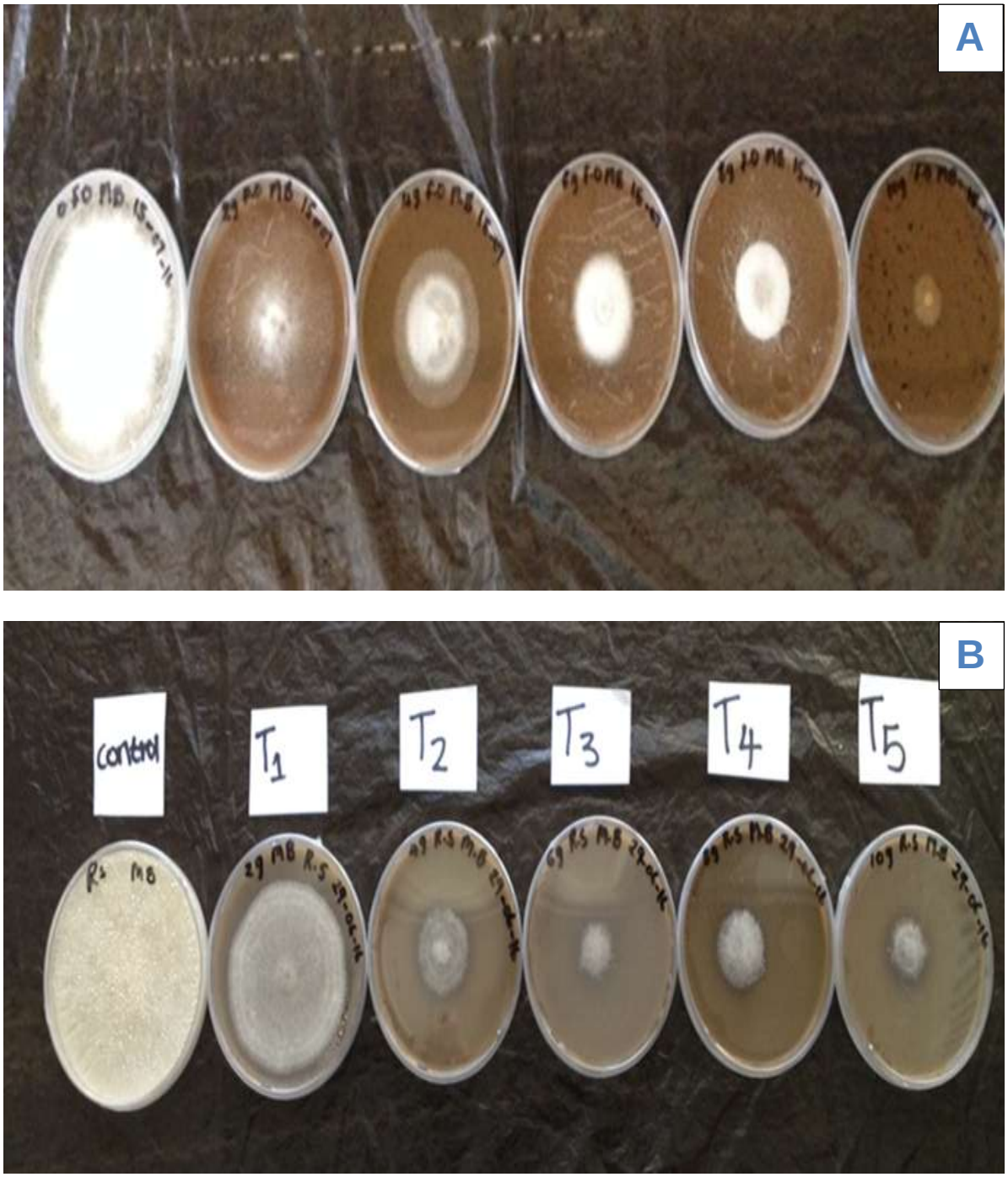

Figure 3.1 Suppression of *Fusarium oxysporum f. sp. lycopersici* (A) and *Rhizoctonia solani* (B) by *Monsonia burkeana* plant extract.

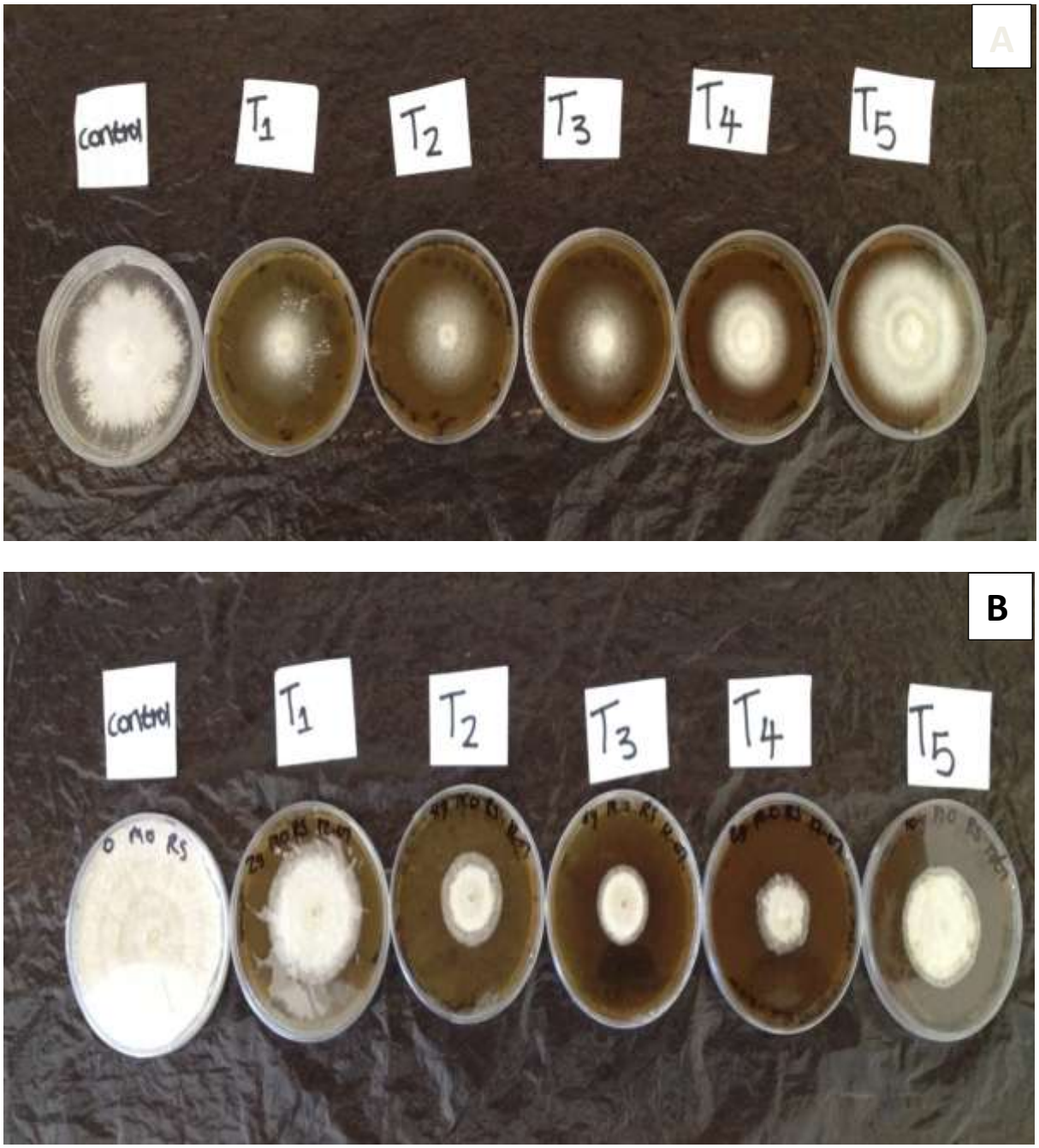

Figure 3.2 Suppression of *Fusarium oxysporum f. sp. lycopersici* (A) and *Rhizoctonia solani* (B) by *Moringa oleifera* plant extract.

In both plant species, suppression of mycelium growth and increasing concentration of *M. burkeana* and *M. oleifera* exhibited negative quadratic relations (Figure 3.3-3.6). The relations between suppression of *F. oxysporum* and *R. solani* versus increasing concentration of *M. burkeana* were explained by 76 % and 97 %

associations, respectively (Figure 3.3-3.4). In contrast, the relations between suppression of *F. oxysporum* and *R. solani* versus increasing concentration of *M. oleifera* were explained by 87 % and 69 % associations, respectively (Figure 3.5-3.6).

Mycelia growth suppression threshold was at different concentrations in *M. burkeana* and *M. oleifera* for both pathogens. For instance, *M. burkeana* concentrations optimised *F. oxysporum f. sp. lycopersici* mycelia growth suppression at concentration 13.03 g/ml with suppression threshold level being 19.09 mm and *R. solani* mycelia growth suppression at concentrations of 8.01 g/ml with suppression threshold level being 23.16 mm (Table 3.3). In *M. oleifera* treatments, *F. oxysporum f. sp. lycopersici* mycelia growth suppression was optimised at a concentration of 5.26 g/ml with suppression threshold level being 48.98 mm and *R. solani* mycelia growth suppression at a concentration of 5.94 g/ml with suppression threshold level being 48.22 mm (Table 3.4).

