DIVERSITY OF FUNGAL AND FUNGI-LIKE PATHOGENS OF TOMATO (*SOLANUM LYCOPERSICUM*) FROM SELECTED PARTS OF LUSAKA PROVINCE

BY

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A dissertation submitted to the University of Zambia in partial fulfilment of the requirements for the award of the degree of Master of Science in Molecular Biology

The University of Zambia
Lusaka

DECLARATION

I, Godfrey Mweemba, do hereby declare that this dissertation represents my own work and that it has not been submitted before for a degree at this or any other University.

Signature

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Date

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CERTIFICATE OF APPROVAL

This dissertation by Godfrey Mweemba is approved as fulfilling part of the requirement for the award of the degree of Master of Science in Molecular Biology by the University of Zambia.

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DEDICATION

To my daughters, Yolanta, Nchimunya, Kareema Gracelyn, and my parents Mr.(late)/Mrs. Elison Mweemba, my brothers Clayford Mweemba, Wiseman Mweemba, Trywell Mweemba, Mazuba Mweemba, my sisters Okel Mweemba, Viginia Mweemba, Cecilia Mweemba, Chilala Mweemba, Eunice Mweemba,and to Zilase .C. Phiri.

ACKNOWLEDGEMENTS

I would like to give thanks to my heavenly father, the Almighty God for His sufficient grace throughout my study. In addition, I would like to express utmost gratitude to my principal supervisor Dr. Evans Kaimoyo for his unwavering fatherly assistance, guidance and encouragement during my research. Special thanks go to Ngoi Chibalange laboratory technologist, from the Plant Science Department, School of Agriculture Science University of Zambia and the HOD, for giving access to the laboratory facilities and guidance. In addition, I would like to give sincere thanks to Mr. Zulu from the mycology laboratory, Department of Biological Sciences, School of Natural Sciences for enabling me to work from his laboratory with the facilities and his guidance. My appreciation goes to Namebo Mululu, my roommate Mwananyambe Mulala, Olga Kamanga, my brother Lumamba Mubbunu (PhD) and Gift Muke Kazika that's for the technical support they rendered during my research and study.

Last but not the least appreciation goes to all the members of staff in the Department of Biological Sciences at the University of Zambia.

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LIST OF ABBREVIATIONS

1.	AFLP	Amplified Fragment Length Polymorphism
2.	AVR	Avirulence
3.	*Cox*	Cytochrome oxidase gene,
4.	β-D-glucan	Beta-D-glucan
5.	DNA	Deoxyribonucleic acid
6.	dNTPs	Deoxynucleoside triphosphates
7.	ECP	Extracellular Proteins
8.	EDTA	Ethylene diamine tetraacetic acid
9.	EMBL	European Molecular Biology Laboratory
10.	HR	Hypersensitive response
11.	IPPC	International Plant Protection Convention
12.	ITS	Internal transcribed spacer
13.	$MgCl_2$	Magnesium chloride
14.	NASREC	Natural and Applied Sciences Research Ethics Committee
15.	NCBI	National Center for Biotechnology Information
16.	PCR	Polymerase chain reaction
17.	PDA	Potato Dextrose Agar
18.	RAPD	Random Amplified Polymorphic DNA
19.	rDNA	Recombinant Deoxyribonucleic acid
20.	RNA	Ribonucleic acid
21.	RPB2	RNA Polymerase subunit B 2
22.	rRNA	ribosomal Ribonucleic acid
23.	SDS	Sodium dodecyl sulfate
24.	SSRs	Simple sequence repeats
25.	Tef1-α	Translation elongation factor-alpha
26.	TPK1b	Tomato Protein Kinase 1b
27.	Tris	(hydroxymethyl)aminomethane

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ABSTRACT

Fungal and fungi-like pathogens including *Cladosporium fulvum, Alternaria alternate,* and *Alternaria solani* class dothideomycetes are major pathogens of solanaceous crops such as tomato *(Solanum lycopersicum*) where they cause significant losses. To contribute to the understanding of the extent to which fungal pathogens affect tomato productivity in a major tomato-growing part of Zambia, the study tested the hypothesis that various fungal and fungi-like pathogens are circulating in tomato-growing areas of Lusaka and show morphological, molecular and virulence diversity.

Tomato tissue showing diverse disease symptoms including leaf spotting, stem rotting and fruit damage were collected from farmers' fields in various parts of Lusaka Province. Surface-sterilised tissue was cultured on potato dextrose agar to analyse the morphological diversity of isolates. Purified isolates were used in infection studies to fulfill Koch's postulates and to analyse the variations in virulence of the isolates. Genomic DNA was extracted from the purified fungal isolates and PCR-amplified using primers targeting the internal transcribed spacer region (ITS). The Sanger dideoxy chain termination sequencing method was used to sequence the PCR-amplified genomic DNA. Taxonomic identification of isolates from this study indicate that *Alternaria alternata, Epicoccum nigrum* and *Cladosporium fulvum* are some of the tomato pathogens circulating in the study area showing diversity in virulence and morphological characteristics. Selected isolates were found to have varying levels of virulence on Tengeru tomato variety. From 20 plants whose leaves were inoculated with pathogens from each study site, the means lesion diameters were found to be 5.9mm for samples from University of Zambia agriculture research field, 8.29mm for isolates from 13Miles and 6.095mm for those from Kafue. Molecular phylogeny analysis of the sequenced ITS amplicons by the maximum likelihood criterion revealed relatedness between isolates from similar regions of the study area as well as those from various regions of the study area. Results from this study will be useful for tomato breeders and farmers as they conduct breeding projects and use fungicides to control the pathogens circulating in the study area.

CHAPTER ONE

INTRODUCTION

1.0 Overview

Chapter 1 aims to establish the background of the study and the statement of the problem. The significance of the study, research objectives, hypothesis, as well as the research questions that gave guide to this study are described. In this chapter, the theoretical framework of the study is been outlined.

1.1 Background to the Study

Tomato (*Solanum lycopersicum)* is a member of the Solanacea family of crop plants grown in all parts of the world where the annual global costs due to yield losses and management of fungal and fungi-like pathogen related foliar diseases were recently estimated to be at least 7 billion euros (Haverkort *et al*., 2008, Haverkort *et al*., 2016, Kokaeva *et al.*, 2020). The crop plant is attacked by viral, bacterial, fungal and fungi-like pathogens (Williamson *et al*., 2021). Fungal and fungi-like pathogens, which are a major production constraint, include early blight caused by *Alternaria alternata*, late blight caused by the fungus-like pathogen *Phytophthora infestans*, leaf lesions caused by *Fusarium oxysporum* (Williamson *et al*., 2021) and tomato leaf mold disease caused by *Cladosporium fulvum.* Fungi pathogens are eukaryotic heterotrophic organisms that grow with elongated, polarized cells (hyphae) or in form of budding cells (yeast-like) (Lűcking *et al*., 2020). They reproduce via meiotic and/or mitotic spores. The true fungi amount to about 99% of known species, 0.7% represent Eumycetozoa and other groups of slime molds in the Amoebozoa (super group Amorphea), and another 0.7% the Oomycota in the Straminopila (Lűcking *et al*., 2020, Stephens *et al*., 2008; Beakes and Thines, 2016; Hawksworth and Lücking, 2018). Tomato leaves and aerial tissues provide niches that may serve as routes to fruit infections (Kokaeva *et al.*, 2020) by several other pathogens including, *Colletotrichum coccodes, A. solani, A. alternata, C. fulvum, P. infestans* that cause a variety of symptoms including leaf spots, stem canker and fruit rot (Kokaeva *et al*., 2020). Some of the fungal and fungi-like pathogens that infect tomato plants are not only phytopathogens, but also saprotrophs and mycotrophs.

In Zambia, tomato farmers rely on heavy uses of fungicides and pesticides particularly in the rainy seasons of tomato growing months. From the standpoint of biology and plant pathology, several methods are used to the study of pathogenic microorganisms particularly of agricultural importance. For diversity and genetic characterization of fungi and fungi-like organisms, traditional approaches rely mostly on morphological characters (Reis *et al*., 2003; Mugao *et al*., 2021 and Ray *et al*., 2017). These, however, tend to have limitations in lower organisms, where variation between colonies may not be easy to visually detect (Lűcking *et al.*, 2020, de Weille, 1964 and Prakash *et al*., 2016). Because of limitations with traditional methods, new methods have had to be developed and chief among these has been the development of molecular approaches. One such approach relies on the use of the polymerase chain reaction (PCR)-based methods to amplify target coding or non-coding sequences, particularly the internal transcribed spacer (ITS) region to analyse diversity in fungi (White *et al.*, 1990; Schoch *et al*., 2012; Stielow *et al*., 2015). These DNA barcoding methods and particularly the ITS region have proven to be useful for taxonomic identification of organisms (Fajarningsih, 2016; Schoch *et al*., 2012; Lindhal *et al*., 2013). Characterization of fungi uses ITS sequencing since the results from studies of over thirty years ago (White *et al.* 1990; Seliger, 1990; Gargas and DePriest, 1996). Apart from the ITS region, several protein-coding loci have also been proposed as barcodes for study of fungi and all are PCR-based. Among these are cytochrome oxidase (*Cox*) gene, translation elongation factor-alpha (Tef1-α) and the two subunits of RNA polymerase II (Fajarningsih, 2016; reviewed in Lűcking *et al*., 2020; Xu, 2016).

PCR-based barcoding methods routinely involve culturing fungi on solid or broth media and extraction of genomic DNA (Samson *et al*., 2010 and Mugao *et al*., 2021). The amplicons are cleaned and sequenced and sequence results are compared to other sequences in public database to examine the percent similarity, and identity, or by generating phylogenetic trees with near relative species (Balajee *et al*., 2009). PCR-based methods for identification enhancement is by accumulation of electronic data in publicly available databases including Mycobank.

1.2 Statement of the Problem

Cultivation of tomato requires substantial investment into disease control and among the diseases known to attack the crop, early blight caused by *Alternaria lycopersci*, late blight caused by *P. infestans* and tomato leaf mold disease caused by *C. fulvum* are major pathogens. The fungal and fungi-like are pathogens of considerable importance both historically and in modern times particularly to farmers of solanaceous crops because they are agents of diseases like early blight , late blight and tomato leaf mold disease. Prevalent climatic conditions that allow year-round cultivation of tomatoes may contribute to high levels of infectious inoculum adversely affecting crop production specifically in moist, cool, rainy, and humid environments (Erwin, 1996). Potato late blight pathogen is the most notorious and devastating organism in recent human history, being responsible for the terrible Irish potato (*Solanum tuberosum*) famine in the 1840s, and together with other foliar and fruit pathogens, they are arguably the most important pathogens of potatoes and tomatoes worldwide (Erwin, 1996).

Studies on the existence and molecular diversity of fungal pathogens of tomato in Zambia remain fragmented. The diversity in foliar or fruit pathogens of fungal nature in morphological, molecular and virulence terms on tomato remain uncharacterized in most parts of Zambia. While Zambian tomato farmers use fungicides to control pathogens, significant losses are recorded particularly in the rainy season while the diversity in virulence, morphological and molecular structures of the pathogens in circulation remains unknown and the impact of fungicide use, remain unstudied. The extent to which the pathogens are a major problem in Zambia is an area where little or no research has been done and is a subject of scientific and agricultural value.

1.3 Significance of the Study

The study is of scientific and economic significance to evaluate the morphological, molecular and virulence diversity of fungal and fungi-like pathogens isolated from farmers' fields in selected parts of Lusaka province. Information generated from the study will help farmers, seed companies and tomato breeders make informed decisions on alternative technologies for managing the pathogens. The information on the local population structure, migrations, and evolution of fungal and fungi-like pathogens are important for the understanding of various diseases that adversely affect tomato production in Zambia. Results from this study are significant in the understanding of the epidemiology, appropriate chemical control, and resistance breeding of potatoes and tomatoes in the country.

1.4 Study Objectives

1.4.1 Objectives

The objective of the research was to gain insights into the challenge posed by fungal and fungi-like pathogens on tomato production in Zambia.

1.4.2 Specific Objectives

The objectives of the research or study were to:

1) Determine the molecular diversity of fungal and fungi-like tomato pathogens from various farmers' fields in selected parts of Lusaka Province.
2) Evaluate the morphological diversity and phylogeny of tomato pathogen isolates.
3) Determine the diversity in virulence characteristics of the pathogen isolates on a popular tomato variety.

1.5 Study hypothesis

The research study tested the hypothesis that various fungal and fungi-like pathogens are circulating in farmers' fields in tomato growing areas of Lusaka and show morphological, molecular and virulence diversity.

1.6 Research questions

1. How diverse is the virulence of tomato fungi and fungi-like pathogens in farmers' fields from selected parts of Lusaka province?
2. How diverse are the tomato pathogen isolates at molecular level when measured at internal transcribed spacer region?
3. Do the purified isolates of fungal pathogens in the study area, show variation in virulence on a popular tomato variety?

CHAPTER TWO

LITERATURE REVIEW

2.0 Overview

Chapter 2 summarises the data, results and findings from related publications on research relevant to fungal and fungi-like pathogens of tomato. The chapter briefly reviews the biology of tomato, describes some of the work conducted by other scholars around the world including what is known about major fungal and fungi-like tomato pathogens such as *Alternaria alternata, Phytophthora infestans and Cladosporium fulvum.* The application of internal transcribed spacer region for taxonomic and phylogenetic evaluation of fungi and fungi-like pathogens is also reviewed.

2.1 Diseases attacking tomato as a vegetable crop

Tomato (*Solanum lycopersicum*) a vegetable is grown in many parts of the world including Zambia. It is a very economically important crop and has been estimated to have a market value ranging between $4.6 billion to $5 billion dollars (Panthee and Chen, 2010; Adhikari *et al*., 2017). The crop plant is cultivated in a wide range of climates such as tropical and temperate (Adhikari and Panthee, 2017) and is the most consumed vegetable after potato in the world (Foolad, 2007; Adhikari and Panthee, 2017). Tomato is consumed as a fresh fruit, or processed and canned as sauce, ketchup, paste, soup, salsa and juice. It is the richest source of vitamin A and C (Adhikari and Panthee, 2017) and is also valued for its high antioxidant lycopene pigment known to have anti-cancer properties as well as protection against heart disease (Boehm *et al*., 2012; Breemen and Pajkovic, 2008; Adhikari and Panthee, 2017). The demand for the crop is high and is constantly increasing thus demanding a corresponding increase in tomato production. However, production of tomato on small, medium and commercial scale faces many challenges including fungal, bacterial, nematode and viral diseases and pests.

