CHARACTERISATION AND AGGRESSIVENESS OF TOMATO EARLY BLIGHT FUNGUS (*ALTERNARIA SOLANI*) IN LIMPOPO PROVINCE

BY

GIFT HLAGEDI MPHAHLELE

MINI-DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE MASTER OF SCIENCE IN AGRICULTURE (AGRONOMY)

IN

DEPARTMENT OF PLANT PRODUCTION, SOIL SCIENCE AND AGRICULTURAL ENGINEERING,

IN THE

FACULTY OF SCIENCE AND AGRICULTURE

(SCHOOL OF AGRICULTURE AND ENVIRONMENTAL SCIENCES)

AT THE

UNIVERSITY OF LIMPOPO, SOUTH AFRICA

SUPERVISOR: DR M.A. KENA (UL)

CO-SUPERVISOR: DR A. MANYEVERE (UFH)

2017

TABLE OF CONTENTS

CHAPTER 4

THE EFFICACY OF VARIOUS FUNGICIDES AGAINST *ALTERNARIA SOLANI* ISOLATES *IN-VITRO*

CHAPTER 5

AGGRESSIVENESS OF *ALTERNARIA* SOLANI LIMPOPO ISOLATES ON TWO SELECTED CULTIVARS OF TOMATO

DECLARATION

I declare that this mini-dissertation is my own work and that all the sources that have been used or quoted have been cited and acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other institution.

_____ _____

Student's signature: GH MPHAHLELE Date

_____ _____

Supervisor's signature: DR M.A. KENA Date

_____ _____

Co-Supervisor's signature: DR A. MANYEVERE Date

DEDICATION

This research is dedicated to my beloved family, especially my late father Simon Kganki Mphahlele and my mother Francinah Raisibe Mphahlele (Boreadi) who always supported, motivated and guided me throughout my studies and my life.

ACKNOWLEDGEMENTS

Firstly and foremost, I would like to thank the Almighty God for giving me life and strength through my studies and my research work, without Him all of this would not be possible.

I would like to express my genuine appreciation to my supervisor Dr M.A. Kena and co-supervisor Dr A. Manyevere for their support, encouragement and assistance throughout the implementation of this research work. Through their comments and strategical thoughts this research work was a success.

I also would like to express my gratitude to National Research Foundation (NRF) for financial support.

To my beloved family, especially my mother Mrs Francinah Raisibe Mphahlele, I gratefully dedicate this work to her and also, I would like to thank her for giving me the courage to study and supporting me in tough and good periods.

To my friends and colleagues, Miss Lillian Mailula, Dr Z.P. Dube, Mr Edward Legong and Miss Mapula Hlokwe, I would like to express my thanks for their support and assistance through my research work. I pray that may God protect you as He provides all your heart's desirer's and may your life be filled with success.

There is no doubt that without the support and assistance of these people I would not have completed my research project, God bless them all.

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Among the fungal diseases infecting tomato crop, early blight caused by *Alternaria solani* (Ellis & G. Martin) is one of the most destructive fungal foliar diseases. The aim of this current study was to document the prevailing pathogenic diversity of *A. solani* populations in Limpopo based on morphological characteristics, fungicide sensitivity, and aggressiveness of *A. solani* isolates. The tested isolates were collected from four different areas across different tomato production sites in the Limpopo Province and pathogen isolations were carried-out under laboratory conditions. The the morphological variation of different *A. solani* isolates was evaluated. The results obtained in this study show that *A. solani* isolates exhibit high variations in mycelial pigmentation, number of septa, beak length and colony diameter. The current study also evaluated the sensitivity of *A. solani* isolates obtained from different areas in the Limpopo to commonly used fungicides. All the tested fungicides (chlorothalonil, copper oxychloride and mancozeb) reduced the mycelial growth of *A. solani* isolates, even at lower concentrations. However copper oxychloride fungicide provides better inhibition of mycelial growth as compared to other tested fungicides. Furthermore, the aggressiveness of the different isolates was investigated using Money-maker and Rodade tomato cultivars. The results obtained in our, study revealed that isolates from different areas differed in terms of their level of aggressiveness on both cultivars. However, all the tested isolates were aggressive in their ability to cause early blight in both cultivars. There was a difference on how both cultivars responded to isolates from different areas with the Money-maker being highly susceptible to all the tested isolates as than the Rodade

Key words: Aggressiveness*, Alternaria solani,* Fungicide sensitivity*,* isolates*,* Limpopo Province*,* Morphological characterization*,* susceptible tomato cultivars**.**

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Tomato (*Lycopersicon esculentum* Mill.) is an economically important crop in South Africa. The Limpopo Province is one of the main tomato growing areas in South Africa, producing approximately 76% of the total annual tonnage of tomatoes (DAFF, 2014). Despite its importance, tomato production is constrained by numerous abiotic and biotic factors. Fungal diseases are major biotic factors affecting tomatoes production resulting in major crop yield losses (Nikam *et al*., 2015). Among the fungal diseases infecting the tomato crop, early blight caused by *Alternaria solani* (Ellis & G. Martin) is one of the most destructive fungal foliar diseases worldwide (Shahbaz *et al*., 2011; Yazici *et al*., 2011). The pathogen attacks the plant and causes damage to all parts of the plant resulting in diseases such as leaf blight; stem collar rot and fruit lesions (Nashwa and Abo-Elyousr, 2012; Abdalla *et al.,* 2014).

Morphological characters are important tools in the identification and classification of fungal pathogens. The Identification of the early blight pathogen is generally based on conidial morphology (Loganathan *et al*., 2016). In South Africa, populations of *A. solani* have been shown to have large morphological variation in agricultural fields (Van der Waals *et al*., 2004). This variation is mainly due to changing climatic conditions, continued application of synthetic fungicides and natural mutation (Van der Waals *et al*., 2004; Chaerani and Voorrips, 2006).

The control of tomato early blight disease is based mainly on the application of agricultural synthetic fungicides (Abdalla *et al.,* 2014; Yazici *et al*., 2011). However due to the ability of *A. solani* to maintain high morphological and pathogenic variability, high incidences of fungicides resistance has been reported (Van der Waals *et al*., 2004; Rosenzweig *et al.*, 2008). In Limpopo Province there is currently lack of information on the current morphological diversity status of *A. solani* pathogen. Therefore understanding the existing morphological variation within *A. solani* population occurring in the Limpopo Province is vital for planning a successful disease management strategy.

Therefore, the aim of this study was to document the prevailing pathogenic diversity of *A. solani* populations in Limpopo based on morphological characteristics, fungicide sensitivity, and aggressiveness of *A. solani* isolates, collected from different tomato production sites in the Province.

1.2 Problem statement

Early blight is one of the most destructive tomato diseases, which causes severe defoliation and yield reduction under favourable conditions worldwide (Abada *et al*., 2008). In South Africa, the disease has been reported in all areas where tomatoes and potatoes are produced (Van der Waals *et al*., 2004). Although various management strategies have been used against early blight, fungicide application has been the most commonly used control measure (Holm *et al.,* 2003; Yazici *et al*., 2011). However, due to the ability of *A. solani* to maintain high morphological and pathogenic variability, the fungicide-resistant strains are developed, rendering fungicide application to be ineffective (Van der Waals *et al*., 2004).