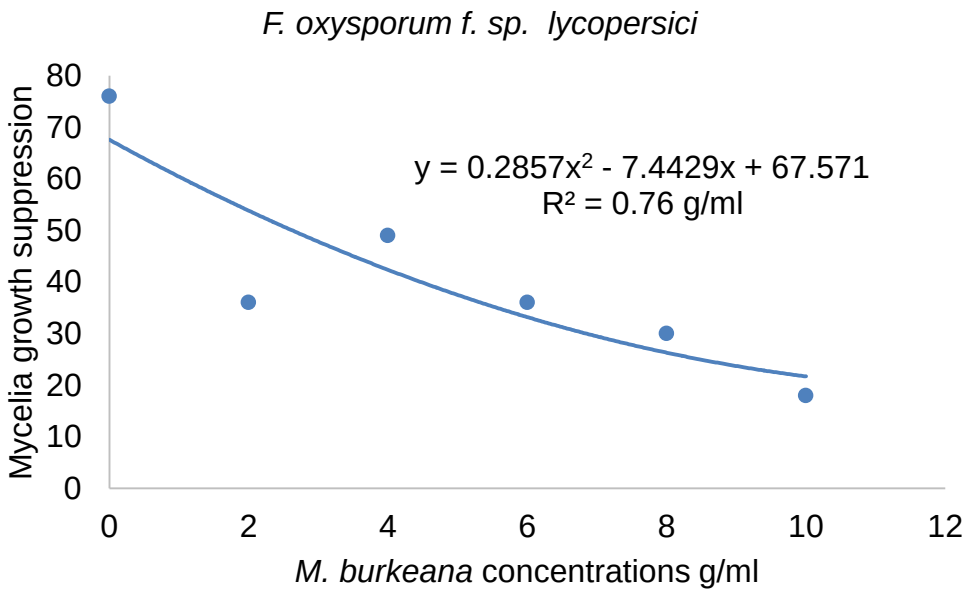

Figure 3.3 Quadratic relationship between mycelia growth of *F. oxysporum f. sp. lycopersici* and *Monsonia burkeana* plant extract concentrations.

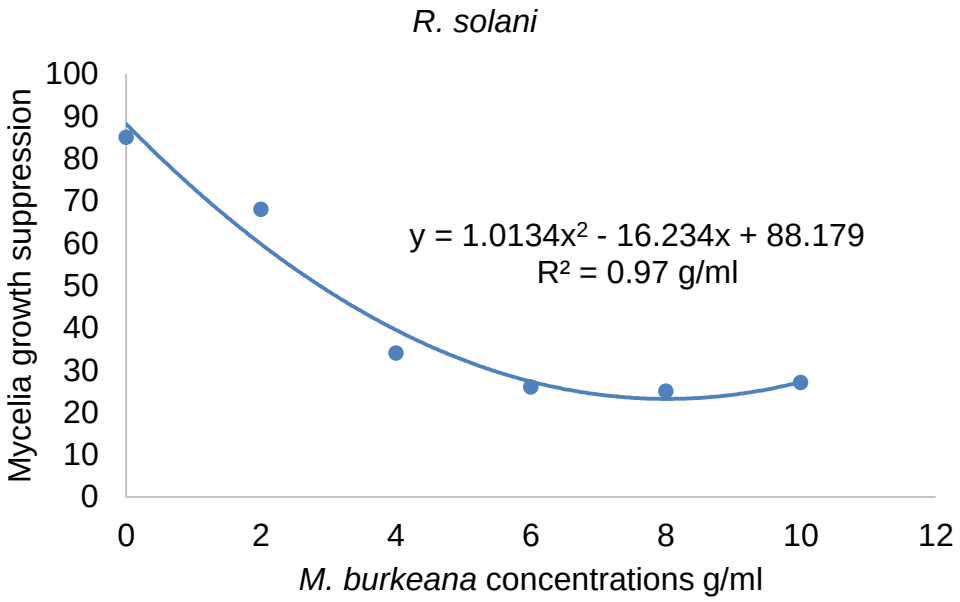

Figure 3.4 Quadratic relationship between mycelia growth of *Rhizoctonia solani* and *Monsonia burkeana* plant extract concentrations.

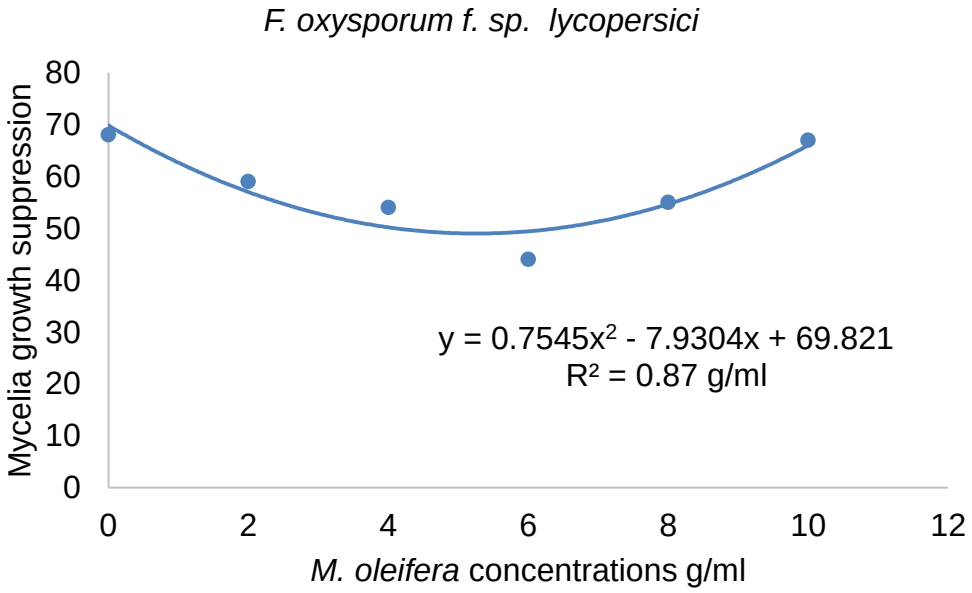

Figure 3.5 Quadratic relationship between percentage mycelia growth of *Fusarium oxysporum f. sp. lycopersici* and *Moringa oleifera* plant extract concentrations.

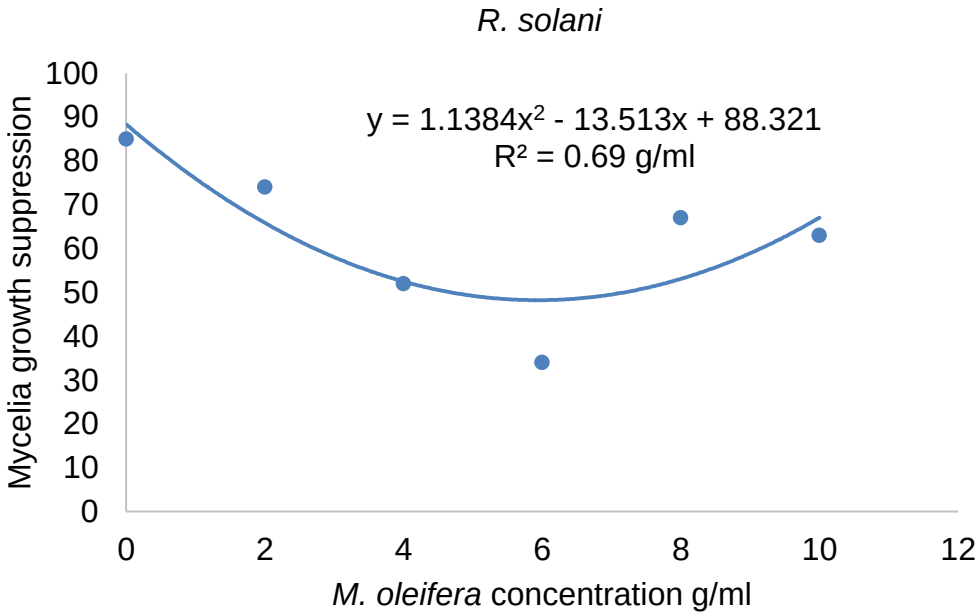

Figure 3.6: Quadratic relationship between percentage mycelia growth of *Rhizoctonia solani* and *Moringa oleifera* plant extract concentrations.

Table 3.3 *Monsonia burkeana* concentrations for optimal mycelia growth suppression of *Fusarium oxysporum f. sp. lycopersici* and *Rhizoctonia solani in vitro.*

Fungal pathogens	Formula	R^2	x	Y	P ≤
F. oxysporum	$y = 0.2857x^2 – 7.4429x + 67.571$	0.76	13.03	19.09	0.05
R. solani	$y = 1.0134x^2 – 16.234x + 88.179$	0.97	8.01	23.16	0.05

Table 3.4: *Moringa oleifera* concentrations for optimal mycelia growth suppression of *Fusarium oxysporum f. sp. lycopersici* and *Rhizoctonia solani in vitro*

Fungal pathogens	Formula	R^2	x	Y	P ≤
F. oxysporum	$y = 0.7545x^2 –7.9304x + 69.821$	0.87	5.26	48.98	0.05
R. solani	$y = 1.1384x^2 –13.513x + 88.321$	0.69	5.94	48.22	0.05

3.4 Discussion

Results of the current study demonstrated that extracts of both *M. burkeana* and *M. oleifera* can suppress mycelia growth of *F. oxysporum f. sp. lycopersic*i and *R. solani.* In comparison, *M. burkeana* was more suppressive than *M. oleifera* towards both pathogens. On average, suppression of both pathogens by *M. burkeana* was above 70 % and below 50 % in *M. oleifera* treatment concentrations. The efficacy of these selected plant extracts is in line with other investigations where extracts obtained from medicinal plants were found to be also effective against plant pathogenic organisms including fungal pathogens (Al-Ramah *et al.,* 2013; Sales *et al.,* 2016). For instance, in studies conducted by Al-Ramah *et al.* (2013) *F. oxysporum* was suppressed by up to 67 % with *Zingiber officinale* extract while *Salvadora persica* was found to be strongly effective against *R. solani* suppressing their mycelia growth by 56.43 %. Report by Sales *et al*. (2016) on the control of *Chalara paradoxa* and *Fusarium guttiforme* fungal pathogens by extracts of *Glycyrrhiza glabra L.* and *Myroxylon balsamum L.* showed mycelia growth being reduced by up to 69 % and 65 %, respectively.