The fungal, bacterial, nematode, viruses and pests that attack tomato vegetable cause diseases such as late blight caused by *P. infestans*, early blight caused by *A. solani*, leaf spots (*A. alternata)*, and leaf mold disease caused by *C. fulvum* (Adhikari and Panthee, 2017). Pests include the recent outbreaks of the South American tomato leaf miner pest (*Tuta absoluta*) which was reported in Asia (Zhang *et al*., 2021) and Africa including Zambia. Control of fungal and fungi-like tomato pathogens is achieved mainly by use of fungicides. On one hand, most fungal pathogens like *A.*

alternate, A. solani however, have high genetic variation that enable them to easily adapt to changes in the environment and develop resistance to fungicides (Pasche, 2004). On the other hand, cultivated tomato has a narrow genetic diversity due to intense selection and inbreeding at the time of evolution and domestication (Zhang *et al*., 2002; Adhikari and Panthee, 2017) making the crop species highly prone to disease epidemics. The major tomato varieties grown in Zambia are Nemonetta, Dominique, Victoria, Karen, Star 9030, Tengeru and Rodade (Mabengwa, 2013) with Tengeru being one of the most widely grown varieties in Zambia (Mabengwa, 2013). Wild relatives of tomato are more resistant to diseases than those cultivated tomato species (Adhikari and Panthee, 2017).

The physiological and molecular defense responses of resistant tomato varieties against fungal and fungi-like pathogens like *A. alternata* compared to susceptible varieties have been attributed to enhanced expression of disease resistance loci such as *TPK1b* (Ray *et al.,* 2015; Adhikari and Panthee, 2017). *TPK1b* is a defense-related gene, which has been linked to accumulation of H_2O_2, phenolic compounds and superoxide anion. Higher percentage of cross-linking and polyphenol activity, lead to decrease in DNA damage and less reduction in cell viability (Ray *et al.,* 2015; Adhikari and Panthee, 2017). Climate change coupled with the intensive exchange of seed material causes the emergence of new types of fungi associated with tomato crops (Kokaeva *et al*., 2020). The solution to the problem would be to develop resistant cultivars through plant breeding approaches using resistance found in wild tomato species (Adhikari and Panthee, 2017).

2.2. Biology of selected fungal and fungi-like tomato pathogens

2.2.1 *Alternaria alternata*

Members of the genus *Alternaria* belong to the Ascomycete group of fungi many of which are saprophytic and subsisting on plant debris and other organic matter in the soil (Mokobi, 2021; Thomma, 2003). Various species occur nearly everywhere including in the soil, air, and on plants especially and other organic materials, water or moist areas (Mokobi, 2021). The genus has 299 species with notable ones being A. *alternata, A. botrytis, A. leptinellae, A. oudemansii, A. scirpinfestans,* and *A. solani*. The sexual stage for *alternaria* remains yet to be identified and members survive as mycelia on plant debris (Thomma, 2003; Rotem, 1994; Mokobi, 2021).Some species cause damage on fruits (Mango rots) and other plants where they exist as endophytes (Carlile *et al*., 2001; Mokobi, 2021). *A. alternata* has dark brown long chain conidiophores when it grows in culture (Mokobi, 2021). They produce asexual conidiospores (conidia) in moist environments with abundant nutrients (Thomma, 2003; Carlile *et al*., 2001; Mokobi, 2021). The conidiophores which bear conidia produce large dark spores, short beaks and fine long septa (Mokobi, 2021). Conidiophores are spread by wind and water onto plant leaves, seeds and fruits at times (Magan *et al*., 1984). Spores germinate in an environment with good amount of moisture with temperature range of 31ºC to 32ºC (Mokobi, 2021; Magan *et al*., 1984). At the hyphae, spores develop into long chains called conidiophore (Mokobi, 2021). In turn, conidiophores release brownish conidia (Mokobi, 2021). *A. alternata* infects plants causing brown and black spots on leaves and fruits and mycotoxicosis developing into necrosis and chlorosis while using plant nutrients (Magan *et al*., 1984; Mokobi, 2021).

Infection of fruit tissue penetrates into lenticels resulting in damaging of the fruit and darkening it. *A. alternata* produces secondary metabolites like mycotoxins and phytotoxins causing spoilage in plants (Magan *et al*., 1984; Mokobi, 2021; Rotem, 1994). These metabolites cause toxicity causing diseases such as tomato black mold, olive black rot, citrus black/grey rot, black rot of apples and carrots (Magan *et al*., 1984; Rotem, 1994; Mokobi, 2021).

2.2.2 Phytophthora infestans

P. infestans is not a true fungus but a fungus-like organism (de *Bary, 1876) whose* name was coined in 1876 by the German mycologist Heinrich Anton de Bary (de Bary, 1876). It is an Oomycete and causes late blight in potatoes and tomatoes (Lévesque, 2011; Nowicki, 2013). Biochemical analyses (Vogel, 1960) and cell wall composition (Bartnicki-Garcia, 1968) led to the reclassification of *P. infestans* from being a true fungus to its inclusion in the Kingdom Chromista that includes oomycetes and other fungi-like organisms (Cavalier-Smith, 1981). The oomycete cell wall is composed mainly of (1→3) and (1→6) β-D-glucans with a small portion of cellulose but lacks chitin, which is the main component of the fungal cell wall (Aronson *et al*., 1967; Bartnicki-Garcia, 1968).

P. infestans complex are not only one of the most damaging pathogens on *Solanaceous* plants but the group also contains recalcitrant organisms affecting crops of high importance (Kamoun *et al*., 2015). The pathogen is a hemibiotroph that initiates infection and keeps the host alive in the process of establishing itself within the host tissue by taking up the nutrients from host cells. Plants become infected when spores fall on the plant surface and start to germinate. The pathogen reproduces both asexually and sexually and in the asexual phase, zoospores or sporangia are produced (Brus-Szkalej, 2019). Each sporangium can release six to eight motile zoospores that can swim for several hours (Brus-Szkalej, 2019). Sporangia can germinate directly and infect the plant and when zoospores are in contact with the leaf, they lose their flagella and build a cell wall forming a cyst, which may germinate in the same manner as sporangia (Brus-Szkalej, 2019). A germ tube forms a specialized structure known as appressorium, arising from either sporangium or cyst to penetrate the leaf surface. Hyphae, which branch from germ tubes, grow in the host intercellular spaces forming haustoria, the feeding structures that invaginate the host cells (Agrios, 1997). When nutrients are depleted *P. infestans* switches from biotrophic to a necrotrophic phase of growth (Brus-Szkalej, 2019). In this phase, host cell death occurs and is observed as brownish lesions on the exterior surface. When the pathogen reaches the exterior surface, sporangiophores are produced that release sporangia into the air and water, which may further infect other plants. The cycle takes four days to be completed, during which many spores are produced in one growing season. Infection is not limited to green parts of the plant but affects stems, roots, and tubers as well (Judelson, 1997; Kamoun *et al*., 2015; Hardham, 2007).

Sexual reproduction is also possible in countries where *P. infestans* is present. The pathogen is heterothallic with both A1 and A2 mating type's necessary for sexual reproduction (Dyer *et al*., 1993; Brus-Szkalej, 2019). The difference in the two mating types lies in the production of hormones and expression of receptors of the reciprocal hormone, which lead to the production of sexual organs known as antheridia in males and oogonia in females (Brus-Szkalej, 2019). Diploid oospore are formed by fusion of haploid nuclei, produced by gametangia. The two-parent isolates produce offspring that carry their genetic material enabling them to exhibit properties of either one or both parents producing a large population diversity (Brus-Szkalej, 2019). Sexual reproduction increases the availability of inoculum in the field by the pathogen living through the cold season while in the soil but does not contribute to the aggressiveness of the strains. (Medina and Platt, 1999). On the other hand, sporangia are only able to survive a few weeks in soil with their infectivity ranging from 15 to 45 days, depending on the pathogen isolate and soil type (Andrivon, 1994). The increase in inoculum means a greater risk of epidemics, as well as a faster rate of disease progression (Hannukkala *et al*., 2007). The majority of the investigated oospores can infect potato and tomato plants after subjection to harsh weather conditions (Mayton *et al*., 2000).

The oomycetes reproduce rapidly in cool and wet areas (McLeod *et al.,* 2001). Each time there is growing of tomato without much use of fungicides; late blight disease will attack plants spreading very quickly in the field. Symptoms include blackening of leaves (Griffith *et al*., 1995) often accompanied by spreading lesions originating from the tip of the leaf (Griffith *et al*., 1995). This leads to death of whole branches of the plant while the fruit, which is still green when infected, becomes marbled brown in appearance without becoming soft. They may be mistaken for lack of nutrients disorders like blossom end rot, which affects the area around the tip of the fruit (Griffith *et al*., 1995). Sporulation is favored by high humidity and tends to be manifested as a zone of grey-white fluff growths around the edge of necrotic lesions, usually on the lower surface of leaves. While the challenges caused by *P. infestans* are recorded worldwide and the molecular biology of the pathogen is known, the status of the oomycete as a pathogen in terms of disease dynamics in Zambia remains unclear.

Spreading black lesions develop on leaves. Under suitable conditions, whole branches are killed and eventually the whole plant is destroyed. Green fruits are susceptible to infection and become marbled brown in appearance and sporulation of *P. infestans* occurs when infected leaves are incubated under high humidity and lesions usually appear visible as zones of grey-white 'fluffy growths' around the edge of darker necrotic lesions, especially on the lower surface of leaves. (Griffith *et al*.,1995).

2.2.3 *Cladosporium fulvum*

C. fulvum a biotrophic fungal pathogen which causes leaf mold disease of tomato (van Esse *et al*., 2008; Joosten and de Wit, 1999; Thomma *et al*., 2005). When it infects plants, host colonization is usually characterized by strict extracellular growth in the apoplastic space surrounding the leaf mesophyll but haustoria are not formed (van Esse *et al*., 2008; Bond, 1938; Lazarovits and Higgins, 1976; de Wit, 1977; Thomma *et al*., 2005). *C. fulvum* secretes effector proteins during host colonization (van Esse *et al*., 2008; Thomma *et al*., 2005) among which are four race-specific avirulence proteins Avr2, Avr4, Avr4E, and Avr9. The pathogen also produces six extracellular proteins (Ecp1, Ecp2, Ecp4, Ecp5, Ecp6, and Ecp7), (van Esse *et al*., 2008; van Kan *et al*., 1991; van den Ackerveken *et al*., 1993; Joosten *et al*., 1994; Lauge´ *et al*., 2000; Luderer *et al*., 2002; Westerink *et al*., 2004; Bolton *et al*., 2008).

Race-specific resistance against *C. fulvum* in tomato occurs in a gene-for-gene manner with the dominant *C. fulvum* (*Cf*) resistance genes that help in the activation of defense cascade, leading to a hypersensitive response (HR) and host immunity (van Esse *et al., 2008* ; Joosten and de Wit, 1999; Rivas and Thomas, 2005). The *C. fulvum* effector protein Avr4 (Joosten *et al*., 1994) leads to full virulence by protecting fungal hyphae against hydrolysis by plant chitinases (van Esse *et al.,* 2008; van den Burg *et al*., 2006; van Esse *et al.,* 2007). It has been hypothesized that protease inhibitors like *C. fulvum* Avr2 and *P. infestans* EPIC2 inactivate basal host defense by suppressing host protease activity, but no demonstration has been done that these protease inhibitors do actually contribute to pathogen virulence. It is also unknown whether basal host defense is required by the targeted plant proteases (van Esse *et al.,* 2008).

Initial disease symptoms of *C. fulvum* show in the first week after the start of infection with pale green or yellowish diffuse spots on the upper leaf surface, later enlarge, forming distinctive yellow spots (Thomma *et al*., 2005). This is because of cell death in the palisade parenchyma. Abaxial part of the leaf appears with distinct symptoms typically white to olive-green patches that turn brown once sporulation starts (Thomma *et al*., 2005). While *C. fulvum* was one of the first tomato pathogens to be used in the analysis of major disease resistance gene in plant-pathogen model systems, the extent to which the pathogen is an economic factor in tomato production in Zambia remains unknown as no reports on the same are available.

2.3 Laboratory culture of fungal and fungi-like pathogens of tomato

Fungal and fungi-like pathogens of tomato are routinely grown under laboratory conditions, though they are quite hard to axenically culture (Griffith *et al*., 1995). In nature, some of the pathogens exist as obligate biotrophs and are quite recalcitrant to common agar culture methods (Pacilly *et al*., 2016 and Mugao *et al*., 2021). Typical culture media for mycological laboratory analysis include RyeA agar and potato dextrose agar (PDA). Frequent subculture and incubation at 15-20°C (Lucas, 1991) are required and to suppress contamination, anti-fungal agents and antibiotics are used effectively and selectively to suppress bacterial contamination (Griffith *et al*., 1995). An antibiotic cocktail (RAN - 50 mg/ml each of nystatin and rifamycin, and 25 mg/ml ampicillin dissolved in dimethyl sulphoxide is routinely used to control bacterial contamination in culture (Caten and Jinks, 1968, Mugao *et al*., 2021 and Griffith *et al*., 1995). The cocktail is used to supplement standard media typically Rye A immediately before dispensing the agar into Petri dishes (Caten, 1960; Black, 2020). Media cultures are typically constituted to contain trace elements, vitamins, and fatty acids required for growth and sporulation while nutrient-poor media are used to prevent the rapid growth of contaminating microorganisms.

2.4 Molecular methods for evaluation of fungi and fungi-like pathogens

Several molecular methods that were historically developed to study the diversity of living organisms in general have been adapted to the analysis of fungal pathogens. These include isozyme analysis and Polymerase Chain Reaction (PCR)-based methods. The latter included analyses of molecular markers such as microsatellites (also known as simple sequence repeats, SSRs), Random Amplified Polymorphic DNA (RAPD), and Amplified Fragment Length Polymorphism (AFLP) markers (Weber and Halterman, 2012; Naik *et al*., 2010; Nandani and Thakur, 2014).

Seminal studies on characterisation of fungal genes and nucleotide sequences by Sanger dideoxy sequencing of PCR-amplified internal transcribed spacer region (ITS) over thirty years ago (White *et al.,* 1990) have led to the development of an entire field of study in taxonomy called DNA barcoding. The DNA barcoding method is one of the most useful DNA-based methods for the identification of organisms (Fajarningsih, 2016). Additional barcoding loci that have been adapted and proposed for mycological diversity analysis are protein-coding genes including the cytochrome oxidase (*Cox*) locus, the two subunits of the RNA polymerase genes (*RPB*1 and *RPB*2) and translation elongation factor 1α (Tef-1α) (Schoch *et al*., 2012; Stielow *et al*., 2015).

These genetic regions and loci have been extensively used in combination with high-throughput sequencing methods facilitated by availability of organism-specific and general primary databases for species delimitation and discovery (Grünwald *et al*., 2011; Fajarningsih, 2016; Stielow *et al*., 2015; Schoch *et al.,* 2012). This research used the ITS region to analyze the pathogens of tomato and a summary review of the ITS barcode is given below.