Despite the fact that tomato production constitutes the highest source of income for both large and smallholder farmers in Limpopo, information on the morphological variation and the aggressiveness status of *A. solani* in the Province is limited.

1.3 Rationale

In South Africa, populations of *A. solani* have been shown to have large morphological variation both within and among fields (Van der Waals *et al*., 2004). This variation is mainly due to changing climatic conditions, continued application of synthetic fungicides and natural mutation (Van der Waals *et al*., 2004; Chaerani and Voorrips, 2006). In various areas of the Limpopo Province, there has been an increase in the incidences of early blight outbreaks despite the increase in fungicide application (Van der Waals *et al*., 2004). This can be attributed to the continued application of fungicides belonging to the same group and possible changes in climatic conditions which result in the development of isolates that are fungicide-resistant (Rosenzweig *et al.*, 2008; Abdalla *et al.,* 2014).

Pathogenic variation and fungicide resistance have been reported to be the major causes of loss in fungicide effectiveness against different pathogens (Yazici *et al*., 2011; Marak *et al.,* 2014). There is currently no documentation on the morphological variation, fungicide sensitivity and aggressiveness of *A. solani* in the Limpopo Province. The availability of such information is crucial in the development of appropriate management strategies. Results obtained in this study are expected to provide a foundation for the development of appropriate early blight management strategies in the Province.

1.4 Aim and Objectives of the study

1.4.1 Aim of the study

The aim of the study was to document the population diversity of *A. solani* isolates collected from different localities in the Limpopo Province using morphological characteristics, fungicide sensitivity and pathogen aggressiveness on commonly cultivated tomato varieties in the Province.

1.4.2 Objectives of the study

The objectives of the study were to:

I. Determine the morphological variation of different *A. solani* isolates in the Limpopo Province.

II. Evaluate the efficacy of various fungicides used against *A. solani* isolates *in vitro*.

III. Determine the pathogenicity of *A. solani* isolates on selected tomato cultivars.

1.5 Hypotheses

I. There is morphological variation among populations of the *A. solani* fungus affecting tomato plants in Limpopo Province.

II. *Alternaria solani* populations in Limpopo Province are resistant to commonly used fungicides.

III. Commonly grown tomato cultivars in Limpopo Province are tolerant to *A. solani* damage.

References

ABADA, K.A., MOSTAFA, S.H. and R. MERVAT. 2008. Effect of some chemical salts on suppressing the infection by early blight disease of tomato. *Egyptian Journal of Applied Science* 23:47-58.

ABDALLA, S.A., ALGAM, S.A.A., IBRAHIM, E.A. and A.M. EL NAIM. 2014. *In-vitro* screening of *bacillus* isolates for biological control of early blight disease of tomato in Shambat soil. *World Journal of Agricultural Research* 2:47-50.

CHAERANI, R. and R.E VOORRIPS. 2006. Tomato early blight (*Alternaria solani*): the pathogen, genetics, and breeding for resistance. *Journal of General Plant Pathology* 72:335-347.

DEPARTMENT OF AGRICULTURE FORESTRY AND FISHERIES (DAFF). 2014. Production guidelines for tomato. *Directorate Plant Production* 1-16.

HOLM, A.L., RIVERA, V.V., SECOR, G.A. and N.C. GUDMESTAD. 2003. Temporal sensitivity of *Alternaria solani* to foliar fungicides. *American Journal of Potato Research* 80:33-40.

LOGANATHAN, M., VENKATARAVANAPPA, V., SAHA, S., RAI, A.B., TRIPATHI, S., RAI, R.K., PANDEY, A.K. and P. CHOWDAPPA. 2016. Morphological, pathogenic and molecular characterisations of *Alternaria* species causing early blight of tomato in Northern India*. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* 86:325-330.

MARAK, T.R., AMBESH, B.S. and S. DAS. 2014. Cultural, morphological and biochemical variations of *Alternaria* solani causing diseases on *solanaceous* crops. *Journal of Plant Pathology* 9:1295-1300.

NASHWA, S.M.A. and K.A.M. ABO-ELYOUSR. 2012. Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. *Plant Protection Science* 48:74-79.

NIKAM, P.S., SURYAWANSHI, A.P. and A.A. CHAVAN. 2015. Pathogenic, cultural, morphological and molecular variability among eight isolates of *Alternaria* causing early blight of tomato. *African Journal of Biotechnology* 14:872-877.

ROSENZWEIG, N., ATALLAH, Z.K., OLAYA, G. and W.R. STEVENSON. 2008. Evaluation of QoI fungicide application strategies for managing fungicide resistance and potato early blight epidemics in Wisconsin. *Plant Disease* 92:561-568.

SHAHBAZI, H., AMINIAN, H., SAHEBANI, N. and M.R. LAK. 2011. Resistance evaluation of potato varieties in comparison with fungal isolates the cause of early blight of *Alternaria solani* and the analysis of total phenol contents in resistant and susceptible varieties. *Seed and Plant Improvement Journal* 27:1-14.

VAN DER WAALS, J.E., KORSTEN, L. and B. SLIPPERS. 2004. Genetic diversity among *Alternaria solani* isolates from potatoes in South Africa. *Plant Disease* 88:959-964.

YAZICI, S., YANAR, Y. and I. KARAMAN. 2011. Evaluation of bacteria for biological control of early blight disease of tomato. *African Journal of Biotechnology* 10: 1573-1577.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In South Africa, tomatoes contribute about 24% of the total vegetable production (DAFF, 2014). Limpopo Province is the largest tomato growing area in South Africa, producing approximately 76% of the total annual tonnage of tomatoes (DAFF, 2014). Despite its importance, tomato yields in South Africa are negatively affected by various factors including, favourable climatic conditions for pathogen survival, poor management practices, cultivar susceptibility, pests and diseases (Van der Waals *et al*., 2004; Rosenzweig *et al.*, 2008; Shahbaz *et al*., 2010). Fungal diseases, particularly early blight are of the most destructive diseases causing great yield loss wherever tomato is grown (Jansky *et al*., 2004; Tewari and Vishunavat, 2012; Nikam *et al*., 2015).

Early blight is more severe during the season with abundant moisture due to frequent rains, followed by warm and dry weather because such conditions encourage conidia germination and accelerate plant infection by the pathogen (Momel and Pemezny, 2006; Ganie *et al*., 2013; Chohan *et al.,* 2015). Poor management practices such as lack of nutrition and water, promote stressful conditions to the plant causing the plant to become more susceptible to pest injury and also contribute to early blight disease infection (Gudmestad *et al*., 2013). The disease is more severe to early maturing cultivars and less severe to medium or late maturing ones (Van der Waals *et al*., 2001).