The difference in fungitoxicity of *M. burkeana* and *M. oleifera* plant extracts against *F. oxysporum f. sp. lycopersic* and *R. solani* may be due to considerable variations in the extracts constituents and fungal pathogen species (Abdalla *et al*., 2009; Narayana and Shukla, 2001). Although this study did not look into the chemical constituents on both tested plant extracts, it can be postulated that their suppressive effect is due to the fungitoxic secondary metabolites responsible for pathogen growth suppression. This postulate is based on the arguments raised by a number of authors who have suggested the fungitoxic effects of medicinal plants as their main

approach in pathogen growth suppression and disease control (Jamil *et al*., 2010; Mohamed *et al*., 2010; Moyo *et al.,* 2012). Amongst others fungitoxic effects, flavonoids, tannins, saponins, alkaloids and glycosides are the secondary metabolites that have been reported to be fungitoxic against *F. oxysporum f. sp. lycopersic* and *R. solani* (Sumathy *et al.,* 2014). These metabolites including various other phenolic compounds are often cited to be very high in both *M. burkeana* organs and leaves of *M. oleifera* (Mamphiswana *et al*., 2011; Sales *et al*., 2016). Biological differences in fungal pathogens can result in varying responses towards different extracts (Kena and Swart, 2011). Such responses may include induced resistance and effector triggered immunity (Stergiopoulos and de-Wit, 2009).

From the tested treatments, an increase of the extracts concentration resulted in the increased inhibition of mycelia growth to a point where the suppression reached a threshold, after which increase in treatment concentrations resulted into stimulation of the pathogen growth. This phenomenon is described as law of the minimum by Liebig (1841) and as the saturation in the limiting factor model of Salisbury and Ross (1992). In both cases, the models state that as the independent factor increases, the effects on the dependent factor also increase to reach a threshold above which the effects become neutral (saturated). Beyond the saturation phase, as the stimulus continues to increase, the responses begin to decrease, which is technically referred to as the inhibition phase. In the studies of Bansal and Gupta (2000) and Srivastava *et al.* (2010), aqueous leaf extracts in certain medicinal plants lost their efficacy against *F. oxysporum f. sp. lycopersici*, which suggested that the concentrations were within the neutral phases. Zaman *et al*. (1997) observed that the efficacy of garlic, neem, ginger and onion extracts on seed borne fungi of mustard decreased

with an increase in concentrations of the extracts, which suggested that the concentrations were already in the inhibitory phases. In the current study, the concentration ranges used were already in the inhibition concentration ranges as depicted by the suppression of mycelia.

In conclusion, the study demonstrated that *M. burkeana and M. oleifera* successfully inhibited growth of the test pathogens and, therefore, have the potential for use in the management of the two soil-borne fungal pathogens as bio-fungicides. The efficacy of the products on pathogen suppression was concentration-specific, with the used concentrations being already in the inhibition phase. However, further work is needed to optimize the efficacy of these extracts against fungal pathogens. Nevertheless, it is important to continue with the identification of natural compounds that are not produced under usual laboratory conditions.

CHAPTER 4
THE EFFICACY OF *MONSONIA BURKEANA*, *MORINGA OLEIFERA* AND *TRICHODERMA HARZIANUM* AGAINST FUSARIUM WILT AND SEEDLING DAMPING-OFF OF TOMATO PLANT UNDER GREENHOUSE CONDITIONS

4.1 Introduction

Tomato is highly susceptible to a high number of soil-borne diseases including Fusarium wilt caused by *F. oxysporum f. sp. lycopersici* and seedling damping-off caused by *R. solani* (Al-Rahmah *et al.,* 2013). Both diseases cause severe losses in susceptible tomato cultivars (Zhou *et al.,* 2017). Management of both diseases is through chemical soil fumigation (Vidyasagar and Tabassum, 2013), seed treatment, use of cultural practices and planting of resistant cultivars (Hossain *et al*., 2016). However, all suggested control measures have high limitation resulting in their inability to provide significant disease control. For example, the detrimental effect of soil fumigants on the environment and human health has resulted in their ban in agricultural production (Gupta *et al.,* 2014). Also, development of resistant cultivars, especially against Fusarium wilt as some selections lack adequate characteristics such as yield, color and appearance (Jendoubi *et al*., 2017). Seed treatment, on the other hand, is more efficient during seed germination and seedling stage (Hossain *et al.,* 2016) and this is lost during maturity when plants are more susceptible to Fusarium wilt (Rongai *et al*., 2017).

Due to the above-mentioned challenges, there is a growing need in research activities geared towards identification and development of less toxic alternative control measures against soil-borne diseases. Plants produce compounds which have been shown to inhibit the growth and development of diseases caused by bacteria, fungi and other disease-causing organisms (Kekuda *et al.,* 2016; Nefzi *et*

al., 2016). The presence of antimicrobial compounds in plants provides an opportunity for their utilization in the management of pest and diseases as environmentally safe alternatives to synthetic pesticides (Sesan *et al*., 2016).

Naturally-occurring compounds derived from plants have been successfully tested against *F. oxysporum* (Kekuda *et al.,* 2016; Rongai *et al*., 2017). For example, Rongai *et al*. (2017) reported a significant reduction in *F. oxysporum* population and wilt severity in naturally infected soils treated with crude extracts of pomegranate peel. *Monsonia burkeana* and *M. oleifera* are medicinal plants used traditionally to heal several ailments on various parts of South Africa including Limpopo (Gopalakrishnan *et al*., 2016; Kena, 2016). Crude extracts of both plants have also been tested and found to effectively suppress pathogen growth and disease development (Choudhury *et al*., 2017). However, reports are still lacking on *M. burkeana* and *M. oleifera* use in the management of Fusarium wilt and tomato seedling damping-off caused by *R. solani*.

Also, biological control has been used successfully in the management of plant diseases as shown in different reports (Jantasorn *et al.,* 2016; Sesan *et al.,* 2016). For example, *Trichoderma spp.* has been used as a seed treatment and soil inoculant to prevent pathogen establishment and suppress disease development in various crops (O'Callaghan, 2016). Another example is the use of bacterium *Bacillus spp.* as biocontrol against both soil-borne and foliage diseases (Shafi *et al.,* 2017). Biological control agents employ various mode of action in the suppression and control plant pathogens and these can include competition, antibiosis, hyperparasitism and many more (O'Callaghan, 2016). The objective of this study

was to compare the efficacy of plant extracts from *M. burkeana* and *M. oleifera* and the biocontrol agent *T. harzianum* in the suppression of fusarium wilt and seedling damping-off in tomato production.

4.2 Materials and methods

4.2.1 Fungal cultures

Isolates of *F. oxysporum f. sp. lycopersici* (PPRI 5457) obtained from an infected tomato plant and *R. solani* (PPRI 13845) isolated from a diseased maize seedling were used in the study. Also, the biological control treatment *T. harzianum* isolate PPRI 8230, was used. The two fungal isolates and bio-control (*T. harzianum*) used in the study were obtained from the Mycology Division, Plant Protection Research Institute, Agricultural Research Council (Pretoria, South Africa). Fungal isolates were maintained on potato dextrose agar (PDA) and stored at 4 °C. Isolates were further sub-cultured on PDA and grown at ± 25 °C for 7-8 days when inoculum was required.

4.2.2 Preparation of plant extracts

Both *M. burkeana* and *M. oleifera* treatment concentrations applied in the greenhouse study were generated previously (Chapter 3) on the basis of low, moderate and high suppressive abilities. Aqueous extracts were prepared by adding selected concentrations of *M. burkeana* (4, 6, 8 g/ml) and *M. oleifera* (2, 4, 6 g/ml) in 100 ml distilled water. The mixture was decocted at 100 °C for 15 minutes and then left to cool before filtering.

4.2.3 Inoculum preparation and procedures

Fusarium oxysporum f. sp. lycopersici

Microconidia of *F. oxysporum f. sp. lycopersici* were harvested from a 7-day-old pure culture by flooding the plates with 10 ml sterile distilled water followed by gentle scrapping of the mycelium with a sterile needle to dislodge spores. The collected conidia were transferred into 250 ml media bottles containing 100 ml sterile distilled water bottles and incubated for 24 hours. The conidial suspension was further adjusted to a final concentration of 1×10^6 conidia/ml under a light microscope.

One-month-old cv. 'HTX14' seedlings were uprooted and roots gently washed under slow running tap water to remove peat debris. Inoculation was done by dipping roots in conidia suspension for 30 minutes. In control treatments, seedlings were dipped in sterile distilled water. Inoculated and un-inoculated seedlings were transferred into 25 cm plastic pots containing a mixture of pasteurized sand and Hygromix in a ratio 3:1 (v/v) ratio and left to stand for seven days. After seven days, growth mixture was treated by incorporating various plant extracts treatments at the rate of 250 ml/plant. The control received the same amount of sterile water thereafter the subsequent applications were done at seven-day intervals.

Rhizoctonia solani

Pathogen inoculum was prepared by soaking 240 g clean quartz in 75 ml of distilled water for 24 hours in 500 litres Erlenmeyer flasks. Thereafter, 6.0 g of yellow maize meal and 75 ml of tomato juice was added to the flasks and autoclaved twice for two

consecutive days. The autoclaved mixture was then inoculated with 20 discs of 7-day-old pure *R. solani* culture and incubated for 14 days at 25 °C. After incubation, the inoculum was oven dried at 30 °C for 14 days.

Money maker cultivar was used as a test plant against *R. solani* seedling damping-off. Tomato seeds were soaked in different concentrations of *M. burkeana* and *M. olifera* extracts solutions for 24 hours after which, they were dried for further 24 hours under a laminar flow bench. For control treatment, seeds were soaked in sterile distilled water. Dried seeds were transferred to 25 cm plastic pots filled with pasteurized sand and Hygromix in a 3:1 (v/v) ratio. Four 80 mm deep and 50 mm wide holes were punched and the media was artificially inoculated with 20 g of the dried *R. solani* inoculum in each hole. Inoculated growth media was moistened with two-hundred ml of sterile distilled water and left to stand for seven days before planting to allow the pathogen to establish in the soil. After seven days, five dry, treated seeds were planted in each pot and left to stand for a further seven days after which treatments were applied once every week.