2.5 Internal Transcribed Spacer (ITS) region

The internal transcribed spacer (ITS) region of nuclear DNA (rDNA) is one of the most sequenced regions and has been used extensively in fungal taxonomy and phylogeny including identification of fungi at the species level, and even within species for over three decades (White *et al*., 1990; Grünwald *et al*., 2011; Schoch *et al*., 2012 and Fajarningsih, 2016). The region is highly polymorphic, has been used to delineate operational taxonomic units, and has been proposed as a barcode marker to classify organisms up to species level (Chase *et al*., 2005; Schoch *et al*., 2012). The International Fungal Barcoding Consortium formally recommended the ITS regions of the nuclear ribosomal RNA gene cluster for use as the primary fungal barcode (Schoch *et al*., 2012; Stielow *et al.*, 2015; reviewed in Raja *et al*., 2016; Fajarningsih, 2016). In fungi, the ITS region is about 600 bp long and contains two variable spacers, ITS-1 and ITS-2 separated by the highly conserved 5.8S rRNA (De Beeck *et al*., 2014; Reviewed by Raja *et al*., 2016) and numerous primers have been designed for its amplification (Schoch *et al*., 2012; Stielow *et al*., 2015). It has been used in many studies and numerous reference sequences are available in the NCBI sequence database, i.e. Genbank, EMBL, as well as in specialized databanks including MycoBank (Samson *et al*., 2010). The method typically entails producing pure cultures on either solid or broth media (Chidiebere, 2018; DifcoTM & BBLTM Manual, 2009; Remel, 2010) or extraction of genomic DNA from the purified cultures. PCR primers are used to amplify the region and amplicons are cleaned up and sequenced. Sequencing results are compared to other sequences in the public database to examine the percent similarity, and identity, or by generating a phylogenetic tree with near relative species (Balajee *et al*., 2009).

The knowledge gap on fungal and fungi-like pathogens that may affect tomato production in a selected part of Zambia was the main area of study for this work. The diversity in morphology, virulence and phylogeny of the pathogens circulating in the study area were analysed. The impact of fungicide use which have remained unstudied particularly in Zambia drove the interest that was taken to contribute to the understanding of the scope of tomato fungal pathogens in the selected study area.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Overview

Chapter 3 describes the materials, methods, approaches and strategies used to generate the results and data in this dissertation under the following subheadings:

(1) Study site and sampling location, (2) Research materials, (3) Fungal and fungi-like pathogens-culturing and identification, (4) Recapitulation of disease symptoms, (5) Disease reaction in uniform blast nursery, (6) Growth of plant material for DNA isolation, (7) Plant genomic DNA extraction, (8) Analysis by ITS markers (9) Polymerase chain reaction amplification, (10) Gel electrophoresis and (11) Analysis of fragment and band intensity.

3.1 Study sites and sampling locations

Tomato tissue samples showing suspected symptoms of fungal infections were collected from three locations selected within Lusaka province (Figure 3.1) as follows: Farmers' fields located in Kafue district to the south of Lusaka (S 15° 48′35.466, E 28° 15′ 25.962), fields located to the north of Lusaka central business district in an area popularly known as 13 miles (S 15°13′26.022, E 28° 15′8.094) and research plots belonging to the School of Agricultural Sciences of the University of Zambia (S 15°23′40.458, E 28°20′9.732).

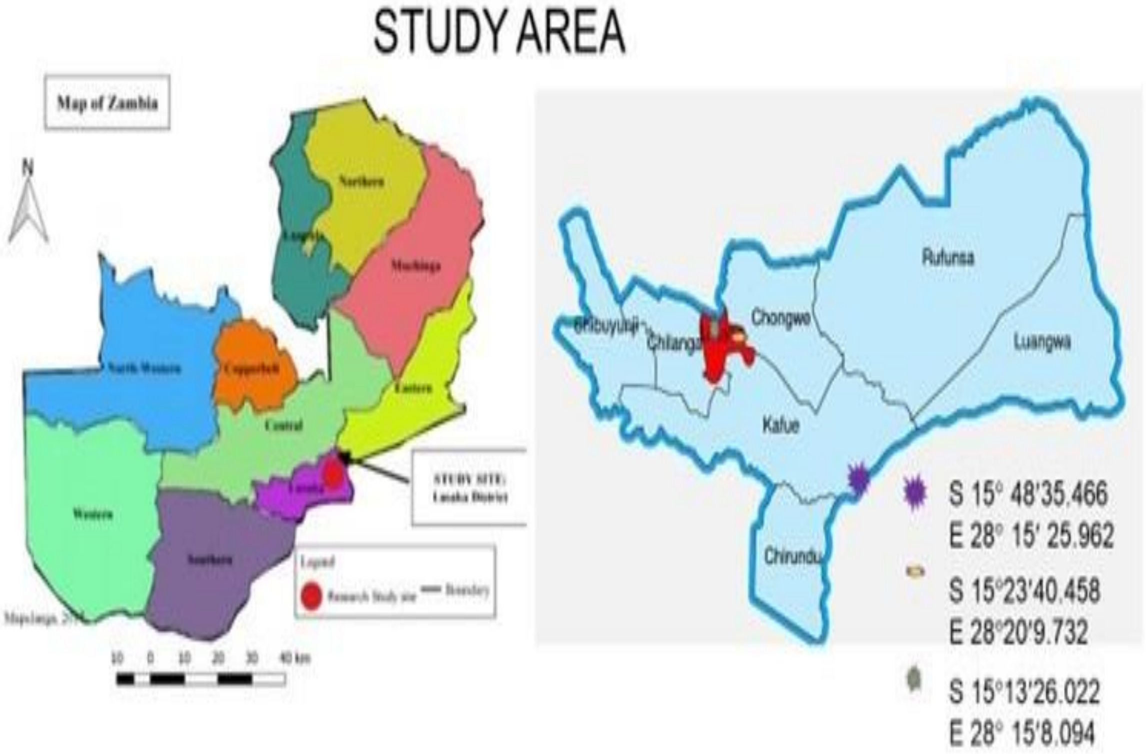


Figure 3.1. Sites and locations of fields from where infected tomato tissue samples were collected.

3.2 Sample collection/ Study design, isolation and purification of pathogens

3.2.1 Sample collection/ Study design

Access to tomato fields for sample collection was gained with the permission of owners of fields after ethical clearance was obtained from the Natural and Applied Sciences Research Ethics Committee (NASREC) of the University of Zambia. Stratified random sampling method was used to collect samples for laboratory analyses within Lusaka province which was divided into three zones for convenience as described above.

Infected tomato tissue samples were collected between 29th March, 2022 and 8th April, 2022. Visual inspections for lesions and symptoms (Figure 3.2) were conducted in each tomato field for leaf, stem and fruit tissue with brown, grey and black lesions. Four samples each were collected in transects from randomly selected five fields in Kafue to make a total of twenty infected samples, four from five tomato fields located at 13 miles north of Lusaka central business district also making twenty samples and four samples from five research plots located at the School of Agricultural Sciences of the University of Zambia. This brought the total number of infected tomato samples analysed in this study to sixty. Samples were transferred into sterile Ziploc™ bags, placed on ice in a cooler box, and transported to the plant pathology laboratory of the Department of Biological Sciences of the University of Zambia for analysis.

All laboratory work on pathogen isolation was conducted at the plant pathology laboratory in Department of Biological Sciences of the University of Zambia, while PCR-amplification and related molecular work were conducted at the Biotechnology laboratory of the School of Agricultural Sciences of the University of Zambia.

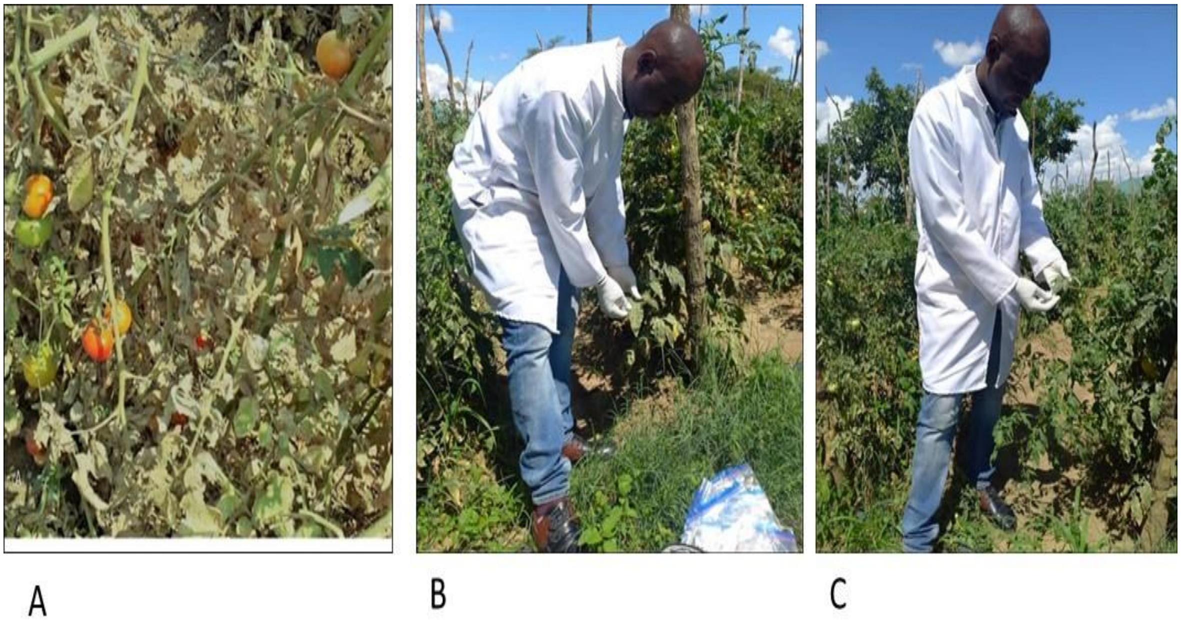


Figure 3.2 Tomato crop affected by leaf and stem tissue pathogens in a farmer's field north of Lusaka city (S 15°13′26.022, E 28° 15′8.094), A, collection of samples from a farmer's field at a sampling site north of Lusaka (13miles), B and C. Pictures taken by Lumamba Mubbunu (Brother) who accompanied me in the fields.

3.2.2 Isolation of pathogens

Potato Dextrose Agar (PDA) culture medium was prepared according to published protocol or following manufacturer's instructions (Chidiebere, 2018; Remel, 2010; Difco$^{\text{TM}}$ & BBL$^{\text{TM}}$ Manual, 2009) with minor modifications. Thirty-nine grams (39 g) of dehydrated PDA was weighed into a conical flask to which 300 ml distilled water added. The suspension was heated on a bench top heating block to melt the agar with constant stirring and once the agar had melted, the culture medium was transferred to a 1000 ml measuring cylinder and distilled water was added to bring the final volume to 1000 ml. This was transferred to a 1-litre glass bottle and autoclaved at 121°C for 15 min at 15 pounds per square inch (psi). The medium was allowed to cool and supplemented with 50 mg/ml each of nystatin and rifamycin, and 25 mg/ml ampicillin to prevent bacterial contamination. Culture media were poured into sterilized Petri-dishes and allowed to gel and solidify (Chidiebere, 2018). All subsequent PDA culture media for fungal culture or purification were prepared according to this protocol. Morphological characterization of isolates was conducted according to published protocols (Samson *et al*., 2010 and Mugao, 2021). Tomato tissue with lesions was washed under running tap water to remove soil and other undesired materials. Lesion areas were surgically cut into smaller pieces ranging from 5-6 mm using a pair of heat-sterilized pair of scissors and surface-sterilized for 3 minutes in 0.5% v/v sodium hypochlorite aqueous solution followed by rinsing twice in sterile distilled water. Excess moisture was removed by blotting the surface-sterilized tissue on a paper towel and infected tissue was transferred onto the PDA plates supplemented with 50 mg/ml each of nystatin and rifamycin, and 25 mg/ml ampicillin. Three to 4 pieces of each of the surface sterilized tissue were inoculated onto freshly prepared PDA medium and incubated at 25°C to allow for colony growth.

3.2.3 Purification and morphological characterization of isolates

Colony development were monitored from the fourth day post-inoculation and various characteristics including pigments produced, colony growth and texture were recorded. Cultures were purified onto secondary PDA plates from single spores or by cutting 3-5 mm agar pieces from primary PDA plates and transferring them onto freshly prepared PDA plates to purify pathogens on secondary PDA plates. Independent experiments were repeated three times. Colony characteristics were recorded visually and microscopically at 400× magnification for sporangia and mycelial characteristics Fig 4.2, and Table 4.1. Samples collected from specific areas were given serial numbers such as K-1, K-2, K-3, etc. from Kafue, 13m-1, 13m-2 and 13m-3etc. from "13 miles" area, and University of Zambia agriculture research fields (U-1, U-2 and U-3). The mycelia that grew on the original PDA petri dishes consisted of a mixed isolates from which sub-culturing was done.

3.3 Virulence tests

To determine the responses of tomato to the purified fungal isolates and fulfill Koch's postulates, seeds of Tengeru variety of tomato were sown in a nursery bed under semi-sterile conditions and transplanted into soil. Five isolates of pure cultures from each of the study areas were selected based on morphological similarities and in some cases differences in colony morphologies giving a total of fifteen cultures. Mycelia and conidia of pure cultures were scrapped from PDA plates and transferred into 5 ml of sterile distilled water in a mortar and homogenized using a pestle to make the inoculum. Three leaves on each of the three sets of twenty tomato plants were inoculated with five pathogen isolates from each of the three study areas using a syringe bringing a total of inoculated plants to sixty the total of inoculated leaf tissue samples to one hundred eighty. Inoculated plants were grown in natural soil under semi sterile conditions without the application of fertilizer and were watered regularly. Seven days after inoculation, infected leaves were detached from plants and evaluated for sporulation. Lesion sizes were measured using a millimeter scale and recorded as means from three leaves each. Pathogens were re-isolated from the infected tissue and cultured on PDA as described. The measured lesion diameter size on each leaf was used to compute means of the lesion sizes from each of the three leaves inoculated on each plant. The higher the mean lesion sizes the more virulent the isolate and the lower the mean lesion sizes the less virulent the isolate from the particular region was deemed to be.

Figure 3.3. Lesion diameter size being measured by the researcher (A) and (B) showing the measured diameter indicating a measure of virulence on the inoculated Tengeru tomato leaf recorded. Pictures taken by Lumamba Mubbunu (Brother).