2.2 Early blight disease

2.2.1 Biology of the causal pathogen, *Alternaria solani*

Alternaria solani is an imperfect fungus that reproduces asexually via multi-cellular conidia with an unknown sexual cycle (Van der Waals *et al.,* 2001; Agrios, 2005; Rogers, 2007). The fungi *A. solani* are classified under *Eukaryota* domain, Kingdom *Fungi,* phylum *Deuteromycota* (formerly) or *Ascomycota* (present), class *Hyphomycetes* and order *Hyphales* (Van der Waals *et al*., 2001; Simmons, 2007).

The conidia of *A. solani* are basically ovoid, but long, narrow whereas broad forms also exist (Simmons, 2000). Normally, as reports indicate, the *A. solani* conidia with long beaks are relatively long-ovoid or ellipsoid, whereas the conidia with 2-3 beaks are short (Simmons, 2000; Simmons, 2007). The conidia are usually muriformed, curved, pear shaped and multicellular with both transverse and longitudinal cross walls (Agrios, 2005; Alhussaen, 2012). The *A. solani* conidia have between 1-5 longitudinal and 5-11 transverse septa. The conidiophores which the conidia arise from are characterised by dark-brown colour, thick-walled, straight to slightly bent, mostly solitary, rarely in groups of 2-3, scarred rounded and slightly geniculate at apex, broader near the base, 2-4 septate, slightly constricted, emerge out through the epidermis or the stomata and measure 37.8 - 67.2 × 5.2 - 6.3µm (Van der Waals *et al*., 2001; Simmons, 2007; Alhussaen, 2012).

Based on morphological characteristics and phylogenetic analysis, *A. solani* is included in the porri-species group with medium to large conidia and long beaks (Woudenberg *et al.*, 2013). Although many *Alternaria* spp, including *A. solani,* lack a sexual state, genetic instability and variability is usually high resulting in changes in

fungal morphology and sensitivity to fungicides (Alhussaen, 2012; Tymon *et al*., 2016).

2.2.2 Factors affecting pathogen growth and disease development

Host susceptibility and favourable climate in the Limpopo Province create conditions conducive for the infection of tomato plants by *A. solani* causing serious yield losses (Van der Waals, 2002). Spore germination and infection of plant tissues is facilitated by climatic conditions such as abundant moisture followed by warm and dry weather (Chaerani and Voorrips, 2006; Tsedaley, 2014). *The A. solani* pathogen applies various mechanisms to survive harsh environmental conditions and can overwinter as conidia in plant debris, infected fruits, seed and other host plants of the same family that have decayed in the soil (Van der Waals, 2002). Tsedaley (2014) indicated that on uncultivated soil the spores of the pathogen remain infective in debris for 5 to 8 months. The spores of this pathogen are dispersed by splashing rain, irrigation water, insects and moving machinery to the new crop in the field (Chaerani *et al*., 2007). The conidia of the pathogen are produced in late spring and dispersed to the lower leaves of the plant where they germinate and penetrate the epidermis directly, through stomata or wounds (Tymon *et al*., 2016). The chlamydospores of *A. solani* act as a source of overwintering inoculum, allowing the pathogen to survive cold temperatures within the soil (Tsedaley, 2014). The dark pigmentation of the mycelium act as a protective agent against adverse environmental conditions, to which then extends the survival time of the pathogen in the soil for several years (Agrios, 2005).

2.3 Morphological variability of *A. solani* and its effect on disease severity

Examining the morphological variability of pathogens within their populations helps in the development of breeding programmes and strategic usage of fungicides (Bessadat *et al*., 2014). In South Africa, populations of *A. solani* have been shown to have large morphological variation in agricultural fields (Van der Waals *et al*., 2004).

Morphological classification of *Alternaria* species is largely based on the assessment of characteristics such as conidia dimension, colour and septa (longitudinal and transverse), wall ornamentation, beak type and size; sporulation patterns and conidiophore type, size, and septa (Simmons, 2007; Nikam *et al*., 2015). Nikam *et al*. (2015) investigated the morphological variability of eight *A. solani* isolates on potato dextrose agar (PDA) media and the variation of the isolates was noted on colony growth, colony diameter, mycelial colour, colony texture, septation, pigmentation and conidia size on medium. Kumar *et al*. (2008) also reported similar variation in conidial morphology of the *A. solani* isolates.

Varying factors including environmental conditions and farming practices such as continuous application of synthetic fungicides, cropping system, planting season and tomato cultivar planted induce the change in the genetic make-up of the pathogen allowing the evolution of new fungal strains within the population with varying morphological characteristics (Van der Waals *et al*., 2004; Chaerani *et al*., 2007; Pachori *et al*., 2016). These affect pathogen reproduction and host-pathogen interaction (Chaerani *et al*., 2007). The newly developed fungal strains are mostly resistant to the existing recommended fungicides making the disease to be more severe in the tomato growing fields (Van der Waals *et al*., 2004; Chaerani and Voorrips, 2006).

2.4 Genetic variability among *A. solani* isolates

Most scholarly works on *A. solani* population variability have focused on genetic characterisation with less attention on morphological changes (Van der Waals *et al*., 2004; Lourenco *et al*., 2011; Leiminger *et al*., 2014). Molecular variation assessment studies on *A. solani* revealed that populations of *A. solani* exhibit large variations both within and among fields, which is unusual for an asexually reproducing fungus (Van der Waals *et al*., 2004; Chaerani and Voorrips, 2006; Odilbekov, 2015). A number of factors such as changing climatic conditions, natural mutation and continued application of synthetic fungicides have been found to induce the genetic variations in *A. solani* (Chaerani *et al*., 2007). However, as reported by Chaerani and Voorrips (2006), high levels of genetic variation in *A. solani* populations might be due to heterokaryosis, which is the occurrence of genetically different nuclei in the same cells as a result of hyphal anastomosis. Furthermore, Okori (2004) emphasised that the genetic variability within the pathogen population is due to the influence of evolutionary forces, mutation, genetic drift, reproduction and mating system, gene flow and natural selection.

This genetic variability usually allows the pathogen to react, adapt and survive in the changing harsh environmental conditions (Calis and Topkaya, 2011). Weber and Halterman (2012), illustrated that the polymorphic life cycle of the *A. solani* pathogen results in the production of many spores at various stages throughout the growing season which then allow the genetic mutation and recombination opportunities that could lead to a relatively high level of variation. Shahbazi *et al*. (2010) used the vegetative compatibility analysis method and reported the genetic variation among *A. solani* isolates from different regions.

Many studies also reported the variation in the genetic make-up of the *A. solani* isolates (Shahbazi *et al*., 2010; Weber and Halterman, 2012; Nikam *et al*., 2015). Loganathan *et al*. (2016) investigated molecular variation of 17 *A. solani* isolates collected from different geographical locations using Polymerase Chain Reaction (PCR) universal primer pairs ITS1/ITS4 and reported the genetic variation among all the tested isolates. According to Alajo (2009) monitoring the genetic variation provides information about the existing structure of pathogen populations that is relevant to breeding programmes for development and deployment of host resistance.