Trichoderma harzianum

Trichoderma harzianum treatment used for *F. oxysporum f. sp. lycopersici* inoculated pots was prepared in the same manner as *F. oxysporum f. sp. lycopersici* inoculum however the treatment was incubated for 72 hours at 25 °C. Pots were then artificially infested with 100 ml of the biocontrol treatment 7 days after the pathogen was established in the pots. In *R. solani* trial, *Trichoderma harzianum* treatment was also prepared following the same procedure used for *R. solani* and received the same amount of dried biocontrol treatment 7 days after *R. solani* was established in

the soil. Pots treated with *T. harzianum* received the same amount of water as in the control.

4.2.4 Study site

The study was conducted in the greenhouse at the Green Biotechnologies Research Centre, University of Limpopo. The greenhouse maximum/minimum temperatures average is 28/21 °C, whereas in winter its average is 24/16 °C. Due to the bigger size of the greenhouse and the wind-blown generated currents, conditions inside the greenhouse were not homogeneous, thereby, dictating that experiments should be properly designed depending on experimental size.

4.2.5 Treatments and experimental design

Fusarium wilt was tested on cv. 'HTX14' as the most susceptible cultivar whilst seedling damping-off was tested on tomato cv. 'Money maker'. Five treatments were used in this study (Table 4.1). The experiment was arranged in a randomised complete block design (RCBD) and each treatment was replicated six times.

Table 4.1 Treatment connotations and levels for both *Fusarium oxysporum f. sp. lycopersici* and *Rhizoctonia solani.*

Treatment		Description
T0	untreated control	
T1	0.4 g/ml *M. burkeana*	0.2 g/ml *M. oleifera*
T2	0.6 g/ml *M. burkeana*	0.4 g/ml *M. oleifera*
T3	0.8 g/ml *M. burkeana*	0.6 g/ml *M. oleifera*
T4	*T. harzianum* inoculum (100 ml / pot)	*T. harzianum* inoculum (100 ml / pot)

4.3 Data collection

Fusarium wilt

The severity of Fusarium wilt on tomato was recorded during harvest by examining shoot, stem and root symptoms. Disease severity was assessed on above and below ground plant parts using a scale of 0-5 (Jarvis and Thorpe, 1976; Shazia *et al*., 2015). Shoot disease was assessed on a severity scale of 0-5 where: 0 = plant well developed with no disease symptoms; 1 = Moderate leaf yellowing at the margin of the leaves 10 %; 2 = Moderate wilting of plant including flowers 30 %; 3 = Plant stunted and yellowing 50 %; 4 = Severe stunting with majority of leaves wilted and dead 70 %; 5 = Complete death of the plant 100 % (Shazia *et al.,* 2015). Lower stem and root disease was assessed on the severity scale of 0-5 scale where: (0 = no symptoms; root well developed with no discolouration; 1 = no internal browning, discrete superficial lesions on taproot or stem base 10 %; 2 = brown taproot with slight internal browning at taproot 30 %; 3 = moderate internal browning of the entire taproot 50 %; 4 = severe internal browning extending from taproot into lower stem above soil surface 70 %; 5 = dead plants 100 %) as described by Jarvis and Thorpe, (1976).

Disease severity was calculated using the formula:

Percentage disease severity = area of plant part affected / total area x 100

Damping-off

Experiment inoculated with *R. solani* was assessed for pre- and post-emergence damping-off at the seedling stage, recording the number of seeds that did not

germinate and plants showing symptoms from the seeds that germinated. Pathogen re-isolation was made from dead seedlings to confirm the presence of *R. solani*.

Pre-emergence damping-off (%) = Number of diseased seeds / total number of seeds planted x 100

Post-emergence damping-off (%) = Number of diseased seedlings / total number of seedlings x 100

Efficacy of plant extracts on each treatment was evaluated by: Relative treatment effect (RTE) = [(treatment / untreated control) –1] × 100, where the reduction was expressed with a negative sign and stimulation or increase was expressed by a positive sign.

4.4 Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SAS statistical programme (Gomez and Gomez, 1984). Severity percent data were transformed to arcsine whilst damping-off incidence data were log-transformed prior to ANOVA. Original data were then recorded for mean separation. Mean separation was achieved by using Fisher's least significant difference (LSD) test at the probability level of 5 %.

4.5 Results

4.5.1 Response of Fusarium wilt to *Monsonia burkeana*, *Moringa oleifera* and *Tricoderma harzianum*

Monsonia burkeana, *M. oleifera* and *T. harzianum* significantly reduced Fusarium wilt severity under greenhouse conditions (Appendices 4.1-4.4). The results of Fusarium

wilt severity in soil amended with different concentrations of *M. burkeana* and *T. harzianum* as biological control are presented (Table 4.2). The results show that amending the infected soil with 0.6 g/ml of *M. burkeana* extract significantly reduced shoot severity resulting in a mean of 0.6. Although the mean was not significantly different to the extracts, the same reduction in shoot disease severity was observed on tomato plants grown in *T. harzianum* amended soil. In stem and root discolouration severity, the highest reduction was recorded in T3 with a mean of 0.5 severity. The same reduction was also recorded in *T. harzianum* treatment. Stem and root severity in T1 and T3 were not significantly different from T0 which represented untreated control. The RTE of the pathogen showed an increase in the efficacy of *M. burkeana* extract and *T. harzianum* as concentration increased. *Monsonia burkeana* extracts reduced shoot symptoms by 32 % in T2 whilst stem and root discolouration was reduced by 40 % relative to control (Table 4.2). *Trichoderma harzianum* also reduced the shoot, stem and root discolouration symptoms by 28 and 49 %, respectively (Table 4.2).

Fusarium wilt severity in soil amended with different concentrations of *M. oleifera* and *T. harzianum* as biological control are presented (Table 4.3). The results show that amending the infected soil with 0.6 g/ml of *M. oleifera* extract significantly reduced both shoot severity and stem and root discolouration resulting in a mean of 0.5 and 0.4. The reduction was also recorded in *T. harzianum* treatment. Amending Fusarium wilt infected soil with *T. harzianum* resulted in significant reduction in shoot and stem and root discolouration symptoms resulting in a mean of 0.5 and 0.6, respectively (Table 4.3). Shoot severity, as well as stem and root severity in T1 and T3, were not significantly different from T0 which represented inoculated, non-

amended control. Relative treatment effect results showed a significant difference in *M. oleifera* extract and *T. harzianum* efficiency amongst concentrations. *Moringa oleifera* extracts reduced shoot symptoms by 49 % at treatment 3 whilst stem and root discolouration was reduced by 54 % on the same treatment relative to control (Table 4.3). Treating Fusarium-infected soil with *T. harzianum* also reduced the shoot and stem and root discolouration symptoms by 44 and 23 %, respectively (Table 4.3). Comparing the two tested extracts and *T. harzianum, M. oleifera* was found to be the most potent to highly reduce the severity of Fusarium wilt on tomato plants.

Table 4.2 Relative treatment effect (RTE) of *Monsonia burkeana* extracts and *Trichoderma harzianum* on shoot symptoms, stem and root discolouration and Fusarium wilt.

	Shoot disease severity		Stem and root discolouration severity	
Treatments g\ml[y]	Mean[x]	RTE (%)	Mean	RTE (%)
T0	0.8[ab]	–	0.9[a]	–
T1	0.7[a]	–6	0.7[bc]	–24
T2	0.6[ab]	–32	0.8[ab]	–15
T3	0.9[a]	4	0.5[cd]	–40
T4	0.5[ab]	–28	0.5[d]	–49

[y]T0 = Untreated control, T1 = 0.4 g/ml, T2 = 0.6 g/ml, T3 = 0.8 g/ml and T4 = *T. harzianum*

[x] Column means followed by the same superscript were not different (P ≤ 0.05) according to Fisher's least significant difference test

Table 4.3: Relative treatment effect (RTE) of *Moringa oleifera* extracts and *Trichoderma harzianum* on above ground, stem and root discolouration of Fusarium wilt.

	Shoot disease severity		Stem and root discolouration	
Treatment (g\ml)[y]	Mean	RTE (%)	Mean	RTE (%)
T0	0.9[a]	–	0.9[a]	–
T1	0.6[b]	–33	0.5[c]	–41
T2	0.5[bc]	–49	0.4[c]	–54
T3	0.6[b]	–36	0.7[b]	–19
T4	0.5[bc]	–44	0.6[b]	–23

[y]Treatments were: T0 = Untreated control, T1 = 0.2 g/ml, T2 = 0.4 g/ml, T3 = 0.6 g/ml and T4=*T. harzianum*

4.5.2 Response of post-emergence damping-off to *Monsonia burkeana*, *M. oleifera* and *T. harzianum*

Monsonia burkeana, *M. oleifera* and *T. harzianum* significantly reduced damping-off incidence under greenhouse conditions (Appendices 4.5-4.8). The results of damping-off incidence in soil amended with different concentrations of *M. burkeana* and *T. harzianum* as biological control are presented (Table 4.4). The results show that amending the infected soil with 0.6 g/ml and 0.8 g/ml of *M. burkeana* extract significantly reduced pre- and post-emergence damping-off incidence resulting in a mean of 0.1 and 0.2, respectively. The *T. harzianum* amended in Fusarium wilt infected soil resulted in significant reduction in pre- and post-emergence damping-off incidence recording mean of 0.2 and 0.4 (Table 4.4). Other treatments were

moderately effective compared to T0 which represented inoculated, non-amended control. Relative treatment effect results showed a significant difference in *M. burkeana* extract and *T. harzianum* efficiency amongst the concentrations. *Monsonia burkeana* extracts reduced pre-emergence damping-off by 78 % in T2 whilst incidence of post-emergence damping-off was reduced by 69 % in T3 relative to control (Table 4.4). Treating *R. solani* infected soil with *T. harzianum* also lead to a relative reduction of pre-emergence (60 %) and post-emergence damping-off (38 %) as presented (Table 4.4).