3.4 Molecular diversity analysis of isolates

3.4.1 Genomic DNA extraction

Sterilized wooden tooth sticks were used to aseptically scrap mycelia of 5 to 7 day-old pathogen cultures and transferred into a sterile porcelain mortar each for genomic DNA extraction. Fungal genomic DNA was extracted according to published protocols (Möller *et al*., 1992; Mugao *et al*., 2021) with minor changes where necessary. Briefly, 500 µl lysis buffer (50 mM Tris [pH 8.5], 20 mM EDTA [pH 8.0], 3% SDS) and 200 mg LG1 of 10 µL proteinase K was added to each sample. Each mycelial tissue sample was ground to a slurry using a porcelain mortar and pestle and slurries were transferred into labeled Eppendorf tubes. The content for each respective tube were mixed by inversion and incubated at 65°C for 60 minutes. To each mixture, an equal volume of phenol-chloroform was added and contents were centrifuged at 13,200 revolutions per minute (rpm) for 5 minutes at 4 °C in a microcentrifuse. Supernatants were transferred to new labeled Eppendorf tubes to which 150 µL of sodium acetate (pH 5.2) was added. An equal amount of isopropyl alcohol was added to each mix and contents were mixed by inverting the tubes gently. Tubes were centrifuged at 13,200 rpm for 10 minutes and the supernatant discarded**.** DNA pellets were washed in 500 µL of 70% ethanol and centrifuged for 1 minute at 10,000 rpm. DNA pellets were air dried for 5 to 10 min on the benchtop after which genomic DNA pellets were re-suspended in 50 µL molecular grade water. The quality of extracted DNA was assessed on horizontal agarose gel slabs supplemented with gel electrophoresis stain. Genomic DNA samples were stored at -20 °C until needed for subsequent work.

3.4.2 PCR amplification, ITS amplicon sequencing and phylogeny analysis

Fungal genomic DNA was PCR-amplified in 25 µL reaction mixtures comprising 1 µL of genomic DNA, 0.5 µL each of ITS1 primer (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 primer (5'-TCCTCCGCTTATTGATATGC-3') (White *et al*., 1990, Schoch *et al*., 2012), 0.125 µL of *Taq* polymerase, 2.5 mM dNTPs (2.5 µL), 0.125 µL of premix *Taq* polymerase buffer and 2.5 µL of 10x dream *Taq* buffer ($MgCl_2$). PCR grade water (17.75 µL) was added to bring the final reaction volume to 25 µL. PCR amplification was performed following published thermal cycling conditions (Schoch *et al*., 2012) with minor modifications and included an initial denaturation at 95 °C for 2 minutes, followed by 35 cycles each of denaturation at 95 ºC for 1 minute, primer annealing at 54 ºC for 1 minute, extension at 72 ºC for 1 minute, and a final extension at 72 ºC for 2 minutes. Amplicons were resolved on a 1.2% agarose horizontal gel slab stained with ethidium bromide (0.5 µg/mL). Results were photographed under UV light and amplicon fragment sizes were estimated by comparing them with a commercial 1 kb ladder. PCR reactions amplified the ITS region giving amplicon fragments of between 500 to 600 bp.

PCR products were purified using a PCR product amplification kit and sequenced in an Applied Biosystems SeqStudio Genetic Analyzer (https://www.thermofisher.com/us/en/) at the School of Veterinary Medicine of the University of Zambia. Sequences were retrieved and contigs assembled through web-based tools followed by multiple sequence alignment algorithms in MEGA 11. Taxonomic identification of isolates was conducted in the Basic Local Alignment Search Tool (BLAST) algorithm (Altschul, 1990) available at NCBI database (https://www.ncbi.nlm.nih.gov/) using the nucleotide sequences as query sequences. Related species were identified based on more than 99% similarity between the isolate sequence and reference sequence.

The maximum likelihood criterion in MEGA 11 (Kumar *et al*., 2021) based on bootstrap value of 1000, was used to examine the phylogeny of the isolates. Briefly, the sequences of the ITS amplicons were processed following the steps in MEGA 11 and according to published reports on molecular taxomy (Kumar *et al*., 2021; Cooke *et al*., 2000).

CHAPTER FOUR

RESULTS AND FINDINGS

4.0 Overview

This chapter presents the results from the study based on the objectives. Results on the morphological, virulence and molecular diversity of isolates are presented. The results from PCR-amplification and sequencing of ITS amplicons are also presented.

4.1 Characterization of fungal pathogens of tomato and their morphological diversity

The fungi isolated from infected tomato tissue were purified on potato dextrose agar and the results recorded as shown, Figure 4.1 and Table 4.1.

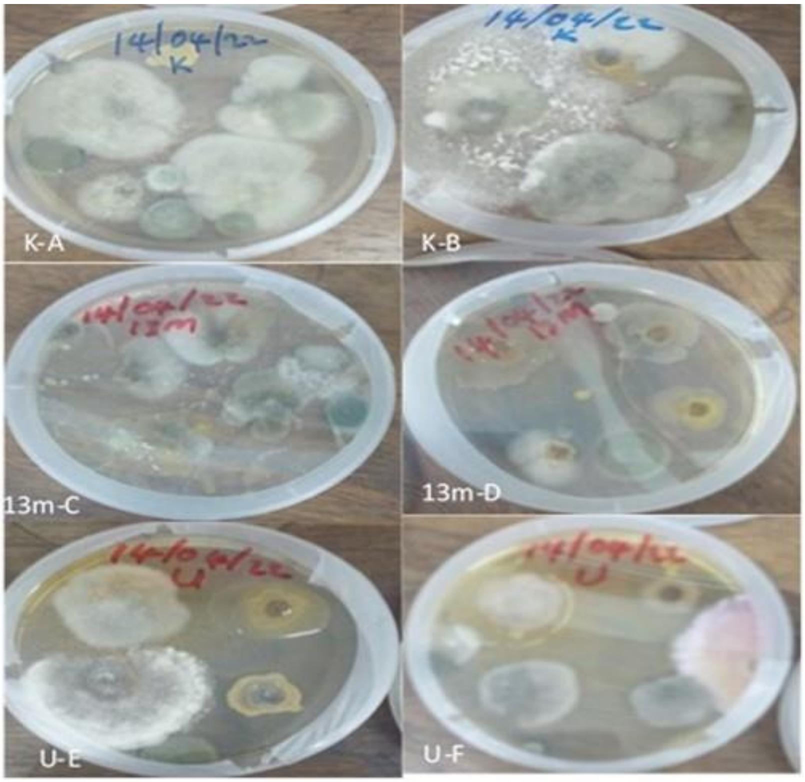


Figure 4.1. Primary cultures of fungal isolates of tomato from the three study areas showing isolates from Kafue, K- A, K-B, (upper row), north of Lusaka, 13-C, 13-D (middle row) and UNZA School of Agriculture Sciences research fields, U-E, U-F (bottom row).

Primary cultures (Fig. 4.1) on PDA plates consisted of mixtures of fungi of varying colony characteristics. Pure cultures were obtained from primary PDA plates and cultured on fresh PDA medium, Figure 4.2 and the details of representative were recorded, Table 4.1. The purified cultures showed variations in colony morphologies, texture and pigments, Fig, 4.2.

Table 4.1. Description of representative purified colony cultures from the three study areas.

Origin	Designation	Description
Kafue	K-1	The mycelia was olive green on top, at the base charcoal grey with yellowish margin and grew with raised colonies. The margins were irregular.
	K-2	Mycelium was cream white, velvet at the top, base view showed pinky and colony had regular margin. No pigment was observed in the PDA medium.
	K-3	Rapidly growing grey with zonations top, while at the base was charcoal grey cracked into three zones and compact mycelium.
13 miles north of Lusaka CBD	13m-1	The mycelia was olive green with zonations on top, at the base was charcoal grey with white zone at the edge and grew with flat colonies. The margins were regular.
	13m-2	Rapidly growing Mycelia pink-whitish coloured on top, base was cream white with regular margin and cottony colony.
	13m-3	The mycelia was olive green top, at the base was cream white.. It was compact with irregular margins.
UNZA School of Agricultural Sciences	U-1	The mycelia was cotton white, raised growth at the top, pinky at the base with regular margins.
	U-2	Rapidly growing mycelia pink coloured top at the base was pinky with irregular margin.
	U-3	The mycelia was purple coloured top view while while at the base was pinky and compact with regular margins.

Figure 4.2. Growth of fungal isolates from tomato tissue samples on PDA. Isolates were from tissue samples obtained from farmers' fields in Kafue, upper row, north of Lusaka central business district, middle row and University of Zambia School of Agriculture Science research fields showing both the top (LHS plate) and base view (RHS plate).

To determine the variations in microscopic details of the isolated fungi, mycelia and conidia were examined under a light microscope (Figure 4.3) and the characteristic features were compared with those from published sources.

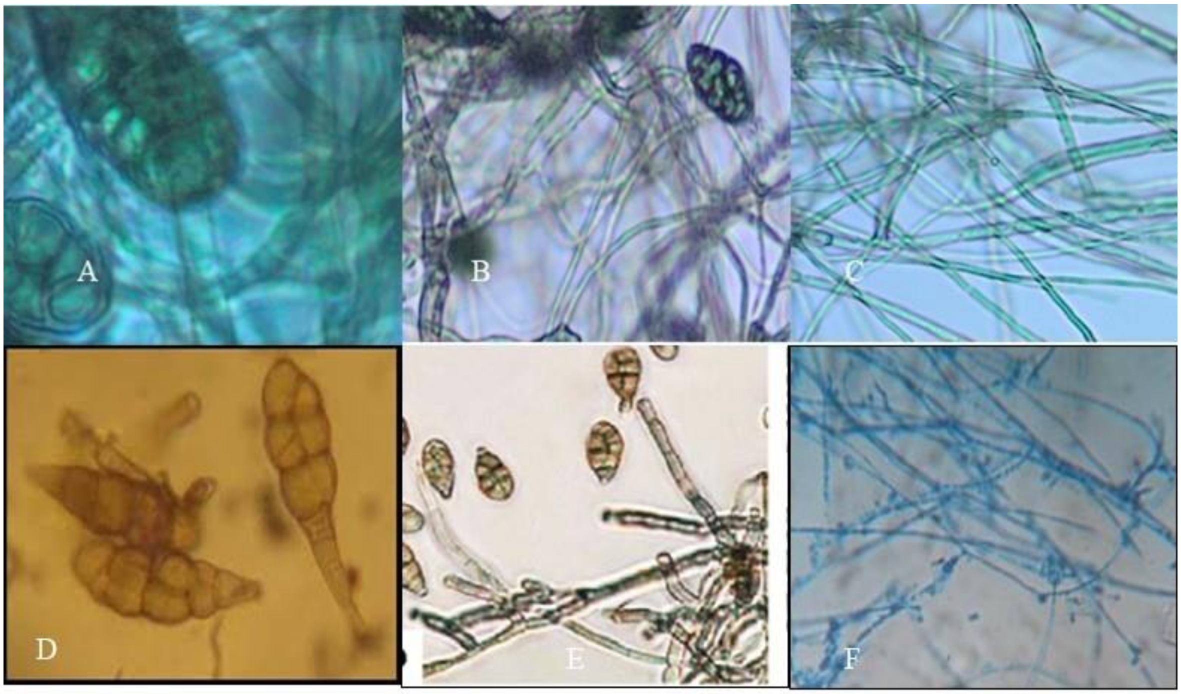


Figure 4.3. Light microscopic examination and identification of pathogen isolates at X40 magnification (A, B and C) compared with the standard keys D (Alternaria brassica), E (Alternaria alternata) and F (Fusarium sp). Valvi et al., (2019).

Isolate A produced conidia with cylindrical to oblong shape, with muriform configuration and produced in chains of 8-10 spores. This isolate was tentatively identified as *Alternaria brassica* according to the standard key D based on its conidia which were dark brown and smooth-walled with the size of up to 60 x 14µm. (Ellis, 1968, Simmons, 2007 and Meena, 2016). The conidia of isolate B were similar to those of standard key E identified as *Alternaria alternata* (Basim *et al*., 2018). Based on comparisons of the shape of conidia of the isolate and those of the standard key, isolate B was identified as *Alternaria alternata*, Figure 4.3 (B versus E). Standard key (Fig 4.3E) showed an isolate that produces large brown, muriform conidia with beaks and borne singly (Sutton, 2009). Isolate C from this study was tentatively identified as *Fusarium sp* when compared with Figure 4.3 of a standard key. *Fusarium sp* typically produces conidia which are often produced in sporodochia appear as slimy dots in the culture (Thrane, 2014).

4.2 Species composition

PCR reactions successfully amplified the ITS region giving amplicon fragments of between 500 to 600 bp, Fig 4.4 which were separated on 1.2% agarose gel. The Basic Local Alignment Search Tool (Blast) algorithm was used to analyse the ITS amplicon sequences at the National Centre for Biotechnology Information(NCBI, www.ncbi.nih.nlm.gov) at 98 to 99% nucleotide sequence identity. Among the genera and species taxonomically identified using the BLAST tool were *Alternaria* and *Fusarium*. The maximum likelihood criterion generated a phylogenetic tree which revealed the relatedness of the isolates from the various study areas, Figure 4.5.

The BLAST algorithm revealed the occurrence of the genus *Alternaria* in all three areas included in this study with A. alternata being isolated from fields in all three areas of study. Additionally, *A.brassicae* was isolated from tissue samples collected from the 13 miles area north of Lusaka, Fig. 4.5. *Fusarium chlamydosporum* was also isolated from samples collected at 13 miles north of Lusaka and research fields at UNZA School of Agricultural Sciences. The rest of the genera and species isolated from tissue samples collected from all the areas are summarized in Fig. 4.5 and a total of thirty DNA sequences generated and analysed by the BLAST algorithm allowed for a tentative identification of the fungi and construction of a phylogenetic tree, Fig. 4.5.

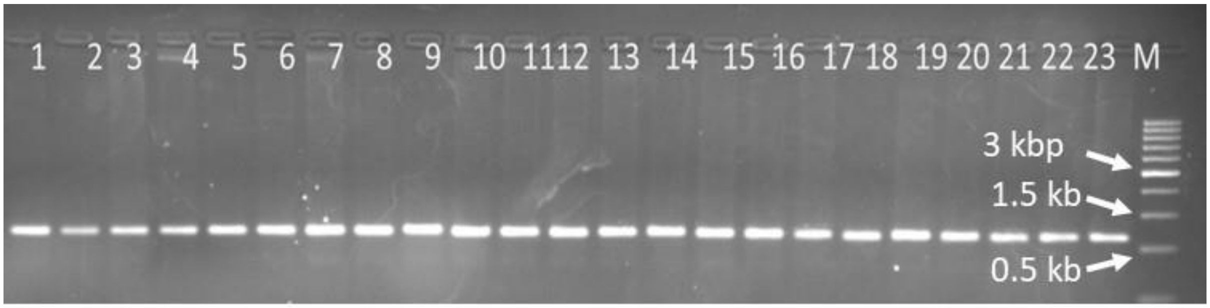


Figure 4.4 Agarose gel electrophoresis analysis of representative PCR-amplified ITS region from fungal isolates from this study. Lanes 1 to 8 were isolates from Kafue, 9-16 were isolates from 13 miles and 17 to 23 were isolates from UNZA School of Agricultural Sciences research fields. M = molecular weight marker.