2.5 Early blight management

2.5.1 Fungicide application

In order to suppress early blight disease and to prevent the losses it causes, tomato fields are sprayed with recommended fungicides (DAFF, 2014). The most common fungicides used to control early blight contain an active ingredients such as captafol, mancozeb, chlorothalonil, benomyl, carbendazim, copper oxychloride and maneb (Pasche and Gudmestad, 2008; Horsfield *et al.*, 2010). For the control of this disease the tomato fields are mostly sprayed four to six weeks after planting at repeated application intervals of seven to ten days to prevent early blight damage (Kemmitt, 2002; Pasche and Gudmestad, 2008; Horsfield *et al.*, 2010). Gondal *et al*. (2012) evaluated the sensitivity of various fungicides on *A. solani* and revealed that copper oxychloride and mancozeb were the most effective fungicides in inhibiting the growth of *A. solani.*

In the Limpopo Province, the use of these fungicides to manage early blight diseases have been reported (Van der Waals *et al*., 2005). However, the sensitivity of these fungicides on *A. solani* isolates is not documented. Other reports have indicated that another group of fungicides that has been used in the management of tomato early blight includes Quinones outside Inhibitors (QoIs) (Van der Waals *et al*., 2005; Tsedaley, 2014). Bartlett *et al.* (2002) and Kemmitt, (2002) found out that due to broad-spectrum activity and low use rates, this group of fungicides is very effective in controlling the early blight disease and decreases the chance of mutation hence reducing fungicide resistance.

2.5.2 Effect of fungicides on *A. solani* genetic variations

The genetic variation within populations of the *A. solani* pathogen gives an indication of disease evolution (Leiminger *et al.*, 2014). Kirk *et al*. (2005) and Gondal *et al*. (2012) reported that fungicides inhibit the spore germination, penetration and growth. So far, conclusive evidence on the effect of fungicides on *A. solani* genetic variation in the Limpopo Province is lacking. However, Rosenzweig *et al.* (2008) and Pasche *et al.* (2005) indicated that the continued application of synthetic fungicides in tomato fields have led to environmental contamination and induced population genetic variations in *A. solani,* causing the development of new fungicide resistant strains in the target pathogen population. The newly developed fungal resistant strains increase the disease severity in the tomato growing fields (Leiminger *et al.*, 2014).

2.5.3 Tomato cultivars susceptibility

The use of tomato resistant cultivars is the most suitable technique to control early blight disease (Duarte *et al.*, 2014). However, as reported by Chaerani and Voorrips (2007), there are limited resistant sources available to produce strong resistant

plants to the fungal pathogen due to quantitative expression and polygenic inheritance of the resistance. The variation of the *A. solani* genetic structure population in Limpopo Province is currently unknown. Consequently, it is difficult to reliably determine the degree of susceptibility on currently cultivars such as Money-maker and Rodade.

All breeding lines and released cultivars range in susceptibility from susceptible to moderately resistant (Peralta *et al*., 2005). Tomato breeding lines exhibiting resistance to early blight have been released to farmers, but most are low-yielding and late-maturing genotypes (Chaerani and Voorrips, 2006). In addition, Warton and Kirk, (2012) recommended that in locations where early blight is more severe, farmers should plant late maturing cultivars that are less susceptible to attack as compared to early maturing cultivars. In order to confirm the aggressiveness and virulence of *A. solani*, Rahmatzai *et al.* (2016) inoculated different isolates collected from different regions on the Super Star cultivar under field conditions. The study revealed that, after two weeks from the inoculation of tomato seedlings, typical symptoms of early blight appeared on the leaves, which were similar to those observed on naturally infected tomato plants. The susceptibility of tomato cultivars to *A. solani* isolates was reported on Pusa ruby, Saint Pierre, Cherry Rio Grande, Sahal, Reograndi, Salar, Roma, Nagina and Packit cultivars (Bessadat *et al*., 2014; Chohan *et al.,* 2015; Nikam *et al*., 2015). Similar findings were reported by Castro *et al*. (2000) who demonstrated susceptibility of 14 tomato genotypes to *A. solani* isolates.

References

AGRIOS, G.N. 2005. *Plant Pathology*. 5th edition. Alsevier Academic Press, New York.

ALAJO, A. 2009. Distribution and characterisation of sweet potato *Alternaria* blight isolates in Uganda. M.Sc. Thesis submitted to the Makerere University, Uganda.

ALHUSSAEN, K. M. 2012. Morphological and physiological characterisation of *Alternaria solani* isolated from tomato in Jordhan valley. *Research Journal of Biological Sciences* 7:316-319.

BARTLETT, D.W., CLOUGH, J.M., GODWIN, J.R., HALL, A.A., HAMER, M. and B. PARRDOBRZANSKI. 2002. The strobilurin fungicides. *Pest Management Science* 58:649-662.

BESSADAT, N., BENICHOU, S., KIHAL, M. and D.E. HENNI. 2014. Aggressiveness and morphological variability of small spore *Alternaria species* isolated from Algeria. *Journal of Experimental Biology and Agricultural Sciences* 22:2320 8694.

CALIS, O and S. TOPKAYA. 2011. Genetic analysis of resistance to early blight disease in tomato. *African Journal of Biotechnology* 10:1871-1877.

CASTRO, M.E.A., ZAMBALIM, L., CHAVES, G.M., CRUZ, C.D. AND K. MATSUOKA. 2000. Pathogenic variability of *Alternaria solani*, the causal agent of tomato early blight. *International Journal of Phytopathology* 24:24-28.

CHAERANI, R. and R.E VOORRIPS. 2006. Tomato early blight (*Alternaria solani*): the pathogen, genetics and breeding for resistance. *Journal of General Plant Pathology* 72:335-347.

CHAERANI, R., GROENWOLD, R., STAM, P. and R.E. VOORRIPS. 2007. Assessment of early blight (*Alternaria solani*) resistance in tomato using a droplet inoculation method. J*ournal of General Plant Pathology* 73:96-103.

CHOHAN, S., PERVEEN, R., MEHMOOD, M.A., NAZ, S. and N. AKRAM. 2015. Morpho-physiological studies, management and screening of tomato germplasm against *Alternaria solani*, the causal agent of tomato early blight. *International Journal of Agriculture and Biology* 17:111-118.

DEPARTMENT OF AGRICULTURE FORESTRY AND FISHERIES (DAFF). 2014. Production guidelines for tomato. *Directorate Plant Production* 1-16.

DUARTE, H.S.S., ZAMBOLIM, L., RODRIGUES, F.A., PAUL, P.A., PADUA, J.G., RIBEIRO, J.I., JUNIOR, A.F.N. and A.W.C. ROSADO. 2014. Field resistance of potato cultivars to foliar early blight and its relationship with foliage maturity and tuber skin types. *Tropical Plant Pathology* 39:294-306.