The results of damping-off incidence in soil amended with different concentrations of *M. oleifera* and *T. harzianum* are presented (Table 4.5). The results show that infected soil amended with 0.2 g/ml and 0.6 g/ml of *M. oleifera* extract significantly reduced pre- and post-emergence damping-off incidence resulting in a mean of 0.2 and 0.3 respectively. Amending Fusarium wilt infected soil with *T. harzianum* also resulted in a significant reduction in pre- and post-emergence damping-off incidence recording means of 0.5 and 0.4, respectively. Relative treatment effect results showed a significant difference in the efficiency of *M. oleifera* extract concentrations and *T. harzianum* treatment. Extracts of *M. oleifera* reduced pre-emergence damping-off by 64 % in T1 whilst post-emergence damping-off was reduced by 31 % in T3 relative to untreated control (Table 4.5). Although damping-off incidence on *T. harzianum* treated pots was slightly reduced, RTE of 18 % and 13 % was recorded for pre- and post-emergence damping-off respectively.

Table 4.4 Effect of *Monsonia burkeana* extract and *Trichoderma harzianum* on pre- and post-emergence damping-off caused by *Rhizoctonia solani* under greenhouse conditions.

	Pre-emergence damping-off		Post-emergence damping-off	
Treatment (g\ml)[a]	Mean	RTE (%)	Mean	RTE (%)
T0	0.4[ab]	–	0.7[a]	–
T1	0.4[ab]	–16	0.6[a]	–7
T2	0.1[c]	–78	0.5[a]	–23
T3	0.4[ab]	–14	0.2[bc]	–69
T4	0.2[bc]	–60	0.4[ab]	–38

[a] T0 = Untreated control, T1 = 0.4 g/ml, T2 =0.6 g/ml, T3 = 0.8 g/ml and T4 = *T. harzianum.*

Table 4.5: Effect of *Moringa oleifera* extract and *Trichoderma harzianum* on pre- and post-emergence damping-off caused by *Rhizoctonia solani* under greenhouse conditions.

	Pre-emergence damping-off		Post-emergence damping-off	
Treatment (g/ml) [a]	Mean	RTE (%)	Mean	RTE (%)
T0	0.6[a]	–	0.3[b]	–
T1	0.2[c]	–64	0.5[a]	–21
T2	0.5[a]	–12	0.4[ab]	5
T3	0.3[bc]	–51	0.3[b]	–31
T4	0.5[ab]	–18	0.4[ab]	–13

[a]T0 = Untreated control, T1 = 0.2 g/ml, T2 = 0.4 g/ml, T3 = 0.6 g/ml and T4 = *T. harzianum.*

4.6 Discussion

Monsonia burkeana and *M. oleifera* plant extracts, separately reduced Fusarium wilt severity and incidence of damping-off under greenhouse conditions in the current study. *Trichoderma harzianum* as a biological control agent was also found to be effective in reducing the two diseases in *vivo*. The efficacy of the products corroborates previous studies which demonstrated the ability of *M. burkeana* (Kena, 2016), *M. oleifera* and *T. harzianum* (Adandonon *et al*., 2006) to reduce the disease severity and disease incidence of fungal soil-borne diseases. This also agrees with Obongonya *et al.* (2010) who reported that neem extract was effective against *F. oxysporum f. sp. phaseoli* and promoted the shoot and root of beans. Shervin *et al.* (2011) found neem powder extract to significantly suppress tomato plant infection by *F. oxysporum f. sp. lycopersici* and this resulted in a significant reduction in wilt severity and vascular discolouration.

Most medicinal plants including *M. burkeana* and *M. oleifera* contain a number of phytochemicals that exhibit antimicrobial activity (Mamphiswana *et al.,* 2011; Mboto *et al.,* 2009). Most of these phytochemicals include secondary metabolites and compounds such as flavonoids and tannins (Mamphiswana *et al.,* 2011; Shafighi *et al*., 2012), which are the main antifungal components associated with disease suppression. Further, these secondary metabolites form complexes with the polysaccharides and proteins associated with the external layer of fungal cells that might result in possible death of the pathogen (Rongai *et al.,* 2017). However, additional work is necessary to determine the mode of action exhibited by the plant extracts on both *F. oxysporum f. sp. lycopersici* and *R. solani.*

The effectiveness of *T. harzianum* might be due to a number of factors including competition, production of antifungal metabolites with fungicidal capabilities, toxic antibiotics and mycoparasitism (Adandonon *et al*., 2006). The degree of reduction of Fusarium wilt and damping-off by *T. harzianum* is possibly attributed to the secretion of antibiotics by the antagonist (Rongai *et al*., 2017) or other inhibitory substances produced by the antagonistic chemical compounds such as geodin, terricin and terric acids (Waing *et al.,* 2015). For example, certain *Trichoderma* species colonise and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant, considered to be part of the plant defense responses, which eventually lead to an induced systemic resistance in the entire plant (Adandonon *et al*., 2006).

Although the main focus of the current study was on the effects of plant extracts and *T. harzianum* on disease severity, it was also observed that the suppressive-ness was concentration-dependent. For example, extracts of *M. oleifera* were more effective against Fusarium wilt than *M. burkeana*, probably due to the different concetrations used. However, *M. burkeana* extracts were more effective on suppression of damping-off incidence than those of *M. oleifera*. A report by Hassenein *et al*. (2010) indicated greater efficacy of neem extracts when compared to other extracts, probably due to different chemical compounds in neem that had greater antifungal activities. Also, biocontrol agents as reported by Waing *et al.* (2015) have had a degree of effectiveness that varied according to the nature, quality and quantity of antibiotics or inhibitory substances secreted.

CHAPTER 5
SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary and conclusion

Soil-borne fungal diseases are the major causes of crop yield losses in tomato production. Although various management practices including application of synthetic fungicides are used as control, their effectiveness are short lived ad compromised. Plant extracts and biological control agents are currently gaining recognition as viable alternatives to fungicides. The aim of this study was to develop alternative management strategy against two most devastating diseases of tomatoes namely, Fusarium wilt and seedling damping-off using extracts of *M. burkeana* and *M. oleifera* as well as biological control agent *T. harzianum.* Application of the extracts and biocontrol agent was found to be the best in reducing severity and disease incidence of soil-borne pathogens tested in this study. There are few references on the efficacy of *M. burkeana* and *M. oleifera* against plant pathogens. Also, information is lacking on their application against both Fusarium wilt and tomato seedling damping-off caused by *R. solani*. The results of this study can therefore form a basis for further and deeper investigations on their mode of action especially taking into consideration the economic importance of these diseases in tomato production.

5.2 Recommendations

From the conclusions, it is recommended that:

i. Antagonistic microbes and plant extracts be incorporated into the integrated crop management programme to reduce accumulation of residues in fresh vegetables and production systems.

ii. Plants and microorganisms with antimicrobial activity need to be formulated into forms that can give long shelf life and ease of application.

iii. More explorations should be made into the natural environment for plants and microorganisms with active compounds which could be used in the place of synthetic fungicides.

iv. The synergistic effects between and among the extracts and antagonistic microorganisms should also be investigated.

v. Awareness should be raised on use of biofungicides in management of diseases especially to the small-scale farmers and a policy on campaigns to promote use of biofungicides.

REFERENCES

ABDALLA, M. A., SIHAM, E., SUMAN, U. and Y. Y. H. A. ALIAN. 2009. Microbial Contamination of Sheep Carcasses at Slaughterhouse in Khartoum State. *Sudan Journal of Veterinary Science and Animal Husbandry*, 48: 51-56.

ABU-TALEB, M. A. KADRIYA, E. and O. A. FATIMAH. 2011. Assessment of antifungal activity of *Rumex vesicarius L*. and *Ziziphus spina-christi L*. wild extracts against two phytopathogenic fungi. *African Journal of Microbiology Research*, 5: 1001-1011.

ADANDONON, A., AVELING, T. A. S., LABUSCHAGNE, N. and M. TAMO. 2006. Biocontrol agents in combination with *Moringa oleifera* extract for integrated control of Sclerotium-caused cowpea damping-off and stem rot. *European Journal of Plant Pathology*, 23: 97-127.

AFROZ, A., KHAN, M. R. and S. KOMATSU. 2010. Proteomic analysis of tomato cultivars treated with growth elicitor's jasmonic acid and salicylic acid. *Protein and Peptide Letters*, 17: 836-846.

AFZAL, S., TARIQ, S., SULTANA, V., ARA, J. and S. EHTESHAMUL-HAQUE. 2013. Managing the root diseases of okra with endo-root plant growth promoting *Pseudomonas* and *Trichoderma viride* associated with healthy okra roots. *Pakistan Journal of Botany,* 42: 118-220.

AGRIOS, G. N. 1997. Plant Pathology. 4th edition. Academic Press: USA Pages 635.

AGRIOS, G. N. 2005. Plant Pathology. 5th edition. Academic Press: USA Pages 3-75.

AJIGBOLA, C. F. and O. O. BABALOLA. 2013. Integrated management strategies for tomato Fusarium wilt. *Biocontrol Sciences*, 18: 117-127.

AKRAMI, M. and Z. YOUSEFI. 2015. Biological control of Fusarium wilt of tomato (*Solanum lycopersici L*.) by *Trichoderma spp.* as antagonist fungi. *Phytopathology*, 7: 887-892.

AL-RAHMAH, A. N., MOSTAFA, A. A., ABDEL-MEGEED, A., YAKOUT, S. M. and S. A. HUSSEIN. 2013. Fungicidal activities of certain methanolic plant extracts against tomato phytopathogenic fungi. *African Journal of Microbiology Research*, 7: 517-524.