The results in Fig. 4.5 showed that members of the genus *Alternaria* (*A. alternate, A. brassicae and A. tamaricis*) were found to band together and included those isolated from 13 miles area north of Lusaka and those isolated from Kafue, Fig. 4.5. *Fusarium chlymydosporum* isolated from

UNZA and 13 miles north of Lusaka also formed a separate clade. Members of the genera *Kalmusia*, *Cladosporium* , *Chaetomium, Fusarium, Sordariomycetes, Geotrichum, Epicoccum, Lasiodiplodia* when analysed by DNA distance-based methods combining 5.8S subunit, ITS1 and ITS 4 regions of the genomic ribosomal RNA tandem gene repeat have been shown to belong to the same clade (Cooke *et al*., 2000). In the phylogenetic tree (Figure 4.5), the numbers at the branch points indicate the percentages of bootstrap values (based on 1000 bootstraps). Thirty isolates appeared to be related based on proximity of regions from which they were isolated while in some cases (Sodaryomycetes 13MS1S3.2 from north of Lusaka) and US6S1.2 from UNZA School of Agricultural Sciences research fields appeared to be closely related as expected. Similar studies (Cooke *et al*., 2000) on molecular phylogeny of fungi-like pathogen and related oomycetes lead to successful construction of a phylogenetic tree though it was based on 500 bootstrap while this study its 1000 bootstrap. Also DNA distance-based analysis of the combined 5.8S subunit and ITS2 regions of the genomic ribosomal RNA tandem gene repeat was used (Cooke *et al*., 2000) was similar though this amplified ITS1 and ITS4 instead.

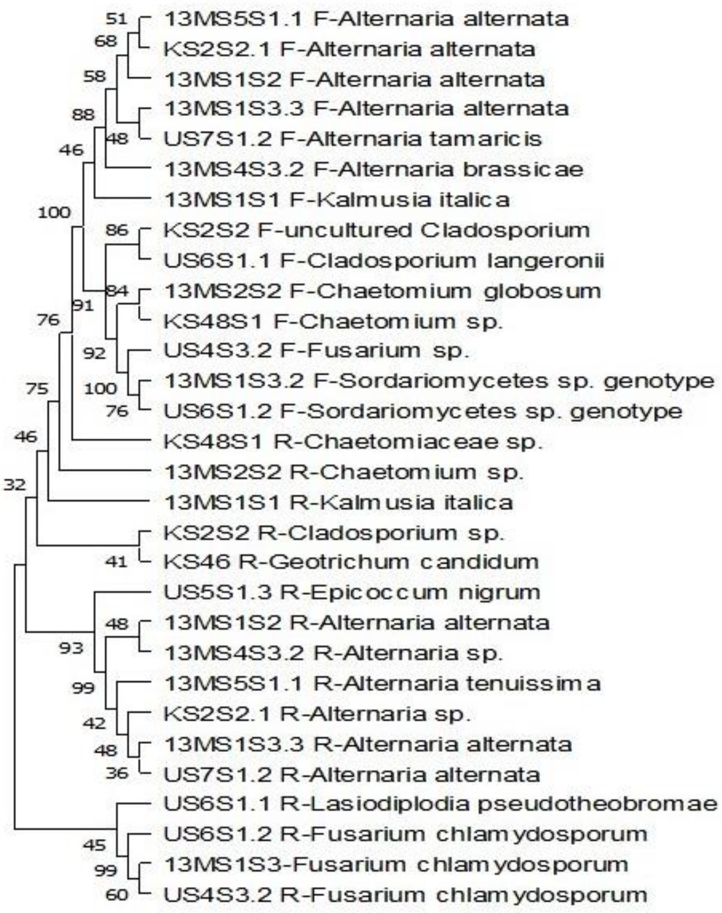


Figure 4.5. Phylogenetic analysis of isolates from the various parts of Lusaka described in this study using the Maximum likelihood method at the bootstrap value of 1000. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis from this study involved 30 nucleotide sequences and all positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). There were 292 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Kumar *et al*., 2021).

4.3 Virulence Diversity

To fulfill Koch's postulates (Byrd, and Segre, 2016) and determine if the isolated pathogens had differences in their virulence, spores and other propagules of isolates were used to inoculate leaf tissue from 3-week-old seedlings of Tengeru tomato variety using published protocols (Reis *et al*., 2005; Grunwald *et al*., 2006). Isolates from each of the three areas of Lusaka induced lesion development on leaves. Lesion sizes showed virulence diversity in some of the identified isolates. For instance, one isolate (KS2S2) from Kafue leaf samples identified as a *Cladosporium clone* induced the development of mean lesion of 12.7 mm. On the other hand, isolate KS46 identified as *Geotrichum candidum* gave mean lesion size of 2.7 mm and KS2S2.1 (*A. alternata*) gave mean lesion size of 6.7 mm.

Three of the isolates from tissue collected from fields north of Lusaka were designated as 13MS1S3, 13MMSS2 and 13MS4S3.2 respectively. 13MS1S3 identified as *Fusarium chlamydosporum* produced the highest mean lesion size (14.7 mm) while 13MS2S2 (identified as *Chaetomium globosum*) produced the lowest mean lesion diameter of 2.7mm. Isolate 13MS4S3.2 (identified as *Alternaria sp*) produced mean lesion of 8.3 mm.

Means of three lesion sizes were recorded at 48-hour intervals from each of the three regions as a measure of virulence Fig. 4.6 and Table 4.2.

Figure 4.6. Lesions development on Tengeru tomato leaves inoculated with pathogen isolates from the University of Zambia School of Agricultural Sciences research field, 13miles and Kafue

Three isolates from UNZA School of Agricultural Sciences research fields were also compared for virulence. The first designated as US5S1.3 and identified as *Epicoccum nigrum* had the highest mean value of 11.3mm and the other, US6S1.1 identified as *Lascodiplodia pseudolheubromae* had the lowest mean lesion size of 2.7 mm signifying least virulence on Tengeru and US4S3.2 identified as *Fusarium sp* produced mean lesion value of 7.7 mm.

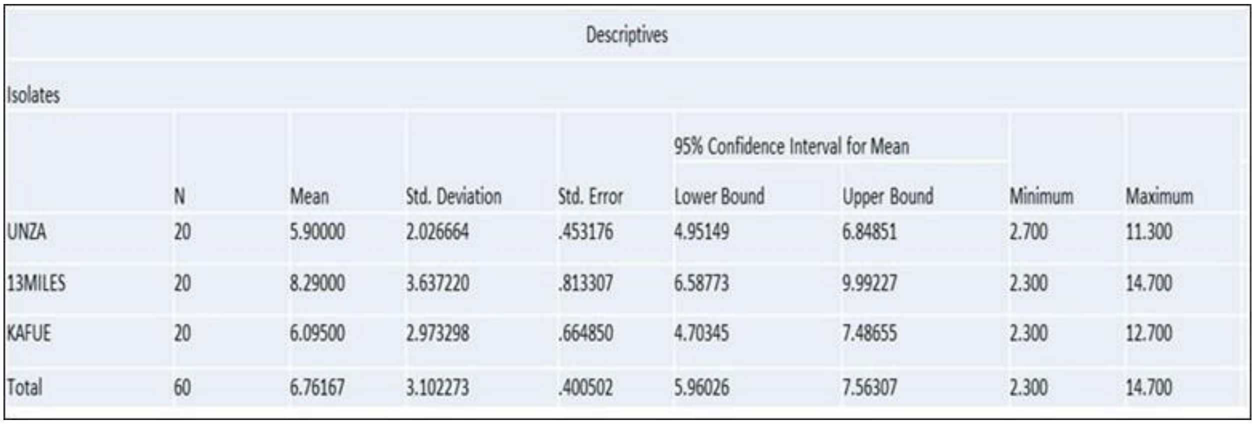
A large number of isolates from north of Lusaka showed the highest virulence while those from UNZA School of Agricultural Sciences research fields had the least virulence as recorded in table 4.2. Raw results for virulence diversity were all recorded (Appendices II to IV).

Table 4.2. Lesions indicating the degree of virulence of the fungal and fungi-like pathogens of tomato variety Tengeru. The mean lesion sizes ranged from 1 to 14 mm and the numbers of isolates from each of the three locations included in the study were enumerated and recorded.

Pathogen isolated from	**Lesion size range (mm)**		
	Lowest	**Medium**	**Highest**
	1-4 mm	**5-9 mm**	**10-14 mm**
UNZA School of Agric. Sci.	6	13	1
13MILES	3	10	7
KAFUE	8	9	3

The lesion sizes from inoculation of Tengeru leaves with isolates from all three sites were analysed using ANOVA and the results presented in table 4.3. From 20 plants whose leaves were inoculated with pathogens from each study site, the means were found to be 5.9mm for University of Zambia agriculture research field, 13Miles 8.29mm and 6.095mm for Kafue.

Table 4.3. Comparison of means of lesion diameters for isolates from the three study sites by ANOVA.



Descriptives

Isolates

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
UNZA	20	5.90000	2.026664	.453176	4.95149	6.84851	2.700	11.300
13MILES	20	8.29000	3.637220	.813307	6.58773	9.99227	2.300	14.700
KAFUE	20	6.09500	2.973298	.664850	4.70345	7.48655	2.300	12.700
Total	60	6.76167	3.102273	.400502	5.96026	7.56307	2.300	14.700

ANOVA multiple comparisons involving the virulence diversity of the isolates from the three study locations were also done. The mean difference was found to be significant at 0.05 level. Specifically, a significant difference in virulence between isolates from UNZA and 13-mile $p<0.05$ on inoculated Tengeru leaves was observed, Fig. 4.6. On the other hand, there was no significant difference in virulence between samples isolated from infected tomato at UNZA School of Agricultural Sciences research fields and those from Kafue at $p< 0.05$ as shown in table 4.4 and between fungal pathogens from tomato samples collected from Kafue and those from 13 miles north of Lusaka $p<0.05$.

Table 4.4: ANOVA multiple comparisons involving the virulence diversity of the isolates from the three study areas.

Multiple Comparisons

Dependent Variable: Isolates

Games-Howell

(I) Location	(J) Location	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
UNZA	13MILES	-2.390000*	.931041	.040	-4.68622	-.09378
	KAFUE	-.195000	.804608	.968	-2.16791	1.77791
13MILES	UNZA	2.390000*	.931041	.040	.09378	4.68622
	KAFUE	2.195000	1.050473	.106	-0.37101	4.76101
KAFUE	UNZA	.195000	.804608	.968	-1.77791	2.16791
	13MILES	-2.195000	1.050473	.106	-4.76101	.37101

Results from this study suggest that tomato pathogen isolates from tissue samples collected from the area north of Lusaka (13Miles) were more virulent followed by Kafue and least the University of Zambia agriculture research field, Fig. 4.8. The results suggested that there was virulence diversity of fungal and fungi-like isolates from different locations in Lusaka province of Zambia.

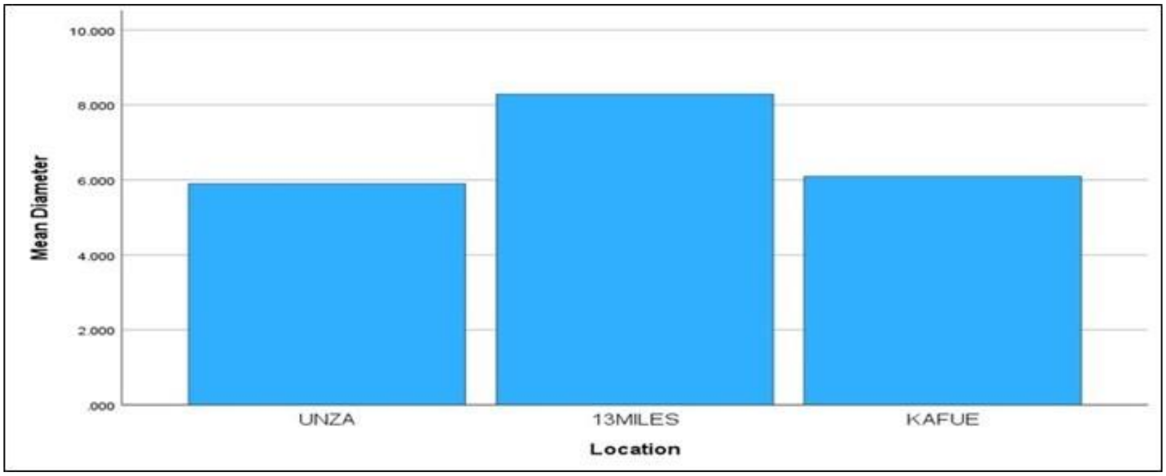


Figure 4.7. Virulence diversity of fungal and fungi-like isolates from infected tomato tissues from the three study locations in Lusaka province.

CHAPTER FIVE

DISCUSSION

5.0 Overview

This chapter discusses the findings of this study in the context of the objectives of the study and results from similar studies conducted elsewhere.

5.1 Discussion

Small and medium scale farmers are the major growers of tomato crop in Zambia. Production is however, constrained by diseases caused by fungal and fungi-like pathogens. Some of the major diseases of tomato that have been reported elsewhere are late blight caused by *P. infestans*, early blight caused by *A. solani*, leaf spots (*A. alternata)*, and leaf mold disease caused by *C. fulvum* (Adhikari and Panthee, 2017). Other major pathogens that have been reported to cause devastating diseases in tomato include *Septoria lycopersici, Passalora fulva, Dydimella lycopersici, Alternaria infectoria, Alternaria alternata* (Kokaeva *et al*., 2020). In addition, Tomato has also been affected by recent outbreaks of the South American tomato leaf miner pest (*Tuta absoluta*) which was reported in Asia (Zhang *et al*., 2021), Africa at large, and Zambia. In this study, fungal pathogens of various mycelial pigments and texture were isolated.

The analysis of pathogens that affect tomato has been conducted using various methods including culture, morphological, molecular, genetic, and pathogenic variations (Naik *et al*., 2010, Gat *et al*, 2012, Kokaeva *et al*., 2020, Mugao *et al*., 2021, Samson *et al*., 2011).

The colony morphological variations in pathogen isolates from various parts of Lusaka were observed. Mycelia that grew on PDA consisted of colonies with colour variations (fig.4.2, 4.3 and Table 4.1). Similar results from studies conducted in Kenya, India and China using morphological identification based on visual observation of pathogen growth patterns, margin colours, mycelia colours, and microscopic assessment of vegetative and reproductive structures have also been reported (Mugao *et al*., 2021; Loganathan *et al*., 2016; Zhao *et al*., 2016). The variations in growth characteristics include those recorded in this study (Fig. 4.3) with colonies showing flattened growth characteristics (13m-1) or raised colony morphology (K-1), cottony mycelium (13m-2) and compact (U-3) mycelium respectively.

Some of the isolates identified in this study include *A. alternata, A. brassicae* and *Fusarium chlamydosporum.* A study in China reported *A. alternata* and *A. solani* as major pathogens of potato blight (Zhao *et al*., 2016). Results from a study in Germany, reported the same pathogens to be causal agents of foliar diseases (Leiminger *et al.*, 2014) whereas *A. alternata* was also isolated and identified to be a major pathogen of solanaceous crop species in India, Sweden and Kenya (Loganathan *et al*., 2016; Odilbekov *et al*., 2016; Mugao *et al*., 2021).