GANIE, S.A., GHANI, M.Y., NISSAR, Q., JABEEN, N., ANJUM, Q., AHANGER, F.A. and A. AYAZ. 2013. Status and symptomatology of early blight (*Alternaria solani*) of potato (*Solanum tuberosum* L.) in Kashmir valley. *African Journal of Agricultural Research* 8:5104-5115.

GONDAL, A.S., IJAZ, M., RIAZ, K. and A.R .KHAN. 2012. Effect of different doses of fungicide (mancozeb) against *Alternaria* leaf blight of tomato in tunnel. *Journal of Plant Pathology and Microbiology* 3:125-128.

GUDMESTAD, N.C., ARABIAT, S., MILLER, J.S., and J.S. PASCHE. 2013. Prevalence and impact of SDHI fungicide resistance in *Alternaria solani*. *Plant Disease* 97:952-960.

HORSFIELD, A., WICKS, T., DAVIES, K., WILSON, D. and S. Paton. 2010. Effect of fungicide use strategies on the control of early blight (*Alternaria solani*) and potato yield. *Australasian Plant Pathology* 39:368-375.

JANSKY, S.H., ROUSE, D.I., and P.J. KAUTH. 2004. Inheritance of resistance to *Verticillium dahlia* in diploid interspecific potato hybrids. *Plant Disease* 88:1075-1078.

KEMMITT, G. 2002. Early Blight of potato and tomato. *The American Phytopathological Society*, St. Paul, MN, USA.

KIRK, W.W., ABU-EL SAMEN, F.M., MUHINYUZA, J.B., HAMMERSCHMIDT, R., DOUCHES, D.S., THILLC, C.A., GROZA, H. and A.L. THOMPSON. 2005. Evaluation of potato late blight management utilising host plant resistance and reduced rates and frequencies of fungicide applications. *Crop Protection* 24: 961-970.

KUMAR, V., HALDAR, S., PANDEY, K.K., SINGH, R.P., SINGH, A.K. and P.C. SINGH. 2008. Cultural, morphological, pathogenic and molecular variability tomato isolates of *Alternaria solani* in India. *World Journal of Microbiology and Biotechnology* 24:1003-1009.

LEIMINGER, J.H., ADOLF, B. and H. HAUSLADEN. 2014. Occurrence of the F129L mutation in *Alternaria solani* populations in Germany in response to QoI application, and its effect on sensitivity. *Plant Pathology* 63:640-650.

LOGANATHAN, M., VENKATARAVANAPPA, V., SAHA, S., RAI, A.B., TRIPATHI, S., RAI, R.K., PANDEY A.K. and P. CHOWDAPPA. 2016. Morphological, pathogenic and molecular characterisations of *Alternaria* Species causing early blight of tomato in Northern India. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* 86:325-330.

LOURENCO, V., RODRIGUES, T., CAMPOS, A.M.D., BRAGANCA, C.A.D., SCHEUERMANN, K.K., REIS, A., BROMMONSCHENKEL, S.H., MAFFIA, L.A. and E.S.G. MIZUBUTI. 2011. Genetic Structure of the Population of *Alternaria solani* in Brazil. *Journal of Phytopathology* 159:233-240.

MOMEL, T.M. and K.L. PEMEZNY. 2006. Florida Plant Disease Management Guide: Tomato. Florida Cooperation Extensive Service, Institute of Food and Agriculture Sciences, Gaineville.

NIKAM, P.S., SURYAWANSHI, A.P. and A.A. CHAVAN. 2015. Pathogenic, cultural, morphological and molecular variability among eight isolates of *Alternaria* causing early blight of tomato. *African Journal of Biotechnology* 14:872-877.

ODILBEKOV, F. 2015. Resistance to early blight in potato and genetic structure of the pathogen population in Southeast Sweden. PhD. Thesis submitted to the Swedish University of Agricultural Sciences.

OKORI, P., RUBAIHAYO, P.R., ADIPALA, H.H., FAHLESON, E. and C. DIXELIUS. 2004. Population studies of fungal plant pathogens: Perspectives for control with specific reference to grey leaf spot. *African Crop Science Journal* 12:327 342.

PACHORI, A., SHARMA, O., SASODE, R. and R.N. SHARMA. 2016. Collection of different isolates of *Alternaria solani* in Bhind, Morena and Gwalior districts of Madhya Pradesh. *International Journal of Advanced Research* 2:217-219.

PASCHE, J.S. and N.C. GUDMESTAD. 2008. Prevalence, competitive fitness and impact of the F129L mutation in *Alternaria solani* from the United States. *Crop Protection* 27:427-435.

PASCHE, J.S., PICHE, L.M. and N.C. GUDMESTAD. 2005. Effect of the F129L mutation in *Alternaria solani* on fungicides affecting mitochondrial respiration. *Plant Disease* 89:269-278.

PERALTA, E., KNAPP, S. and O.M. SPOONER. 2005. New species of wild tomato (Solanum section *Lycoperisicon*: *Solanaceae*) from Northern Peru. *Systematic Botany* 30:424-434.

RAHMATZAI, N., AHMED, A. Z., MOHAMED, H.M., AHMADY, A., HAZIM Z. and M A. A. MOUSA. 2016. Morphological, pathogenic, cultural and physiological variability of the isolates of *Alternaria solani* causing early blight of tomato. *International Journal of Advanced Research* 4:808-817.

ROGERS, P. M. 2007. Diversity and biology among isolates of *Alternaria dauci* collected from commercial carrot fields. PhD. Thesis Submitted to University of Wisconsin-Madison.

ROSENZWEIG, N., ATALLAH, Z.K., OLAYA, G., and W.R. STEVENSON. 2008. Evaluation of QoI fungicide application strategies for managing fungicide resistance and potato early blight epidemics in Wisconsin. *Plant Disease* 92:561-568.

SHAHBAZI, H., AMINIAN, H., SAHEBANI, N. and D. HALTERMAN. 2010. Biochemical evaluation of resistance responses of potato to different isolates of *Alternaria solani*. *Journal of Phytopathology* 100:454-459.

SIMMONS, E.G. 2000. *Alternaria* themes and variations. *Mycology taxonomy* 55:55 163.

SIMMONS, E.G. 2007. *Alternaria* identification manual. CBS Fungal Biodiversity Center, Netherlands.

TEWARI, R. and K. VISHUNAVAT. 2012. Management of early blight (*Alternaria solani*) in tomato by integration of fungicides and cultural practices. *International Journal of Plant Protection* 5:201-206.

TSEDALEY, B. 2014. Review on early blight (*Alternaria* spp.) of potato disease and its management options. *Journal of Biology, Agriculture and Healthcare* 4: 2224-3208.

TYMON, L. S., CUMMINGS, T. F. and D. A. JOHNSON. 2016. Pathogenicity and aggressiveness of three *Alternaria* species on potato foliage. *Journal of Phytopathology* 25:451-462.