ANJORIN, T.S., IKOKOH, P. and S. OKOLO. 2010. Mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions in Abuja, Nigeria. *International Journal of Agriculture and Biology*, 12: 431-434.

ASHFAQ, M., BASRA, S. M. A. and U. ASHFAQ. 2012. Moringa: A miracle plant of Agro-forestry. *Journal of Agricultural Social Science,* 8: 115-122.

ASHWANI, T., SHARMA, Y. P. and T. N. LAKHANPAL. 2004. Effect of volatile compounds released by *Gliocladium virens and Trichoderma spp.* on growth of *Dematophora necatrix. Journal of Mycology and Plant Pathology*, 34: 37-40.

BABALOLA, O. O. 2010. Pectinolytic and cellulolytic enzymes enhance *Fusarium compactum* virulence on tubercles infection of Egyptian broomape. *International Journal of Microbiology*, 6: 1-7.

BANSAL, P. K. and P. K. GUPTA. 2000. Evolution of plant extracts against *Fusarium oxysporum* wilt pathogen. *Indian Journal of Phytopathology,* 53: 107-108.

BAWA, I. 2016. Management strategies of Fusarium wilt disease of tomato incited by *Fusarium oxysporum f. sp. lycopersici* (Sacc.): A REVIEW. *International Journal of Advanced Academic Research*, 2: 32-42.

BENÍTEZ, T., RINCÓN, M. A., LIMÓN, M. C. and C. A. CODÓN. 2004. Bio-control mechanisms of *Trichoderma* strains. *International Microbiology,* 7: 249-260.

CAI, G., GALE, L. R., SCHNEIDER, R. W., KRISTLER, H. C., DAVIS, R. M. and K. S. ELIAS. 2013. Origin of race 3 of *Fusarium oxysporum f. sp. lycopersici* at a site in California. *Phytopathology*, 93: 1014-22.

CERESINI, P. 1999. *Rhizoctonia solani*. North Carolina State University, Carolina Plant Pathology Publication, Page 728.

CERKAUSKAS, R. 2001. *Fusarium* Stem and Fruit Rot of Greenhouse Pepper. Ministry of Agriculture and Food. Fact sheet. Government of Ontario, Canada. Pages 1- 8.

CHOHAN, S., PERVEEN, R., MAHMOOD, M. A. and A. U. REHMAN. 2016. Fungi colonizing different parts of tomato plant (*Lycopersicon lycopersici L*.) Karst in Pakistan. *Pakistan Journal of Phytopathology*, 28: 25-33.

DAN, O. C., ERIN, N. R. and N. KOKALIS-BURELLE. 2013. The effect of transitional organic production practices on soil-borne pests of tomato in a simulated micro-plot study. *Phytopathology,* 8: 792-801.

DEPARTMENT OF AGRICULTURE, FORESTRY and FISHERIES. 2011. A Profile of the South African Tomato Value Chain, *Department of Agriculture, Forestry and Fisheries, Pretoria*, South Africa.

DEPARTMENT OF AGRICULTURE, FORESTRY and FISHERIES. 2015. A Profile of the South African Tomato Value Chain, *Department of Agriculture, Forestry and Fisheries, Pretoria*, South Africa.

DISSANAYAKE, M. L. M. C. 2014. Inhibitory Effect of Selected Medicinal Plant Extracts on phytopathogenic fungus *Fusarium oxysporum* (Nectriaceae) Schlecht. Emend. Snyder and Hansen. *Annual Research and Review in Biology*, *4*: 133-175.

DORRANCE, A. E., KLEINHENZ, M. D., McCLURE, S. A. and N. T. TUTTLE. 2003. Temperature, moisture and seed treatment effects on *Rhizoctonia solani* root rot of soybean. *Plant Disease*, 87: 533-538.

DORRANCE, A. E., LIPPS, P. E. and D. R. MILLS. 2001. *Rhizoctonia* Damping-off and Stem Rot of Soybeans. Extension Fact-sheet. Plant Pathology, Columbus, Ohio State University. Pages 1-2.

DWIVEDI, S. K. and A. ENESPA. 2012. Effectiveness of extract of some medical plants against soil borne fusaria causing diseases on *Lycopersicon esculantum* and *Solanum melongena*. *International Journal of Pharmacy and Biological Sciences,* 3: 1171-1180.

DWIVEDI, B. P., and D. N. SHUKLA. 2000. Effect of leaf extracts of some medicinal plants on spore germination of some Fusarium species. Karnataka Journal of Agricultural Science, 13:153-154.

EL–MOHAMEDY, R. S. R. and A. M. ABDALLA. 2014. Evaluation of antifungal activity of *Moringa oleifera* extracts as natural fungicide against some plant pathogenic fungi *in vitro*. *Journal of Agricultural Science and Technology*, 10: 963-982.

EL-MOHAMEDY, R. S. R., ABDEL-KADER, M. M, ABD-EL-KAREEM, F. and N. S EL-MOUGY. 2013. Essential oils, inorganic acid and potassium salts as control measures against the growth of tomato root rot pathogens *in vitro*. *Journal of Agricultural Technology*, 9: 1507-1520.

FAOSTAT, 2011. Food and Agricultural Organisation of United Nations, Statistical database. Rome, Italy.

FAOSTAT, 2016. Food and Agricultural Organisation of United Nations, Statistical database. Rome, Italy.

FAOSTAT. 2017. Food and Agricultural Organisation of United Nations, Statistical database.

FRAVEL, D., OLIVIAN, C. and C. ALABOUVETTE. 2003. *Fusarium oxysporum* and its biocontrol. *Phytopathology*, 154: 493-502.

GOPALAKRISHNAN, L., DORIYA, K. and D. S. KUMAR. 2016. *Moringa oleifera:* A review on nutritive importance and its medicinal application. *Food Science and Human Wellness,* 5: 49-56.

Gomez, K. A. and A. A. Gomez. 1984. Statistical procedures for agricultural research 2nd edition, John Wiley and sons, New York, page 680.

GUPTA, S., SINGH, G. and N. SHARMA. 2014. *In vitro* screening of selected plant extracts against *Alternaria alternata*, *Journal of Experimental Biology and Agricultural Sciences*, 2: 344-351.

HAFIZA, A. S., VIQAR, S., SYED, E. and A. MOHAMMAD. 2016. Management of soil-borne diseases of organic vegetables. *Journal of Plant Protection Research,* 56: 221-230.

HAGGAG, K. H. E. and G. E. NADIA. 2012. In *vitro* study on *Fusarium solani* and *Rhizoctonia solani* isolates causing damping-off and root rot diseases in tomatoes. *Journal of Nature and Science,* 10:16-25.

HANAA, R. M. F., ABDOU, Z. A., SALAMA, D. A., IBRAHIM, A. R. and H. A. M. SROR. 2011. Effect of neem and willow aqueous extracts on Fusarium wilt disease in tomato seedlings: Induction of antioxidant defensive enzymes. *Annual of Agricultural Sciences*, 56: 1-7.

HARARI, H. 2016. Bio-control of tomato Fusarium wilt by *Trichoderma* species under *in vitro* and *in vivo* conditions, *Cercetări Agronomice în Moldova*, 1: 91-98.

HARIKRISHNAN, R. and X. B. YANG. 2004. Recovery of anastomosis groups of *Rhizoctonia solani* from different latitudinal positions and influence of temperatures on their growth and survival. *Plant Disease,* 88: 817-823.

HARMAN, G. E. 2006. Overview of mechanisms and uses of *Trichoderma spp. Phytopathology,* 96: 190-194.

HARMAN, G. E., HOWELL, C. R., VITERBO, A., CHET, I. and M. LORITO. 2004. *Trichoderma* species-opportunistic, avirulent plant symbionts. N*ature Review Microbiology*, 2: 43-56.

HASSANEIN, N. M., ALI, M. M., YOUSSEF, K. A. and D. A. MAHMOUD. 2010. Control of tomato early blight and wilt using aqueous extract of neem leaves. *Phytopathology,* 49: 143-151.

HERMOSA, R. A., VITERBO, L., CHET, A. and E. MONTE. 2012. Plant beneficial effects of *Trichoderma* and its genes. *Microbiology Journal,* 158: 14-25.

HIBAR, K., DAAMI-REMADI, M. and M. EL-MAHJOUB. 2007. Induction of resistance in tomato plants against *Fusarium oxysporum f. sp. radicis-lycopersici* by *Trichoderma spp. Tunisian Journal of Plant Protection,* 2: 47-58.

HOSSAIN, M. T., KHAN, A., CHUNG, EJ., RASHID, M. H. and Y. R. CHUNG. 2016. Biological control of rice Bakanae by an endophytic *Bacillus oryzicola* YC7007. *Plant Pathology Journal,* 32: 228-241.

IJATO, J. Y., OYEYEMI, S. D., IJADUNOLA J. A. and J. A. ADEMUYIWA. 2010. Allelopathic effect of leaf extract of *Azardirachta indica* and *Chromolaena odorata* against post-harvest and transit rot of tomato (*Lycopersici esculentum L.). Journal of American Science,* 6: 1595-1599.

JABNOUN-KHIAREDDINE, H., DAAMI-REMADI, M., PLATT, H. W., AYED, F. and M. EL-MAHJOUB. 2010. Variation in aggressiveness of Tunisian *Verticillium dahliae* races 1 and 2 isolates and response of differential tomato cultivars to Verticillium wilt. *International Journal of Plant Breeding and Genetics*, 4: 63-70.

JAHROMI, G. M, POURMIRZA, A. A. and H. M. SAFARALIZADEH. 2012. Repellent activity of sirinol (garlic emulsion) against *Lasioderma sernicorne* (Coleoptera:

Anobiidae) and *Tribolum castaneum* (Coleoptera: Terebrionidae) by three laboratory methods, *African Journal of Biotechnology,* 11: 280-288.