One observation finding from this study was absence of *Phytophthora infestans*. The late blight pathogen was expected to be isolated but none of the samples from all the three study sites was identified to be the late blight pathogen. This finding though surprising may be explained by the limited sample size, limited sampling area or inability to culture the pathogen. Instead, *Chaetomium globosum strain, Fusarium chlamydosporum, Sordariomycetes sp, A. brassicate* from the isolates collected in the north of Lusaka were identified. Others were *A. alternata COLI, Geotrichum candidum, Chaetomium sp,* were isolated from samples collected from south of Lusaka-Kafue and *A. alternata, Fusarium chlamydosporum, Epicoccum nigrum, and Cladosporium langeronii* were isolated from samples from the University of Zambia School of Agricultural Sciences research fields. Mugao *et al.,* (2021) attempted to isolate *P. infestans* in tomato using sexual and asexual characteristics but were unsuccessful. Instead, they isolated other fungal pathogens (Mugao *et al*., 2021). The study showed high morphological variations within a particular study area and among the three study areas. The features, which were, viewed macroscopically included, colony (upper) colour, substrate (lower) colour, growth margin and growth pattern Fig.4.2, Table 4.1, revealed variations in the fungal and fungi-like pathogens. While morphological classification of fungal species has contributed to the understanding of both pathogenic and non-pathogenic fungi, it is however not precise.

The isolates in the study were identified to species level by the amplification of the ITS region followed by BLAST algorithm analysis search using sequences at an established database. The ITS1 and ITS4 primers (White *et al*., 1990) were used to generate amplicons ranging between 500 to 600bp from the products of PCR. The region is known to show a high probability of successful identification for a wide range of fungi showing well defined inter- and intraspecific variation of barcode gape (Schoch *et al*., 2012). Similar results using ITS- specific primer pair have been reported (Gardes and Bruns, 1993; Kokaeva *et al*., 2020; White *et al*., 1990). In addition, similar outcomes have also been reported in studies on potato and bittersweet nightshade leaves (Kokaeva *et al*., 2018; Kokaeva *et al*., 2019; Kokaeva *et al*., 2020). The ITS region has also been used in the characterization of *Fusarium spp*. (Pothiraj *et al*., 2021).

In this study, the success of PCR amplification of the ITS region, was from 75% to 89%. The PCR amplifications of ribosomal RNA genes are more reliable across the fungal and fungi-like pathogens (Schoch *et al*., 2012) making it easy for sequencing. The ITS has been proposed as a key fungal barcode as it combines high resolving power to discriminate species which are closely related with a high PCR and sequencing rate of success (Schoch *et al*., 2012). Results from this study have revealed that *A. alternata, Chaetomium globosum strain, Fusarium chlamydosporum, Sordariomycetes sp, A. brassicae* (North of Lusaka Zambia, 13miles). *A. alternata COLI, Geotrichum candidum, Chaetomium sp,* were among the species circulating in the tomato cultivating areas south of Lusaka. Samples from the research fields at the University of Zambia, School of Agricultural Sciences revealed presence of *A. alternata, Fusarium chlamydosporum, Epicoccum nigrum, Cladosporium langeronii* at 98% to 99% identity. Similar results were obtained from a study in China on variation of *A. alternata* where ITS region was sequenced and used as query sequence to identify the pathogen in the GenBank (Guo *et al*., 2004). *Fusarium ssp*. was also characterized as a tomato pathogen (Pothiraj *et al*., 2021) while a study identified endophytes *Sordariomycetes, Fusarium, Chaetomium* as fungi that interacted with tomato using phylogenetic analysis of the internal transcribed spacer (Zhang *et al*., 2021). These findings from the cited related studies are in agreement with those from this study.

Koch's postulates (Byrd, and Segre, 2016) was fulfilled at least for the fungal isolates which could be tentatively identified in that the isolated fungi were demonstrated to be pathogenic on a popular tomato variety grown extensively in Zambia. The isolates had differences in their virulence, spore morphology and other propagules. The results showed that pathogen in plate KS2S2 (*Cladosporium clone*) had highest virulence giving mean lesions size of 12.7mm, while the lowest virulence recorded was that of KS46 (*Geotrichum candidum isolate*) with mean of 2.7mm. Moderate virulence was recorded for isolate KS2S2.1 (*A.alternata*) with mean of 6.7mm. This showed that there was virulence diversity of the pathogens affecting tomato in Kafue as can be seen in differences of the means, fig 4.7

The findings of this study clearly showed that various fungal and fungi-like pathogens are circulating in farmers' fields in tomato growing areas of Lusaka, Zambia and the isolates showed molecular and virulence diversity. Results from a similar study in Antioquia, Colombia in 2009 focusing on *P. infestans* found diversity in the virulence of the various isolates of the late blight tomato pathogen (Ramelli *et al*., 2009). In another study the virulence and aggressiveness of *P.infestans* on potato and tomato plants identified no strong specificity (Michalska *et al*., 2016). Differences between potato and tomato virulence spectra were observed and showed more frequent virulence to of *P. infestans* on differentials of their own hosts ((Michalska *et al*., 2016). It was reported that isolates of potato sporulation intensity was higher on their own hosts compared to the one on tomato hosts, however for tomato isolates the sporulation intensity was high on both hosts ((Michalska *et al*., 2016). This showed that there was virulence diversity just like this study is showing.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.0 Overview

Chapter six presents the conclusion and the recommendations.

6.1 Conclusions

Molecular diversity was observed to occur among the isolates from this study and colonies differed in growth characteristics. Growth characteristics included flat or raised, cottony and compact mycelium respectively. In addition, mycelial and colony colour variations included olive green, charcoal grey, pink top view at the base were red, cream white, red, reddish respectively showing signified molecular diversity. Isolates from 13 miles north of Lusaka13MS1S3 identified as *Fusarium chlamydosporum* produced the highest mean lesion size (14.7 mm) with the highest virulence followed by those from Kafue and University of Zambia agriculture research fields was least. There were significant differences in virulence between isolates from University of Zambia agriculture research fields and those from 13-miles at $p<0.05$. While there was no significant difference in virulence between isolates from University of Zambia research agriculture fields and those from Kafue at $p< 0.05$. Further there was no significant difference between isolates from Kafue and 13 miles at $p<0.05$. Fungi were more virulent than the fungi-like pathogens. The results indicated that there were various fungal and fungi-like pathogens circulating in tomato growing areas of Lusaka and show molecular and virulence diversity.

6.2 Recommendations

Some of the recommendations that should be considered by stakeholders relevant to the tomato-growing sector are as follows:

(i) Additional studies should be conducted covering large parts of Zambia where tomato is grown as a commercial crop to determine the diversity of fungal pathogens including the potential presence of other pathogens that were not isolated in this study including *P. infestans*. Such a study could also use other barcodes to aid in the accurate taxonomic identification of fungal pathogens of tomato.

(ii) Different parts of tomato-growing areas of Zambia should be treated differently from other localities in terms of the fungicides used by farmers, due to the observed molecular and virulence diversity in the isolates.

(iii) Farmers in tomato-farming areas should be encouraged to destroy the remains of the tomato plants after every harvest to prevent carry-over inoculum from re-infecting the new tomato crop. Burying of the tomato plant remains after every harvest should be discouraged because the pathogens like *A. alternata* are known to survive in the soil for prolonged periods resulting in infesting the new crops.

(iv) Tomato seed producing and breeding companies should be encouraged to breed for disease resistant tomato varieties especially specifically with alleles with strong resistance against leaf spot and early blight diseases being the most prevalent in Lusaka province areas like 13miles, University of Zambia research agriculture fields and Kafue.

(v) Farmers should be encouraged to practice crop rotation and Ministry responsible for crop agriculture must sensitize and educate farmers periodically on the best ways of treating the remains of the tomato plants after every harvest.

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APPENDICES

APPENDIX IA: The fungi-like pathogen common in all the study areas *A. alternata*

>13MS4S3.2 R

GTCCCCCGGGGTGGCCCCTACTGCAGGAGGGGAACCGTACGGCGGAAGGT
GGCATATAAGGGTTAATGGATGCTAGACCTTTGCTGATAGAGAGTGCGAC
TTGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCG
AGTCTCCAGCAAAGCTAGAGACAAGACGCCCAACACCAAGCAAAGCTTGA
GGGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCG
CAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACT
ACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCC
GTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTA
CAAAAGGTTTATGTTTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAA
GAAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTAACCCCGA
GAGGTTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCA
CCTACGGAAGG

APPENDIX IB: DNA sequence of ITS PCR region from *Cladosporium sp.* from south of Lusaka-Kafue

>KS2S2 R

CCGACAGCGATTCTTCACATACGTCCTGATCCGTAGTCAAGCGTGTAGGA
AAAGAGGGTTGTTTTACGGCGTAGCCTCCCGAMAACCCTGTTAGCGAATA
GTTTCCACAACGCTTAGGGGACAGAAGACCCAGCCGGTCGATTTGAGGCA
CGCGGCGGACCGCGTTGCCCAATACCAAGCGAGGCTTGAGTGGTGAAATG
ACGCTCGAACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTT
CAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCAT
TTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTAAAAG
TTTTAATTTATTAATTAAGTTTACTCAGACTGCAAAGTTACGCAAGAGTT
TGAAGTGTCCACCCGGAGCCCCCGCCCGAAGGCAGGGTCGCCCCGGAGGC
AACAGAGTCGGACAACAAAGGGTTATGAACATCCCGGTGGTTAGAACGGG
GTCACTTGTAATGATCCCTCCCGCAGGTTCACCTACGGAGAGG

APPENDIX IC: pathogen isolate (*Epicoccum nigrum)* from the University Of Zambia Agriculture Research fields

>US5S1.3 R

GTTCCCCCGGGGTCCCCTACGTGCAGGGGGTCCGAGGTCAGAGTGTCACT

TTTGGACTTTTGGAACGTCGTCGTTATGAGTGCAAAGCGCGAGATGTACT

GCGCTCCGAAATCAATACGCCGGCTGCCAATTGTTTTAAGGCGAGTCTGC
GCGCGGAGGCGAGACAAAACACCCAACACCAAGCAGAGCTTGAAGGTACA

AATGACGCTCGAACAGGCATGCCCCATGGAATACCAAGGGGCGCAATGTG
CGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTACTTATC
GCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTG
AAAGTTGTAACTATTAAGTTTTTTCAGACGCTGATTGCAACTGCAAAGGG
TTTGAATGTTGTCCAACCGGCGGGCGGACCCGCCGAGGAAACGAAGGTAC
TCAAAAGACATGGGTAAGAGGTAGCAGACCGAAGTCCACAAACTCTAGGT
AATGATCCTTTCCGCAGGTTTCACCTACCGGAAGG

Epicoccum nigrum was the pathogen sample collected from UNZA with mean of 11.3mm after it was inoculated on tomato leaves Tengeru variety.

APPENDIX ID: Pathogen isolate (*Fusarium chlamydosporum*) from North of Lusaka-13miles.

>13MS1S3
TCYCAAAGGGGTTTCCCGTAAGTGCCACCTGATCCGAGGTCAACATTCAG
CACTGTTGGGGGTTTAACGGCTTGGCCGCGCCGCGTTCCAGTTGCGAGGT
GTTAGCTACTACGCAATGGAGGCTGCAGCGAGACCGCCACTAGATTTCGG
GGCCGGGACGCGGTAGAACCGCAGCCCGATCCCCAACACCAAACCCGGGG
GCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATACTGG
CGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATT
CACATTACTTATCGCATTTTGCTGCGTTCTTCATCGATGCCAGAACCAAG
AGATCCGTTGTTGAAAGTTTTGATTTATTTGTTTTTTAAACTCAGAAGTT
ACAATAAAATTCAGGGTTTGTGGGTCCTGCGGCGGGCCGTCCCGTTTTAC
GGGGCGCGGGCTGATCCGCCGAGGCAACGTATAGGTATGTTCACAGGGGT
TTGGGAGTTGTAAACTCGGTAATGATCCCTCCGCAGGTTCCCCTACGGAA
G

APPENDIX IE: Pathogen isolate (*Kalmusia italica*) from North of Lusaka-13miles.

>13MS1S1 F
GGAAAAAAAAGATATTCCTGGCGGCGGGAAGGGGAGGTCAGGAGCACACG
CTCCCCTGACCCTTTCGCTCCCCACCCTCTTTTACTATCACCTTTCCTTC
TTCCGGGCGGGGCGACCCGCCGGCGGAAACATCCATGAACTCTTTTTGCA
TCTAAGAAGTACACTGTTCTGATAAACCAAAATCGTTACAACTTTCAACA
ATGGATCTCTTGGCTCGGGCATCAATGAAAAACGCAGGGAAATGCGATAC
GTAGTGTGAATTGCAGAATCCAGTGAATCATCAAATCTTTGAACGCCCCT
TGCTCCCTTTGGTATTCCATGGGGCATGCCTGTTCAAGCGTCATTTACAC
CCTGGGGGCTGTGGTTGGTGTTGGGCGTCCGTCCGCGCCCCCGCGTGCAA
ACTAGTTCAAAAAGGCGTTGCCAGCCCCCGTCGACCACAACAATAGAAAA
AAAAGTTGCGAACCGGGGTGCGACGGGCCGTGACATTCCCACCATTCTCC
TCTTCCACGTTTGACGTGGGAGGGGGGGAGGGGATACCCGCTGAATTTAA
GCGAAAAAAAATAAAGCGGAGGAAA

APPENDIX IF: Pathogen isolate (*Kalmusia italica*) from North of Lusaka-13miles.

>13MS1S1 R

GGGGGAAATCCCGGGGCATATACAGACCGGATCCAAGGTCAAACGTGGAA
GATGACACTTGTTGGAACGCCACGGACGTCGGACCCTGTTCCCAACTGTG
CTGCGAGGGCTGTCGTCAACCGGGGCTGCAAATGCCTTTGGGGCGAGTCC
GCACGCGGGGGCGCGGAGTTCGCCCAACACCAAGCAGATCTTGAGGGTGT
AAATGACTCTCTAGCCGGCGTGCCCCATGAAAAACCAAGGGGCGCGCTGT
GCGTTCAAAGATTTGATGAAACCCTGTGATTCTGCAATTCCTACTACCTA
TCGTATTTCCCTGCGTTCTTCATCGATGCCTGAGCGTAGAAATCCATTGT
TGAACGTTGTAACCATTTTGGTTTAGGAGAACTGTGTACTTCCTAGATGC
AAAAAGAGTTCGTGGATGGTTCCGCCGGCGGGTCGCCCCGCCGAGGAAGA
AGGAAATGTGCTCCTGGGACAGAGTGGGGAGCCAAAGGGTCAAGTCGACT
CTTTGATCTTTGACATCCCATGTTGTAGGGATACAGGTTATAATCCTTCC
TATCGGTCAGCGGAGGAA

APPENDIX IG: Pathogen isolate (*Chaetomium globosum*) from North of Lusaka-13miles.