VAN DER WALLS, J.E., KORSEN, L. and T.A.S. AVELING. 2001. A review of early blight of potato. *African Plant Protection* 70:91-102.

VAN DER WAALS, J.E. 2002. A review of early blight on potatoes. *University of Pretoria*.

VAN DER WAALS, J.E., KORSTEN, L. and B. SLIPPERS. 2004. Genetic diversity among *Alternaria solani* isolates from potatoes in South Africa. *Plant Disease* 88:959-964.

VAN DER WAALS, J.E., KORSTEN, L. and F.D.N. DENNER. 2005. Early blight in South Africa: knowledge, attitudes and control practice of potato growers. Journal of *Potato Research* 46:27-37.

WARTON, P. and W. KIRK. 2012. Early Blight. Potato Disease, *Michigan State University*.

WEBER, B. and D.A. HALTERMAN. 2012. Analysis of genetic and pathogenic variation of *Alternaria solani* from a potato production region. *European Journal of Plant Pathology* 134:847-858.

WOUDENBERG, J.H.C., TRUTER, M., GROENEWALD, J.Z. and P.W. CROUS 2014. Large-spored *Alternaria* pathogens in section *Porri* disentangled. *Journal of Mycology* 79:1-47.

CHAPTER 3

MORPHOLOGICAL CHARACTERISATION OF *ALTERNARIA SOLANI* ISOLATES FROM THE LIMPOPO PROVINCE

3.1 Introduction

Early blight caused by *Alternaria solani* is one of the most common and destructive tomato diseases in most tomato growing areas (Abada *et al*., 2008). The disease affects mainly tomatoes and potatoes and is distributed worldwide where both crops are planted (Abada *et al*., 2008; Kamble *et al*., 2009). The pathogen attacks the crop at all developmental stages and can significantly reduce the economic value of the crop. The use of symptoms and pathogen morphological characterisation is common and remains an important practice in the identification of the disease causal agent (Nikam *et al.*, 2015). However, morphological and pathogenic variability occurs frequently among isolates of many fungal pathogens including *A. solani* (Marak *et al.,* 2014).

In South Africa, populations of *A. solani* have been shown to have large morphological variations (Van der Waals *et al*., 2004). These variations have been attributed to changing climatic conditions, different cropping systems, continued application of synthetic fungicides and natural mutation which lead to genetic change (Van der Waals *et al*., 2004; Chaerani *et al*., 2007). These changes can affect cultivar resistance stability resulting in increased early blight infection and severity (Naik *et al*., 2010). Furthermore, these variations have a serious implication in the management of early blight as they can result in pathogen resistance to fungicides.

Therefore, understanding the morphological variations arising within the population of *A. solani* isolates is important in planning a successful disease management strategy (Bessadat *et al*., 2014). Although morphological variation has been previously evaluated across some of the areas in South Africa (Van der Waals *et al*., 2004), there is still little information about the morphological variation of *A. solani* in Limpopo Province. Therefore, the objective of this study was to determine the morphological variation among *A. solani* isolates causing early blight on tomatoes in the Limpopo Province, South Africa.

3.2 Materials and methods

3.2.1 Study location and experimental design

The study was conducted at the University of Limpopo plant pathology laboratory, at Turfloop campus, South Africa (23° 53′ 10″ S, 29° 43 ′05″ E). The experiment was arranged in complete randomised design (CRD) with four replications of each tested isolate per area.

3.2.2 Collection of diseased tomato leaf samples

Tomato leaves showing early blight symptoms (Figure 3.2) were collected during March 2016 from four tomato producing areas with different climatic conditions across Limpopo Province (Figure 3.1). Sampled areas were Blood-river, Mankweng, Tzaneen and Venda (Table 3.1). A total of 12 fields in these four areas were surveyed for early blight disease, with three fields per each area. The plant samples were collected from both smallholder and commercial farmers' fields across all the areas. Infected plant leaves were cut off from the point where the petiole is attached to the stem using a sharp sterilised blade.

Leaf samples were then placed into a brown paper bag placed in a cooler box and brought to the University of Limpopo plant pathology laboratory where they were kept at 4 °C before pathogen isolation and verification was done.

Table 3.1 Climatic condition of various areas across Limpopo Province where leaf samples were collected

Area	Geographical locations	Climatic conditions	
		Annual average rainfall (mm)	Average temperature (°C)
Blood-river	23°49'60" S , 29°25'60" E	495	21-22
Mankweng	23° 53′ 10″ S, 29° 43 ′05″ E	403	18.9-26.1
Tzaneen	24° 6' 0" S, 30° 23' 0" E	621	21.9-29.1
Venda	22° 58' 0" S, 30° 35' 0" E	752	22.9-30.3

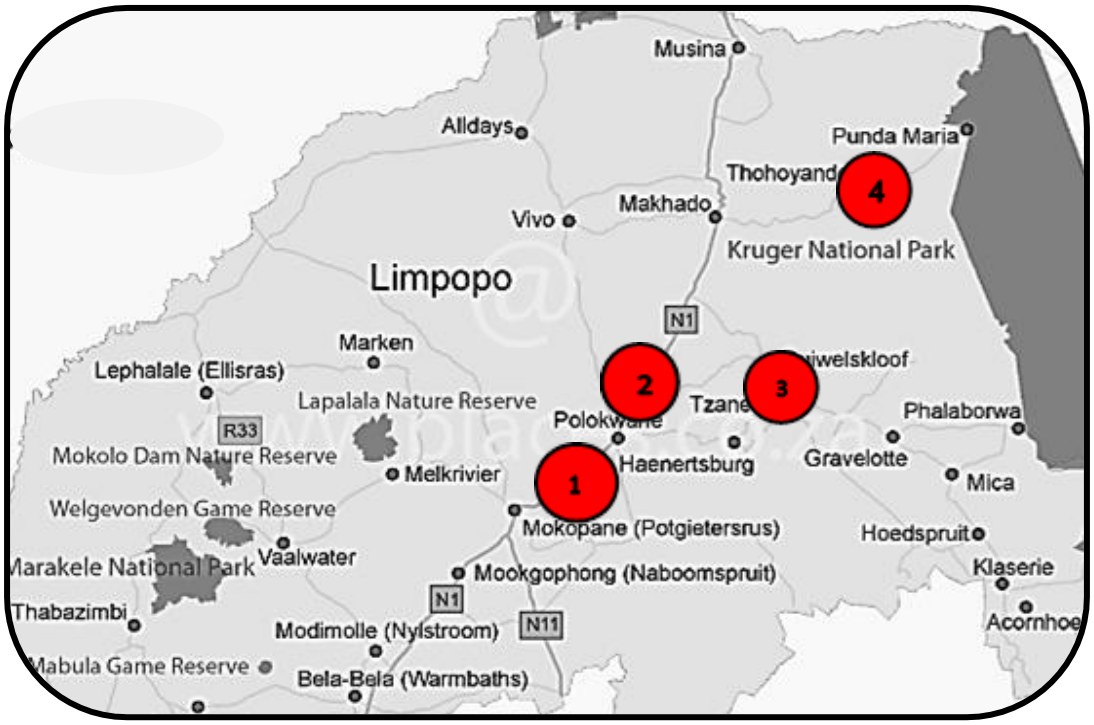


Figure 3.1 Four areas sampled for tomato early blight disease (Circles 1, 2, 3 and 4 represents: Blood-river, Mankweng, Tzaneen and Venda respectively)