JAMIL, M., IQBAL, W., BANGASH, A., REHMAN, S., IMRAN, Q. M. and E. S. RHA. 2010. Constitutive expression of OSC3H33, OSC3H50 and OSC3H37 genes in rice under salt stress. *Pakistan Journal of Botany*, 42: 4003-4009.

JANTASORN, A., MOUNGSRIMUANGDEE, B. and T, DETHOUP. 2016. In vitro activity evaluation of five plant extracts against five plant pathogenic fungi causing rice and economic crop diseases, *Journal of Biopesticides*, 9: 1-7.

JARVIS, W. R. and H. J. THORPE. 1976. Susceptibility of *Lycopersicon* species and hybrid to the foot and root rot pathogen *Fusarium oxysporum. Plant Disease,* 60: 1076-1031.

JENDOUBI W., BOUHADIDA, M., BOUKTEB, A., BÉJI, M. and M. KHARRAT. 2017. Fusarium wilt affecting chickpea crop, review. *Agriculture*, 7: 1-16.

JONES, J. B. JONES, J. P. STAL, R. E. and T. A. ZITTER. 1997. Compendium of Tomato Diseases: The American Phytopathological Society, United States of America. Pages 20-25.

JOSHI, B. B., VISHWAKAEMA, M. P., BAHUKHANDI, D. and R.P. BHATT. 2012. Studies on strains and *Trichoderma spp.* from high altitude of Arhwal Himalayan region. *Journal of Environmental Biology*, 33: 843-847.

KAGALE, S., MARIMUTHU, T., THAYUMANAVAN, B., NANDAKUMAR, R. and R. SAMIYAPPAN. 2005. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani*

and *Xanthomonas oryzae pv oryzae*. *Physiological and Molecular Plant Pathology,* 65: 91-100.

KEKUDA, P. T. R., AKARSH, S., NAWAZ, S. A. N., RANJITHA, M. C., DARSHINI, S. M. and P. VIDYA. 2016. In *vitro* antifungal activity of some plants against *Bipolaris sarokiniana* (Sacc.) *Shoem*, *International Journal of Current Microbiology and Applied Sciences,* 5: 331-37.

KENA, M. A. 2016. Antifungal activities of *Monsonia burkeana* and *Euphorbia ingens* extracts against *Penicillium digitatum*, the causal agent of citrus green mould. *African Plant Protection,* 19: 1-3.

KENA, M. A. and W. J. SWART. 2011. Effects of plant extracts on *in vitro* suppression of fungal pathogens and control of damping-off of vegetable seedlings in the greenhouse. *Botswana Journal of Agriculture and Applied Sciences, 7: 36-44.*

KOWSARI M., MOTALLEBI M. and R. M. ZAMANI. 2014. Construction of new GFP-tagged fusants for *Trichoderma harzianum* with enhanced biocontrol activity. *Journal of Plant Protection and Research*, 54: 122-131.

KUBICEK, C. P., MACH, R. L., PETERBAUER, C. K. and M. LORITO. 2001. *Trichoderma*: From genes to bio-control. *Plant Pathology*, 83: 11-23.

LAROCHE, J. P. HABAJI-HARE, S. H. and P. M. CHAREST. 1992. Differentiation of two anastomosis groups of *Rhizoctonia solani* by isozyme analysis. *Phytopathology,* 82: 1387-1393.

LEEDY, P. D. and J. E. ORMROD. 2005. Practical research: Planning and design (8th edition). Upper Saddle River, New York: Prentice Hall.

LEWIS, J. 2003. Tomato notes: Missouri Environment and Garden. News for Missouri Garden, Yards and Resources. *Journal of Plant Protection,* 9: 8-10.

LIEBIG, J. 1841. Organic chemistry in its applications to agriculture and physiology. John Owen, Cambridge. First American edition. Page 577.

LOGANATHAN, M., SIBLE, G. V., MARUTHASALAM, S., SARAVANAKUMAR, D., RAGUCHANDER, T., SIVAKUMAR, M. and R. SAMIYAPPAN. 2010. *Trichoderma* and chitin mixture based bioformulation for the management of head rot (*Sclerotinia sclerotiorum* (Lib.) de Bary) root-knot (*Meloidogyne incognita* Kofoid and White; Chitwood) complex diseases of cabbage. *Archives of Phytopathology and Plant Protection,* 43: 1011-1024.

LOWELL, L. B. 2001. Tomato diseases: AVRDC International Cooperators Fact sheet.

MAMPHISWANA, N. D., MASHELA, P. W. and L. K. MDEE. 2010. Distribution of total phenolic and antioxidant activity in fruit, leaf, stem and root of *Monsonia burkeana*. *African Journal of Agricultural Research,* 5: 2570-2575.

MAMPHISWANA, N. D., MASHELE, P. W. and L. K. MDEE. 2011. Distribution of selected essential nutrient elements and secondary metabolites in *Monsonia burkeana. African Journal of Agricultural Research,* 18: 2570-2575.

MARTINEZ, A. 2007. Georgia plant disease loss estimates. cooperative extension, College of Environmental Science and Family Consumers Sciences, University of Georgia, USA.

MBOTO, C. I., EJA, M. E., ADEGOKE, A. A., IWATT, G. D., ASIKONG, B. E., TAKON, I., UDO, S. M. and M. AKEH. 2009. Phytochemical properties and

antimicrobial activities of combined effect of extracts of the leaves of *Garcinia kola*, *Vernonia amygdalina* and honey on some medically important microorganisms. *African Journal of Microbiology and Research,* 3: 557-559.

McGOVERN, R. J., ELMER, W. H., GEISER, D. M. and B. K. HARBAUGH. 2001. Biology, epidemiology, and integrated management of diseases caused by *Fusarium* in potted ornamentals. Department of Plant Pathology, University of Florida, Gainesville. United States of America. Pages 1-5.

MIZUBUTI, G. S. E., JUNIOR, V. L. and G. A. FORBES. 2007. Management of late blight with alternative products, *Pest Technology,* 1: 106-116.

MOHAMED, M., SIRAJUDEEN, K. N. S., SWAMY, M., YAACOB, N. S. and S. A SULAIMAN. 2010. Studies on the antioxidant properties of tualang honey of Malaysia. *African Journal of Traditional, Complementary and Alternative Medicines*, 7: 59-63.

MOUSSA, T. A. A. 2002. Studies on biological control of sugar beet pathogen *Rhizoctonia solani Kühn. Journal of Biological Science,* 2: 800-821.

MOYO, B., MASIKA, P. J. and V. MUCHENJE. 2012. Antimicrobial activities of *Moringa oleifera* Lam leaf extracts. *African Journal of Biotechnology*, 11: 2797-2802.

MUI-YUN, W. 2003. Soil-borne Plant Pathogen Class Project. Pages 728.

NARAGHI, L., HEYDARI, A., REZAEE, S., RAZAVI, M., JAHANIFAR, H. and E. M. KHALEDI. 2010. Biological control of tomato *Verticillium* wilt disease by *Talaromyces flavus*. *Journal of Plant Protection and Research* 50: 360-365.

NARAYANA, B. M. and B. K. SHUKLA. 2001. Evaluation of some leaf extracts against *Pythium aphanidermatum in vitro* and pot culture. *Indian Phytopathology Journal*, 54: 395-397.

NASEBY, D. C., PASCUAL, J. A. and J. M. LYNCH. 2000. Effect of bio-control strains of *Trichoderma* on plant growth, *Pythium ultimum* populations, soil microbial communities, and soil enzyme activities. *Journal of Applied Microbiology*, 88: 161-169.

NEFZI, A., ABDALLAH, B. A. R., JABNOUN-KHIAREDDINE, H., SAIDIANA-MEDIMAGH, S., HAOUALA, R. and M. DANMI-REMADI. 2016. Antifungal activity of aqueous and organic extracts from *Withania somnifera L*. against *Fusarium oxysporum f. sp*. *radias-lycopersici*, *Journal of Microbial and Biochemical Technology*, 8: 144-150.

O'CALLAGHAN, M. 2016. Microbial inoculation of seed for improved crop performance: issues and opportunities. *Applied Microbiology and Biotechnology*, 100: 5729-5746.

OBONGOYA, B., WAGAI, S. and G. ODHIAMBO. 2010. Phytotoxic effect of selected crude plant extracts on soil-borne fungi of common bean. *African Crop Science Journal*, 18: 15-22.

OLSEN, M. W. and D. J. YOUNG. 1998. Damping-off. Cooperative Extension. College of Agriculture, The University of Arizona, Tucson, Arizona, USA Pages 1-3.

PHIRI, C. and D. N. MBEWE. 2010. Influence of *Moringa oleifera* leaf extracts on germination and seedling survival of three common legumes. *International Journal of Agriculture and Biology*, 12: 315-317.

RONGAI, D., PULCINI, P., PESCE, B. and F. MILANO. 2017. Antifungal activity of pomegranate peel extract against Fusarium wilt of tomato. *European Journal of Plant Pathology*, 147: 229-238.

SALES, M. D. C., COSTA, H. B., FERNANDES, P. M. B., VENTURA, J. A. and D. D. MEIRA. 2016. Antifungal activity of plant extracts with potential to control plant pathogens in pineapple. *Asian Pacific Journal of Tropical Biomedicine*, 6: 26-31.

SALISBURY, F. B. and C. W. ROSS. 1992. Plant physiology, 4th edition. Wadsworth, Belmont, California. Page 682.

SALLAM, M. A. N. and A. M. A. KAMAL. 2012. Evaluation of various plant extracts against the early blight disease of tomato plants under green house and field conditions, *Plant Protection Science,* 48: 74-79.

SALLY, A. M., RANDAL, C. R. and M. R. RICHARD. 2006. *Fusarium* and *Verticillium* wilts of Tomato, Potato, Pepper and Egg-plant. The Ohio State University Extension Bulletin, Columbus, USA, Page 139.

SARAVANAKUMAR, D., VIJAYAKUMAR, C., KUMAR, N. and R. SAMIYAPPAN. 2007. PGPR-induced defence responses in the tea plant against blister blight disease. *Crop Protection*, 26:556-565.