>13MS2S2 F

GGAGAAAATAAAGAGGCAGCAGGAATCCCTAAACCATTGTGAACGTTACC
TAAACCGTTGCTTCGGCGGGCGGCCCCGGGGTTTACCCCCCGGGCGCCCC
TGGGCCCCACCGGGGGCGCCCGCCAGAGGTCACCAGACTCTTGATAATTT
ATGGCCTCTCTGATTCTTCTGTACTGAATAAGTCAAAACTTTCAACAACG
GATCTCTTGGTTCTGGCATCGATGAAAAACGCAGCGAAATGCGATAAGTA
ATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGG
GCCCGCCAGTATTCTGGCGGGCATGCCTGTTCAAACGTCATTTCAACCAT
CAAGCCCCCGGGCTTGTGTTGGGGACCTGCGGCTGCCGCAGGCCATGAAA
AGCAGTGGCGGGCTCGCTGTCCCACCGAGCGTAGTAGCGTATATCTCGCT
CTGGGCGTGCTGGGGGTTCCGGCCGTTAAACGGYCTTTTAACCCAAGGTT
GAGCTCGGATCAGGTAGGAAAACCCGCTGAACTTAAGCATATCAATAAGG
CGGAAGAA

APPENDIX IH: Pathogen isolate (*Chaetomium sp.)* from North of Lusaka-13miles.

>13MS2S2 R

GGGGAAMTMAAGAGTAGCTACAAACTGTATCCGATGTCACCTTGGGCCTAAAGGTG
GTTTAACGGCCGGAACCCCCAGCATTCACAAACCGAGATATATGCTACTACGCTCGG
TGTGACAGCCAGCCCGCCACTGCTTTTCAAGGCCTGCTGGACCCGCAGGTCCCCCAC
ACAAGCCCGGGGGCTTGATGGTTGAAATGACCCTCTAACAGGCATGCCCGCCAGAA
TACTGGCGGGCGCAATGTGCGTTCTGTGATTCTATGATTCACTGAATTCTGCAATTTC
CATTACCTATCCTATTTCCCTGCCTTCTTCTTCAATGCCTGAACCAAGAAATCCGTTG
TTGAAAGTTTTGACTTATTCCCTACGTAAGACTCAGAGAGGCCATAAATTATCAGAG
TTTGGTGACCTCCGGCGGGCGCCAGCGGTGGGGCCCAGGGGCGCCCGGGGGGTGAC
CTGCGGGGCCGCCCGCCGAAGCAACGGTTTAGGTAACGTTGGCTATGGTTTAGGCA
GTTTTGAAAATCTGTTATGATTCAGCCTCTCGATCACCGGGAAGA

APPENDIX I-I: Pathogen isolate (*Alternaria alternata*) from North of Lusaka-13miles.

>13MS1S2 F

CCCCCTCTATCCGAAGAATGGGACGGGCTGGACCCTCTCGGGGGTCGTTA
GGGGGTGCTGAATTATTCACCCTTGTCTTTTGCGTACTTCTTGTTTCCTT
GGTGGGTTCGCCCACCACTAGGACAAACATAAACCYTTTGTAATTGCAAT
CAGCGTCAGTAACAAATTAATAATTACAACTTTCAACAACGGATCTCTTG
GTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATT
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGG
TATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCTTTG
CTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAG
TAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCT
CTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTTCAACTTTTGACC
TCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAG
GAA

APPENDIX I-J: Pathogen isolate (*Alternaria alternata*) from North of Lusaka-13miles.

>13MS1S2 R

CKKCCCGGCGGACCCTCCCGGCGACATGGGATCCGTACGTCAGGAGTTGCCAAAA
AAGGCTTAATGGATGCTAGACCTTTGCTGATAGAGAGTGCGACTTGTGCTGCGCTCC
GAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAG
ACAAGACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGC
ATGCCCTTTGGAATACCAAAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTG
AATTCTGCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAAC
CAAGAGATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAA
TTACAAAAGGTTTATGTTTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAA
GTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTAACCCCGAGAGGTTCCA
GCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCCTACGGAAGG

APPENDIX I-K: Pathogen isolate (*Sordariomycetes sp. genotype*) from North of Lusaka-13miles.

>13MS1S3.2 F

TTCGCCAGTCGTTACAGCGGCAAGCAGCTCCCAACCCCTGTGAACATACCTATATAG
AAGCCTCGGCGGATCAGCCCGCGCCCCGTAAAACGGGACGGCCCGCCGCAGGACCC
ACAAACCCTGAATTTTATTGTAACTTCTGAGTTTAAAAAACAAATAAATCAAAACTT
TCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAA
GTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCC
GCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCCCGG
GTTTGGTGTTGGGGATCGGGCTGCGGTTCTACCGCGTCCCGGCCCCGAAATCTAGTG
GCGGTCTCGCTGCAGCCTCCATTGCGTAGTAGCTAACACCTCGCAACTGGAACGCGG
CGCGGCCAAGCCGTTAAACCCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGA
ATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA

APPENDIX I-L: Pathogen isolate (*Fusarium chlamydosporum*) from North of Lusaka-13miles.

>13MS1S3
TCYCAAAGGGGTTTCCCGTAAGTGCCACCTGATCCGAGGTCAACATTCAG
CACTGTTGGGGGTTTAACGGCTTGGCCGCGCCGCGTTCCAGTTGCGAGGT
GTTAGCTACTACGCAATGGAGGCTGCAGCGAGACCGCCACTAGATTTCGG
GGCCGGGACGCGGTAGAACCGCAGCCCGATCCCCAACACCAAACCCGGGG
GCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATACTGG
CGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATT
CACATTACTTATCGCATTTTGCTGCGTTCTTCATCGATGCCAGAACCAAG
AGATCCGTTGTTGAAAGTTTTGATTTATTTGTTTTTTAAACTCAGAAGTT
ACAATAAAATTCAGGGTTTGTGGGTCCTGCGGCGGGCCGTCCCGTTTTAC
GGGGCGCGGGCTGATCCGCCGAGGCAACGTATAGGTATGTTCACAGGGGT
TTGGGAGTTGTAAACTCGGTAATGATCCCTCCGCAGGTTCCCCTACGGAA
G

APPENDIX I-M: Pathogen isolate (*Alternaria alternata*) from North of Lusaka-13miles.

>13MS1S3.3 F
TGCCCCCCTGGGGACCCTGCATTGGTGCGGGCTGGGAACCTCTCGGGGGAATAGMA
GGTGCTGAATTATTCACCCTTGTCTTCCTGCGTACTTCTTGTTTCCTTGGTGGGTTCGC
CCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATT
AATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAG
CGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAAC
GCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTACC
CTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTA
AAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTAT
CAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTTCAACTTTTGACCTCGGATCAGGTA
GGGATACCCCGCTGAACTTAAGCA
TATCAATAAGGGGAGGAA

APPENDIX I-N: Pathogen isolate (*Alternaria brassicae*) from North of Lusaka-13miles.

>13MS4S3.2 F
GGGCGCCCAAATTCCGTCGGCATGAGGACGGATGGACCTCTGGGGACAAT
AAGGGGGCTGATTATTCACCCTTGTCTTTAGAAGAAACTTCTTGTTTCCT
TGGTGGGTTCGCCCACCACTAGGACAAACATAATCCTTTTGTAATTGCAA
TCAGCGTCAGTAACAAATTAATAATTACAACTTTCAACAACGGATCTCTT
GGTTCTGGCATCGATGAAGAACGCAGCGAAATGCCATAAGTAGTGTGAAT
TGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTG
GTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCTTT
GCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAA
GTAATTGGCAGCCGGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACT
CTCTATCAGCAAAGGTCTAGCATCCATTAAGCCCTTTTTTTCAACTTTTG
ACCTCGGATCAGGTAGGGATGACCCGCTGAACTTAAAGCATATCAATAAA
GCGGGAGGAA

APPENDIX I-O: Pathogen isolate (*Alternaria tenuissima*) from North of Lusaka-13miles.

>13MS5S1.1 R

GRRTTACGGCATTGGTCCCCCTACGTGCCGGATCCGAGGTCAAGAGTTGC
CATTTAAAGGCTTAATGGATGCTAGACCTTTGCTGATAGAGAGTGCGACT
TGTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGA
GTCTCCAGCAAAGCTAGAGACAAGACGCCCAACACCAAGCAAAGCTTGAG
GGTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGC
AATGTGCGTTCGAAGATTCGATGATTCACTGAATTCTGCAATTCACACTA
CTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCG
TTGTTGAAAGTTGTAATTATTAAGTTGTTACTGACGCTGATTGCAATTAC
AAAAGGTTTATGTTTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAG
AAGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTAACCCCGAG
AGGTTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCAC
CTACGGAAG

APPENDIX I-P: Pathogen isolate (*Alternaria alternata*) from North of Lusaka-13miles.

>13MS1S3.3 R

GTCACCTATGGTGGCCGCCGTACCTGAACCGTAGGTCAGAAGGTGCRCCTTTAAGGC
TTAATGGATGCTAGACCTTTGCTGATAGAGAGTGCGACTTGTGCTGCGCTCCGAAAC
CAGTAGGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAG
ACGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCC
TTTGGAATACCAAAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCT
GCAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGA
GATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAA
AAGGTTTATGTTTGTCCTAGTGGTGGGCGAACCCACCAAGGAAAACAAGAAGTACG
CAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTAACCCCGAGAGGTTCCAGCCCG
CCTTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAAAG

APPENDIX IQ: DNA sequence of ITS PCR region from *uncultured Cladosporium* from south of Lusaka-Kafue

>KS2S2 F

GGGGGGGGTCCTTTCAATAGCGGGGGGGTCAACCACCGGGATGTTCATAC
TGCAAGGATTGTCCGACTCTGTTGCCTCCGGGCCGACCCTGCCTTGGGGC
GGGGGCTCCGGGTGGACACTTCAAACTCTTGCGTAACTTTGCAGTCTGAG
TAAACTTAATTAATAAATTAAAACTTTTAACAACGGATCTCTTGGTTCTG
GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA
TTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGTATTCC
GGGGGGCATGCCTGTTCGAGCGTCATTTCACCACTCAAGCCTCGCTTGGT
ATTGGGCAACGCGGTCCGCCGCGTGCCTCAAATCGACCGGCTGGGTCTTC
TGTCCCCTAAGCGTTGTGGAAACTATTCGCTAAAGGGTGTTCGGGAGGCT
ACGCCCGTAAAACAACCCCCATTTCTAAGGTTGACCTCGGATCAGTAGGG
ATACCCCGCTGAACTTTAGCATATCAATAAGCGGAGGAA

APPENDIX IR: DNA sequence of ITS PCR region from *Alternaria alternata* from south of Lusaka-Kafue

>KS2S2.1 F

TGCTGGCCCCTTTCCCAAGGAGTGAGGGCGGGCTGGAAACCTCTCGGGGGAAAGAA
GGGTGCTGAATTATTCACCCTTGTCCTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCG
CCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAAT
TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCA
GCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTAC
CCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTT
AAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTA
TCAGGCAAAAGGTCTAGCATCCATTAAGCCTTTTTTTCAACTTTTGACCTCGGATCAG
GTAGGGATACCCGCTGAAACTTAAGCATATCAATAAGCGGAGGAA

APPENDIX IS: DNA sequence of ITS PCR region from *Alternaria sp.* from south of Lusaka-Kafue

>KS2S2.1 R

CCTTATCGCMAGGCTTCCCTACCGGCCTGGGTCCGAGGTCAGAGTTGCCTTAAAGGC
TTAATGGATGCTAGACCTTTGCTGATAGAGAGTGCGACTTGTGCTGCGCTCCGAAAC
CAGTAGCCGGCTGCCAATTACTTTAAGGCGAGTCTCCAGCAAAGCTAGAGACAAGA
CGCCCAACACCAAGCAAAGCTTGAGGGTACAAATGACGCTCGAACAGGCATGCCCT
TTGGAATACCAAAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTG
CAATTCACACTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAG
ATCCGTTGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACAAA
AGGTTTATGTTTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGAAGTACGCA
AAAGACAAGGGTGAATAATTCAGCAAGGCTGTAACCCCGAGAGGTTCCAGCCCGCC
TTCATATTTGTGTAATGATCCCTCCGCAGGTTCACCTACGGAGAGG

APPENDIX IT: DNA sequence of ITS PCR region from *Geotrichum candidum* from south of Lusaka-Kafue

>KS46 R

CCCTGAGGGGAGGGCATGCATGCAGGATCTGAGGTCGATAAYGTTGTTTT
TCAACGAATTTGATTCCTATTTTAGACAAGCAATGCAATTCCAAGAGAGA
AACAACGCTCAAACAAGTATACTTTGGGGGATACCCCAGGGTGCAATGTG
CGTTCAAAAACTGATGATTCACTTCTGCAATTCGCAAGAAATATCGCGTT
TCGCTGCGTTCTTCATCGATACGAGAACCAAGAGATCCATTGTTAAAAGT
TTTAATTTTTTTTTGTTTTGAAAAAAAAAATAATTGTTGCCGTTTAATTT
TCACCAATTAATTATAATTCTAAAGGCCCCTGCCGGGGGTGCGCGGGGGG
GAGAGG

APPENDIX IU: DNA sequence of ITS PCR region from *Chaetomium sp.* from south of Lusaka-Kafue

>KS48S1 F

ATGAGATCTTCACGCGGCGCCGGCTCCCTAACCATTGTGAACTCCCTTCT

AACTGTTGCTTCTGCGGGTTCGGCGCGCTCGCGCGCGGCCGGTCCCGCCT
CGACGCGGCCCGGCGGACGCCCCTAAATCTTGAACTTTGTATGGCCTCTC
TGAGTCTTCCGTACTGAATAAATAAAAACTTTCAAACCTGGATCTCTTGG
TTCTGGGATCGATGAAGAAAGGAGCGACATGGGATAAGGAATGTGAATTG
CAGAATTCAGTGAATCATCGAATCTTTGAACGCTCATTGCGCCCGCCAGT
ATTCTGGCGGGCATGCCTGTTCGACCTTCATTTCAACCATCAAGCCCCGG
GCTTGTGTTGGGGACCTGCGGCTGCCCGCCGGCCCTGAAAACCAGGGGCG
AGCTCGCTAGTCCCACCCAGCGTAGTAGATCTCCTCTCGCTCACGGCGTG
CTGCGTGTTCCAGCCCGTTACCAACCCCTTACACCAAAGGTTTGACTTCG
GATCAGGGTAGGAAGACCCGCTAAAACTTAAGCATGCCAATAAAGCGGAAGGAAG
G

APPENDIX IV: DNA sequence of ITS PCR region from *Chaetomium sp.* from south of Lusaka-Kafue