Figure 3.2 *Alternaria solani* symptoms on a tomato leaf. Adopted from Johnson *et al*. (2015)

3.2.3 Pathogen isolation and identification

The infected leaves were cut into approximately 2 cm pieces, surface sterilised with 1% sodium hypochlorite solution for 2 minutes and rinsed three times with sterile distilled water. After rinsing, plant pieces were dried on filter paper and placed in 90cm petri dishes (four pieces per petri dish) containing Potato Dextrose Agar (PDA) medium. Petri plates were then incubated at 25 ± 2 °C for seven days. Seven days after incubation, a small piece of mycelia growing on infected leaves was transferred to fresh PDA plates using a flame sterilised inoculation needle. Inoculated plates were incubated as above for seven days to obtain a pure culture of *A. solani.*

3.2.4 Data collection

Seven days after incubation, cultures were studied for morphological characterisation. Measurements of colony diameter (mm) and visual observations on mycelial pigmentation were made on PDA medium. Colony diameter (mm) was measured using a clear transparent ruler (Sallam and Kamal, 2012). Variations on other morphological characteristics including conidial length, number of septa, presence or absence of horizontal and transverse septa and beak length were determined using a light microscope (10×20 Boeco microscope) at 60x magnification as described by, Simmons (2007); Alhussaen (2012) and Nikam *et al.* (2015).

3.2.5 Data analysis

Colony diameter data were subjected to analysis of variance using Statistix 10.0 software. Separation of means to determine differences in diameter growth on each isolate was carried out through Tukey's HSD at $P \leq 0.05$.

3.3 Results

3.3.1 Morphological characterisation *of A. solani* isolates from different areas

A total of 61 isolates were collected and identified from diseased tomato plants. Out of the 61 isolates only 34 were confirmed as *A. solani* based on their morphological characteristics. These represented 18 isolates from Blood-river, 14 from Mankweng, 15 from Tzaneen and 14 from Venda. Isolates confirmed as *A. solani* are presented in Table 3.2.

Table 3.2 Confirmed *A. solani* isolates collected from various areas in Limpopo Province

Areas	Isolate number
Blood-river	B1; B2; B5; B6; B7; B8; B9; B10; B13; B20
Mankweng	M1; M2; M4; M5; M8; M9; M10; M12
Tzaneen	T1; T4; T5; T8; T9; T10; T12; T13
Venda	V1; V2; V3; V7; V8; V9; V10; V12

Blood-river isolates

The morphological characteristics of *A. solani* isolates from Blood-river are presented in Table 3.3. *Alternaria solani* isolates from this area displayed three mycelia pigmentation which were mainly; greyish-brown, black and grey. The greyish-brown pigmentation was observed on isolates B2, B5 and B13 whereas isolates B1, B10 and B20 displayed black pigmentation. The remaining isolates from the same area namely B6, B7 and B9 displayed grey pigmented. Both horizontal and transverse septa were observed in all confirmed *A. solani* isolates. Maximum number of horizontal septa were recorded in isolate B1 (4-8) followed by B8 (4-7) whilst isolate B5 (2-3) had the least septation.

The number of vertical septa was higher in isolate B8 and B13 (1-4), whereas the least number of vertical septa was recorded in isolate B7 (0-2). Isolates also displayed variations in beak length ranging from short, medium and long. Four isolates, namely B1, B6, B8 and B20 had long beak, while three namely B5, B9 and B13 displayed short conidial beak. Medium beak lengths were witnessed in isolates B2 and B6.

The significant ($P \leq 0.05$) colony diameter growth was observed among isolates from this area. The colony diameter of all the tested isolates ranged from 68.75 to 76.75 mm. In all examined isolates, the highest colony diameter was recorded on isolate B10 (76.75 mm). The least colony growth was observed in isolates B1, B2, and B9 all with the growth diameter of 68.75 mm.

Table 3.3 Morphological characteristics of *Alternaria solani* isolates from Blood-river, seven days after incubation at 25 ± 2°C

Isolate	Mycelial pigmentation	Number of septa		Beak	Colony diameter [z]
		Horizontal	Transverse		
B1	Black	4-8	2-3	Long	68.75b
B2	Greyish-brown	2-4	1-2	Medium	68.75b
B5	Greyish-brown	2-3	1-3	Short	71.00ab
B6	Grey	4-6	1-3	Medium	69.75b
B7	Grey	2-4	0-2	Short	74.50ab
B8	Black	4-7	1-4	Long	71.75ab
B9	Grey	2-4	2-3	Long	68.75b
B10	Black	2-4	1-2	Short	76.75a
B13	Greyish-brown	3-5	1-4	Long	70.50ab
B20	Black	3-5	1-3	Long	70.25b

[z] Column means followed by the same letter are not significantly different at Tukey's HSD at P ≤ 0.05.

Mankweng isolates

The morphological characteristics of Mankweng isolates are presented in Table 3.4. The tested isolates from this area displayed greyish-brown and black mycelial pigmentation on PDA medium. The greyish-brown mycelial pigmentation was observed on M2 and M4 isolates only, while the rest of the isolates were black. Both horizontal and transverse septa were observed in all confirmed *A. solani* isolates. There was variation between isolates with regards to septation. The maximum number of horizontal septa were observed in isolate M4 (5-7) followed by M1 (4-7) with the least septation observed in isolate M2 (2-5) and M10 (3-5). The number of transverse septa was higher in the isolate M1, M9, M10 and M12 (3-4) whereas the least number of transverse septa was noticed in the isolate M8 (0-1). All the isolates possessed short and long beaked conidia. The conidia of isolates M5, M8, M9, M10 and M12 had short beaks, while M1, M2 and M4 had long beaks.

The colony diameter growth differed significantly ($P \leq 0.05$) across all the examined isolates. Colony diameter growth results revealed that isolate M1 had the higher mean colony diameter of 76.50 mm. The lowest mean colony diameter was observed in isolate M2 (62.50 mm) followed by isolate M10 with the diameter of 65.25 mm.

Table 3.4 Morphological characteristics of *Alternaria solani* isolates from Mankweng, seven days after incubation at 25 ± 2°C

Isolate	Mycelial pigmentation	Number of septa		Beak	Colony diameter (mm) [z]
		Horizontal	Transverse		
M1	Black	4-7	2-3	Long	76.50a
M2	Greyish brown	2-5	1-3	Long	65.25e
M4	Greyish brown	5-7	1-3	Long	67.50de
M5	Black	4-6	1-2	Short	72.50bc
M8	Black	3-7	0-1	Short	74.50ab
M9	Black	4-6	2-3	Short	71.25bc
M10	Black	3-5	2-3	Short	66.00de
M12	Black	3-6	2-3	Short	69.50cd

[z] Column means followed by the same letter are not significantly different according to Tukey's HSD at P ≤ 0.05.