SESAN, T. E., ENACHE, E., IACOMI, M., OPREA, M., OANCEA, F. and C. IACOMI. 2016. Antifungal activity of some plant extract against *Botrytis*

cinerea Pers. in the blackcurrant crop (*Ribes nigrum L*.), *Phytopathology*,14: 29-43.

SHAFI, J., TIAN, H. and M. JI. 2017. *Bacillus* species as versatile weapons for plant pathogens: a review, *Biotechnology and Biotechnological Equipment*, 31: 446-459.

SHAFIGHI, M., AMJAD, L. and M. MADANIIN. 2012. Antifungal activity of methanolic extract of various parts of *Punica granatum (L*.). *International Journal of Scientific and Engineering Research*, 3: 1-4.

SHAFIQUE, H. A., NOREEN, R., SULTANA, V., ARA, J. and S. EHTESHAMUL-HAQUE. 2015. Effect of endophytic *Pseudomonas aeruginosa* and *Trichoderma harzianum* on soil-borne diseases, mycorrhizae and induction of systemic resistance in okra grown in soil amended with *Vernonia anthelmintica* (*L*.) seed's powder. *Pakistan Journal of Botany,* 47: 2421-2426.

SHALINI, V. and N. P. DOHROO. 2005. Novel approach for screening different antagonists against *Fusarium oxysporum f. sp. pisi* causing Fusarium wilt of Autumn Pea. *Plant Disease*, 20: 58-61.

SHAZIA, S., MUHAMMAD, A. and S. SOBIYA. 2015. Management of *Fusarium oxysporum f. sp. Capsici* by leaf extract of *Eucalyptus citriodora. Pakistan Journal of Botany,* 47: 1177-1182*.*

SHERVIN, H., KAMRAN, R., SALAR, J. and E. ALI. 2011. Comparing neem extract with chemical control on *Fusarium oxysporum* and *Meloidogyne incognita* complex of tomato. *Advances in Environmental Biology,* 21: 77-431.

SINGH, S. P. 2012. Effect of consortium of *Trichoderma harzianum* isolates and growth attributes and *Sclerotinia sclerotiorum* of brinjal. *Vegetable Science,* 39: 144-148.

SRIVASTAVA, S., SINGH, V. P., KUMAR, R., SRIVASTAVA, M., SINH, A. and S. SIMON. 2010. *In vitro* evaluation of Carbendazim 50 % WP, antagonists and botanicals against *Fusarium oxysporum f. sp. psidii* associated with rhizosphere soil of Guava. *Asian Journal of Plant Pathology,* 20: 1- 8.

STEPHEN, A. F. and A. K. G. NDRE. 2003. *Fusarium oxysporum*: Department of Plant pathology, CTAHR University of Hawaii at Manoa, 2A-FUOXY Page 96.

STERGIOPOULOS, I., and P. J. de-WIT. 2009. Fungal effector proteins. *Annual Review of Phytopathology*, 47: 233-263.

STRANGE, R. N. and P. R. SCOTT. 2005. Plant diseases: A threat on global food security. *Annual Review of Phytopathology*,43: 83-116.

SUMATHI, C., MOHANAPRIYA, D., SWARNALATHA, S., DINESH, M. G., and G. SEKARAN. 2014. Production of prodigiosin using tannery fleshing and evaluating its pharmacological effects. *Science World Journal,* 20: 145-185.

TALREJA, T. 2010. Screening of crude extract of flavonoids of *Moringa oleifera* against bacterial and fungal pathogen. *Journal of Phytology*, 2: 31-35.

TSHIVHANDEKANO, I., NTUSHELO. K., NGEZIMANA, W., TSHIKALANGE, E. and N. F. MUDAU. 2014. Chemical compositions and antimicrobial activities of *Athrixia phylicoides* DC. (bush tea), *Monsonia burkeana* (special tea) and synergistic effects of both combined herbal teas. *Asian Pacific Journal of Tropical Medicine,* 7: 448-453.

TSUKIBOSHI, T. N. 2002. Microbial systematic laboratory, Japanese fungi on plants. Natural Resources Inventory Centre NIAES, Japan. Page 1-8.

VIDYASAGAR, G. M. and N. TABASSUM. 2013. Antifungal investigations on plant essential oils; A Review. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5: 19-28.

WAING, K. G. D., ABELLA, E. A., KALAW, S. P., WAING, F. P. and C. T. GALVEZ. 2015. Antagonistic interactions among different species of leaf litter fungi of Central Luzon State University. *Plant Pathology and Quarantine,* 5: 122-130.

ZAMAN, M. A., SALEH, A. K. M., RAHMAN, G. M. M. and M. T. ISLAM. 1997. Seed-borne fungi of mustard and their control with indigenous plant extracts. *Bangladesh Journal of Plant Pathology*, 13: 25- 28.

ZHOU, J., WANG, M., SUN, Y., GU, Z., WANG, R., SAYDIN, A., SHEN, Q. and S. GUO. 2017. Nitrate increased cucumber tolerance to Fusarium wilt by regulating fungal toxin production and distribution. *Toxicology Journal*, 9: 1-20.

APPENDICES

Appendix 3.1 Analysis of variance of six different concentrations of *Monsonia burkeana* plant extracts on *Fusarium oxysporum f. sp. lycopersici in vitro*.

Source	DF	SS	MSS	F	P ≤
Replication	3	13.33	4.44	2.07	0.1469
Treatment	5	8095.83	1619.17	755.05	0.0000
Error	15	32.17	2.14		
Total	23	8141.33	1625.75		

Appendix 3.2 Analysis of variance of six different concentrations of *Monsonia burkeana* plant extracts on *Rhizoctonia solani in vitro*.

Source	DF	SS	MSS	F	P≤
Replication	3	65.5	21.82	1.27	0.3205
Treatments	5	13375.4	2675.08	155.65	0.0000
Error	15	257.8	17.19		
Total	23	13698.6	2714.09		

Appendix 3.3 Analysis of variance of six different concentrations of *Moringa oleifera* plant extracts on *Fusarium oxysporum f. sp. lycopersici in vitro*.

Source	DF	SS	MSS	F	P≤
Replication	3	347.67	155.889	3.47	0.0430
Treatments	5	1617.50	323.500	9.69	0.0003
Error	15	500.83	33.389		
Total	23	2466.00	512.778		

Appendix 3.4 Analysis of variance on six different concentrations of *Moringa oleifera* plant extracts on *Rhizoctonia solani in vitro.*

Source	DF	SS	MSS	F	P≤
Replication	3	817.79	272.60	1.52	0.2509
Treatments	5	6315.88	1263.18	7.03	0.0014
Error	15	2695.96	179.73		
Total	23	9829.63	1715.51		

Appendix 4.1 Analysis of variance of six different concentrations of *Monsonia burkeana* plant extracts on shoot disease severity caused by *Fusarium oxysporum f. sp. lycopersici.*

Source	DF	SS	MSS	F	P≤
Replication	5	0.12272	0.02454		
Treatment	5	0.82317	0.16463	2.64	0.0479
Error	25	1.56191	0.06248		
Total	35	2.50779	0.25165		

Appendix 4.2 Analysis of variance of six different concentrations of *Monsonia burkeana* plant extracts on stem and root discolouration caused by *Fusarium oxysporum f. sp. lycopersici.*

Source	DF	SS	MSS	F	P≤
Replication	5	0.07217	0.01443		
Treatment	5	2.89649	0.5793	27.13	0.00
Error	25	0.53377	0.02135		
Total	35	3.50242	0.61508		

Appendix 4.3 Analysis of variance of six different concentrations of *Moringa oleifera* extracts on shoot severity caused by *Fusarium oxysporum f. sp. lycopersici.*

Source	DF	SS	MSS	F	P≤
Replication	5	0.29506	0.05901		
Treatment	5	0.98217	0.19643	12.96	0.00
Error	25	0.379	0.01516		
Total	35	1.65623	0.2706		

Appendix 4.4 Analysis of variance of six different concentrations of *Moringa oleifera* extracts on stem and root discolouration caused by *Fusarium oxysporum f. sp. lycopersici.*

Source	DF	SS	MSS	F	P≤
Replication	5	0.00072	0.00014		
Treatment	5	0.13727	0.02745	71.45	0.00
Error	25	0.00961	0.00038		
Total	35	0.1476	0.02797		

Appendix 4.5 Analysis of variance of six different concentrations of *Monsonia burkeana* extracts on Pre-emergence damping-off caused by *Rhizoctonia solani*.

Source	DF	SS	MSS	F	P≤
Replication	5	0.22033	0.04407		
Treatment	5	0.07359	0.01472	0.9	0.4938
Error	25	0.4069	0.01628		
Total	35	0.70083	0.07507		

Appendix 4.6 Analysis of variance of six different concentrations of *Monsonia burkeana* extracts on Post-emergence damping-off caused by *Rhizoctonia solani.*

Source	DF	SS	MSS	F	P≤
Replication	5	0.31498	0.063		
Treatment	5	1.23533	0.24707	8.23	0.0001
Error	25	0.75017	0.03001		
Total	35	2.30047	0.34008		

Appendix 4.7 Analysis of variance of six different concentrations of *Moringa oleifera* extracts on Pre-emergence damping-off caused by *Rhizoctonia solani.*

Source	DF	SS	MSS	F	P≤
Replication	5	0.07395	0.01479		
treatment	5	1.45646	0.29129	4.6	0.0041
Error	25	1.58201	0.06328		
Total	35	3.11242	0.36936		

Appendix 4.8 Analysis of variance of six different concentrations of *Moringa oleifera* extracts on Post-emergence damping-off caused by *Rhizoctonia solani.*

Source	DF	SS	MSS	F	P≤
Replication	5	0.70024	0.14005		
treatment	5	0.88512	0.17702	2.16	0.0906
Error	25	2.04464	0.08179		
Total	35	3.63	0.39886		