>KS48S1 R

CCCCGGGGCCCGACTGCACCATGATCCGAGTCACCTTGTCTGAAATGGGG
GTGTAACGGCTGGACGGGCGGGACGCCCTGAGGAGAGAAATCTACTACGC
CCGGAGTGACTGGCGAGCCCGCCACTGTTTTTTTGGGGCTGCGCCCCGCC
GAACGTCCGCAACACCCACACAGGGAGGATGGTTTAAAGGACGCTCGAAC
AGGCATGCCCGCCAGAAACTGGAAGGCGGGTGTGCATGTACATTCAATGA
CTCACTGAAGTCTGAATTGCATAACTATACAATGCTTTTTTTGTCKTCAC
GAACCAGAGAAAGTTTTGATGGGTGATTTTCTAATTAATCTCACAGAGGC
TTAAATTATATAGTTTCGACGCCGGCCGCGCACGGGTGGACCGGGAACCG
CGGGCGCCAAAAGGGGGCACGGTTAAGGTGGTTCATATGTTTTGGGATTG
GACTATTAATCTCATCGCAGTCCCCTAAGAAGTCAAAAGGAAGT

APPENDIX IW: pathogen isolate (*Fusarium sp.)* from the University Of Zambia Agriculture Research fields

>US4S3.2 F

CGGGCGGCAAATTACCGAGCTTTAACTCCCTACCCCTGTGAACATACGAGAAAGAG
GGCCTCGGCGGATCAGCCCGCGCCCCGTAAAACGGGACGGCCCGCCGCAGGACCCA
CAAACCCTGAATTTTATTGTAACTTCTGAGTTTAAAAAACAAATAAATCAAAACTTT
CAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAG
TAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCG
CCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCCCGGG
TTTGGTGTTGGGGATCGGGCTGCGGTTCTACCGCGTCCCGGCCCCGAAATCTAGTGG
CGGTCTCGCTGCAGCCTCCATTGCGTAGTAGCTAACACCTCGCAACTGGAACGCGGC
GCGGCCAAGCCGTTAAACCCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAAT
ACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA

APPENDIX IX: pathogen isolate (*Fusarium chlamydosporum*) from the University Of Zambia Agriculture Research fields

>US4S3.2 R

GTGTTCAGCCGGGGTATCCTACCTGATCCGAGGTCACATTCACCAAGTTG
GGGGTTTAACGGCTTGGCCGCGCCGCGTTCCAGTTGCGAGGTGTTAGCTA
CTACGCAATGGAGGCTGCAGCGAGACCGCCACTAGATTTCGGGGCCGGGA
CGCGGTAGAACCGCAGCCCGATCCCCAACACCAAACCCGGGGGCTTGAGG
GTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATACTGGCGGGCGCA
ATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTAC
TTATCGCATTTTGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGT
TGTTGAAAGTTTTGATTTATTTGTTTTTTAAACTCAGAAGTTACAATAAA
ATTCAGGGTTTGTGGGTCCTGCGGCGGGCCGTCCCGTTTTACGGGGCGCG
GGCTGATCCGCCGAGGCAACGTATAGGTATGTTCACAGGGGTTTGGGAGT
TGTAAACTCGGTAATGATCCCTCCGCAGGTTCACCCTACGGAAG

APPENDIX IY: pathogen isolate (*Lasiodiplodia pseudotheobromae*) from the University Of Zambia Agriculture Research fields

>US6S1.1 R

TTTCCCGGGGGCCCTACTGCAGCGTGATCCGAGGTCACCTTGACAATGGT
TCAGAATGTTCGTCCGGCGGGCGACGCCCACCACTCCAAAGCGAGGTGTA
TTCTACTACGCTTGAGGGCTGAACAGCCACCGCCGAGGTCTTTGAGGCGC
GTCCGCATTGAGGACGGTGCCCAATTCCAAGCAGAGCTTGAGGGTTGTAA
TGACGCTCGAACAGGCATGCCCCCCGGAATACCAAGGGGCGCAATGTGCG
TTCAAAGATTCCATGATTCACTGAATTCTGCAATTCTCATTACTTATCGC
ATTTCTCTGCGTTCTTCATCGATGCCAAAACCAAGAGATCCGTTGTTGAA
AGTTTTATTTTATTAACTTGATTATCAGACGTCTGCGTTTACTGACTGGA
GTTTGGAGGTCCTTTGGCGGCCGGAGCCGCCAAACCAACAGAGGTACATT
CACAAAGGGTGGGAGAGTCAAGCCGAAGCCCGAAAACTCGGTAATGATCC
TTCCGCACGTTCACCTACTGAAGCTACGGAA

APPENDIX IZ: pathogen isolate (*Sordariomycetes sp. genotype*) from the University Of Zambia Agriculture Research fields

>US6S1.2 F

CTTCTTCTCGGTCAGCAGTTTCGGCTCCCAACCCCTGTGAACATACCCAC
TTCGATTGCCTCGGCGGATCAGCCCGCGCCCCGTAAAACGGGACGGCCCG
CCGCAGGACCCACAAACCCTGAATTTTATTGTAACTTCTGAGTTTAAAAA
ACAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGAT
GAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA
ATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCA
TGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCCCGGGTTTGGTGTTGG
GGATCGGGCTGCGGTTCTACCGCGTCCCGGCCCCGAAATCTAGTGGCGGT
CTCGCTGCAGCCTCCATTGCGTAGTAGCTAACACCTCGCAACTGGAACGC
GGCGCGGCCAAGCCGTTAAACCCCCAACTTCTGAATGTTGACCTCGGATC

AGGTAGGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAA

APPENDIX IAB: pathogen isolate (*Fusarium chlamydosporum*) from the University Of Zambia agriculture research fields

>US6S1.2 R

CTCGGAGGCTCTACTGCGAGCGGGATCGAGTCAGATTCGAAGAAAAGGGT
TTAACGGCTTGGCCGCGCCGCGTTTCCAGTTGCGAGGTGTTAGCTACTAC
GCAATGGAGGCTGCAGCGAGACCGCCACTAGATTTCGGGGCCGGGACGCG
GTAGAACCGCAGCCCGATCCCCAACACCAAACCCGGGGGCTTGAGGGTTG
AAATGACGCTCGAACAGGCATGCCCGCCAGAATACTGGCGGGCGCAATGT
GCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTAT
CGCATTTTGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTT
GAAAGTTTTGATTTATTTGTTTTTTAAACTCAGAAGTTACAATAAAATT
AGGGTTTGTGGGTCCTGCGGCGGGCCGTCCCGTTTTACGGGGCGCGGGCT
GATCCGCCGAGGCAACGTATAGGTATGTTCACAGGGGTTTGGGAGTTGTA
AACTCGGTAATGATCCCTCCGCAGGTTCACCTACGGAAG

APPENDIX IAC: pathogen isolate (*Alternaria tamaricis*) from the University Of Zambia agriculture research fields

>US7S1.2 F

CTGTGGCGCATTCAATAAGCGGGAGGCCGCCCTGGACCTCACGGGGCTAC
AAGAAGGGCTGAATATTACCCTTGTCCTCTGGGTACTTCTTGTTTCCTTG
GTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGGAATC
AGCGTCAATATAAATTAATAATTACAGCTTTCAACAACGGATCTCTTGGT
TCTGGCATCGATGAAGAACGCAGGCGAAATGCGATAAGTAGTGTGAATTG
CAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGT
ATTCCAAAGGGCATGCCTGTTCTAGCGTCATTTGTACCCTCAAGCTTTGC
TTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGT
AATTGGCAGCCGGCCTACTGGTTTCGGACCGCAGCACAAGTCGCACTCTC
TATCAGCAAAGGTCTAGCATCCCATTAAGCCTTTTTTTCAACTTTTGACC
TCCGGATCAGGTAAGGGATACCCCGCTGAACTTAAGCATATCAATAAACC
GAAGGAA

APPENDIX IAD: pathogen isolate (*Alternaria alternata*) from the University Of Zambia agriculture research fields

>US7S1.2 R

GTCTCCCCTAMAGGTTCCCTCCGGGCCAGGATCGAGGTCAAAAAGTTGCC
AAATTGAGGGTTAATGGATGCTAGACCTTTGCTGATAGAGAGTGCGACTT
GTGCTGCGCTCCGAAACCAGTAGGCCGGCTGCCAATTACTTTAAGGCGAG
TCTCCAGCAAAGCTAGAGACAAGACGCCCAACACCAAGCAAAGCTTGAGG
GTACAAATGACGCTCGAACAGGCATGCCCTTTGGAATACCAAAGGGCGCA
ATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACACTAC
TTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGT
TGTTGAAAGTTGTAATTATTAATTTGTTACTGACGCTGATTGCAATTACA

AAAGGTTTATGTTTGTCCTAGTGGTGGGCGAACCCACCAAGGAAACAAGA
AGTACGCAAAAGACAAGGGTGAATAATTCAGCAAGGCTGTAACCCCGAGA
GGTTCCAGCCCGCCTTCATATTTGTGTAATGATCCCTCCGCAGTTCACCT
ACGGAAAGG

The isolate sample collected from 13miles, which is North of Lusaka after inoculation on the tomato leaves of Tengeru variety the one with the highest virulence was *Fusarium chlamydosporum* with a mean of 14.7mm

APPENDIX II: Mean lesion sizes caused by pathogen isolates from Kafue. Fungi were purified and cultured PDA media. Isolates were used to inoculate three leaves on each plant. KS2S2 identified as *Cladosporium clone* showed highest virulence based on mean lesion size of 12.7 mm while, *Geotrichum candidum* designated as KS46 showed the lowest mean lesion size of 2.7 mm. Intermediate lesion size of 6.7 mm was induced by *A. alternate* identified as KS2S2.1.

Table AII. Mean lesion sizes on Tengeru leaves inoculated with isolates from Kafue.

SAMPLES FROM KAFUE					
PLANT NUMBER	PLATE WITH PURE FUNGI COLONY	LESION SIZE DIAMETER IN MILIMETERS			MEAN
		LEAF 1	LEAF 2	LEAF 3	
1	KS1S1.1	11	13	8	10.7
2	KS1S1.2	12	6	4	7.3
3	KS1S1.3	14	8	11	11
4	KS1S1.4	5	4	6	5
5	KS1S1.5	5	8	10	7.7
6	KS2S2	16	18	4	12.7
7	KS2S2.1	6	2	12	6.7
8	KS2S2.2	3	3	4	3.3
9	KS2S2.3	10	11	8	9.7
10	KS2S2.4	7	6	2	5
11	KS2S2.5	2	2	3	2.3
12	KS2S2.6	4	2	4	3

13	KS2S2.7	6	8	6	6.7
14	KS3S1.2	4	5	2	3.7
15	KS3S1.3	2	6	4	4
16	KS4S1.2	8	4	5	5.7
17	KS4S1.3	3	10	4	5.7
18	KS45S1	3	6	4	4.3
19	KS46	2	4	2	2.7
20	KS47S1	5	4	5	4.7

This is the degree of virulence of the fungal and fungi-like pathogens collected from Kafue after inoculation on the commonly grown tomato variety Tengeru.

APPENDIX III: The isolates that were collected from northern part of Lusaka Zambia cultured and inoculated the leaves as well as pure colonies to determine virulence. The outcome was that plate 13MS1S3 (*Fusarium chlamydosporum* isolate) showed to have highest virulence with mean of 14.7. The least virulent was 13MS2S2 (*Chaetomium globosum* strain) with mean of 2.7 and the medium virulent 13MS4S3.2 (*Alternaria sp*) with mean of 8.3 as shown in the table.

Table AIX. Mean lesion sizes on Tengeru leaves inoculated with isolates from 13 miles north of Lusaka.

	SAMPLES FROM 13MILES				
PLANT NUMBER	PLATE WITH PURE FUNGI COLONY	LESION SIZE (DIAMETER) IN MILIMETERS			MEAN
		LEAF 1	LEAF 2	LEAF 3	
1	13MS1S1	7	7	2	5.3
2	13MS1S2	2	10	16	9.3
3	13MS1S2.1	15	20	2	12.3
4	13MS1S2.2	8	4	3	5
5	13MS1S3	13	15	16	14.7
6	13MS1S3.1	9	11	2	7.3
7	13MS1S3.2	11	16	4	10.3

8	13MS2S1.1	2	2	6	3.3
9	13MS2S1.2	19	6	5	10
10	13MS2S2	2	2	4	2.7
11	13MS2S2.1	7	10	2	6.3
12	13MS2S2.2	11	6	12	9.7
13	13MS2S2.3	9	4	3	5.3
14	13MS2S2.4	13	8	10	10.3
15	13MS3S1.3	13	15	13	13.7
16	13MS4S1.1	19	9	15	14.3
17	13S4S1.2	15	4	2	7
18	13MS4S3.1	6	11	3	6.7
19	13MS4S3.2	4	2	19	8.3
20	13MS5S1.1	4	5	3	4

The table shows the degree of virulence of the fungal and fungi-like pathogens (isolates) that were collected from northern part of Lusaka Zambia after inoculation on the commonly grown tomato variety Tengeru.

APPENDIX IV: The isolates sampled from the University of Zambia agriculture research fields, cultured to get pure cultures were used to inoculate the tomato leaves to determine their virulence. The following was observed; plate US5S1.3 (*Epicoccum nigrum*) had the highest mean value of 11.3mm implying that it was the most virulent among all samples collected from the University of Zambia Agriculture research field. The lowest mean value of 2.7mm signifying least virulent was plate US6S1.1 (*Lascodiplodia pseudolheubromae*) and the medium virulent plate US4S3.2 (*Fusarium sp*) mean value of 7.7mm was recorded.

Table AIV. Mean lesion sizes on Tengeru leaves inoculated with isolates from research fields at the UNZA School of Agricultural Sciences.

SAMPLES FROM THE UNIVERSITY OF ZAMBIA SCHOOL OF AGRICULTURE SCIENCES RESEARCH FIELDS					
PLANT NUMBER	PLATE WITH PURE FUNGI COLONY	LESION SIZE DIAMETER IN MILIMETERS			MEAN
		LEAF 1	LEAF 2	LEAF 3	
1	US1S1	10	6	10	8.7
2	US1S1.1	10	4	2	5.3
3	US1S1.2	2	5	9	5.3
4	US3S1.1	5	4	4	4.3
5	US4S1	2	10	3	5
6	US4S1.1	2	8	2	4
7	US4S1.2	5	3	5	4.3
8	US4S2.1	7	3	1	3.7
9	US4S3	6	4	7	5.7
10	US4S3.1	6	6	2	4.7
11	US4S3.2	8	2	13	7.7
12	US5S1.1	9	8	3	6.7
13	US5S1.2	19	2	4	8.3
14	US5S1.3	6	14	14	11.3
15	US6S1	3	7	5	5
16	US6S1.1	3	2	3	2.7
17	US6S1.2	8	2	8	6
18	US7S1	12	4	3	6.3
19	US7S1.1	6	2	15	7.7
20	US7S1.2	6	2	8	5.3

The table shows the degree of virulence of the fungal and fungi-like pathogens (isolates) that were collected from the University of Zambia agriculture research fields on commonly locally grown tomato variety Tengeru by small, medium and large scale farmers.