Tzaneen isolates

The morphological characteristics of Tzaneen isolates are presented in Table 3.5. There was an observed variation in the pigmentation of *A. solani* isolates collected from this area. Isolates T1, T5 and T13 exhibited black pigmentation and greyish-brown mycelial pigmentation was observed on T8, T10 and T12 isolates. Two isolates namely T4 and T9 displayed grey pigmentation. The significant differences were observed for colony diameter among all the isolates ($P \leq 0.05$), with the highest colony diameter of 73.00 mm detected on T13 isolate followed by T4 (72.50 mm). The lowest colony growth was obtained on isolates T8 (65.75 mm) followed by isolate T12 (66.00 mm), Horizontal and transverse septation was observed in all *A. solani* isolates from Tzaneen. The highest number of horizontal septa was recorded in isolates T8 and T10 (3-7) followed by isolate T12 (3-6).

Table 3.5 Morphological characteristics of *Alternaria solani* isolates from Tzaneen, seven days after incubation at 25 ± 2°C

Isolate	Mycelial pigmentation	Number of septa		Beak	Colony diameter (mm) [z]
		Horizontal	Transverse		
T1	Black	2-3	0-2	Short	69.50ab
T4	Grey	3-5	2-3	Long	72.50a
T5	Black	2-4	1-3	Long	67.75ab
T8	Greyish brown	3-7	1-4	Long	65.75b
T9	Grey	2-4	1-3	Long	67.75ab
T10	Greyish brown	3-7	2-3	Short	71.50ab
T12	Greyish brown	3-6	2-4	Short	66.00b
T13	Black	3-5	1-4	Long	73.00a

[z] Column means followed by the same letter are not significantly different according to Tukey's HSD at P ≤ 0.05.

Venda isolates

Results of mycelial pigmentation, colony diameter, conidial length, presence or absence of both transverse and horizontal septa and beak length for *A. solani* isolates collected from Venda area are presented in Table 3.6. The results showed three mycelial pigmentation variations and these were mainly black (isolates V1, V7, V9 and V10), greyish brown (isolates V2, V8 and V11) and grey pigmentation in isolate V3. The growth rate of the isolates as determined by measuring colony diameter varied significantly ($P \leq 0.05$), with the highest colony diameter recorded on isolates V3 and V7 (71.75 mm), and followed by V8 and V10 isolates with 70.00 mm. The lowest colony diameter was observed on V1 and V11 isolates with the diameter of 67.25 and 66.25 mm respectively. The conidia in all isolates had both horizontal and transverse septa; however there was a variation in the number of septa for different isolates. For example, isolate V9 had a total of 3-6 horizontal septa and this number was different from 2-5 per conidia recorded in isolates V2, V10 and V8. The same variation was observed in the number of transverse septa in all isolates. The highest number of transverse septa was recorded on isolates V7 (2-4) with the lowest transverse septa recorded on isolate V10 (0-2). Beak length of the conidia varied from short (V1, V2, V3, V8 and V11) to long (isolates V7, V9 and V10).

Table 3.6 Morphological characteristics of *Alternaria solani* isolates from Venda, seven days after incubation at 25 ± 2°C

Isolate	Mycelial pigmentation	Number of septa		Beak	Colony diameter (mm) [z]
		Horizontal	Transverse		
V1	Black	3-5	2-3	Short	67.25cd
V2	Greyish brown	2-5	1-2	Short	69.50abc
V3	Grey	3-5	1-3	Short	71.75a
V7	Black	3-5	2-4	Long	71.75a
V8	Greyish brown	2-5	1-2	Short	70.00a
V9	Black	3-6	1-3	Long	68.25bcd
V10	Black	2-5	0-2	Long	70.00ab
V11	Greyish brown	3-5	2-3	Short	66.25d

[z] Column means followed by the same letter are not significantly different according to Tukey's HSD at P ≤ 0.05

3.4 Discussion

Pathogen biology, especially morphological characteristics is used to determine the changes in pathogen populations occurring in different areas (Pachori *et al*., 2016). In this study, variations in the morphology were used to determine the changes in *A. solani* populations in different areas of Limpopo with more emphasis placed on climatic differences and farming systems. Variations in the morphological appearance of *A. solani* isolates from different areas have been reported by different authors (Chaerani *et al*., 2007; Kumar *et al*., 2008; Nikam *et al*., 2015). A number of factors including climatic conditions (Pachori *et al*., 2016), farming practices (Marak *et al.,* 2014) and continuous application of synthetic fungicides (Rosenzweig *et al*., 2008) have been shown to induce morphological changes in *A. solani*.

In this study collection area did not have any effect in isolate pigmentation and most collected isolates displayed greyish-brown, grey and black pigmentation. These results are in agreement with those of Kumar *et al*. (2008), Singh *et al*. (2014) and Nikam *et al*. (2015) who reported that the area of isolate collection does not affect mycelial pigmentation. In their studies, colony pigmentation ranged from grey, greyish brown and black. These are variations used in the identification of *A. solani* as described by Simmons, (2007). However contrary to the finding of the current study, Pachori *et al*. (2016) reported that *A. solani* isolates can also exhibit yellow, greenish black and reddish black pigmentation on PDA. In the same study variations in pigmentation were found to be influenced by the area of plant sample collection.

Colony diameter is another characteristic applied in determining *A. solani* population variability (Nikam *et al*., 2015; Pachori *et al*., 2016). This is mainly due to adaptation to temperature changes resulting in the ability to grow faster or slower when exposed to standard temperature ranges (Sodlauskiene *et al*., 2003; Singh *et al*., 2014). *Alternaria solani* isolates in the present study showed variation in colony growth when cultured at ± 25°C. These variations were based on the area of collection. For example, representatives of fast growing isolates were mainly found in Blood-river and Mankweng areas, whilst the slow growing isolates were those collected from Tzaneen and Venda. These variations can be due to climatic adaptation of *A. solani*. Climatic variability has been shown to play a role in the growth patterns of *A. solani* resulting in reduced or increased growth rate of the pathogen. These can also influence the genetic makeup of the isolates which further influence the changes in pathogen morphological structure (Singh *et al*., 2014; Nikam *et al*., 2015; Pachori *et al*., 2016).

Marak *et al*. (2014) reported that *A. solani* isolates collected from different agro-climatic areas produce different colony diameter and variations increased with an increase in incubation period. Differences in agro-climates conditions such as humidity and temperatures have also been shown to affect conidia development and septation (Naik *et al*., 2010; Bessadat *et al*., 2014). In the current study, *A. solani* isolates from the four areas exhibited variations in beak length and number of longitudinal and transverse septa. Isolates also displayed short, medium and long beaks within the range of 2-8 horizontal septa and 0-4 transverse septa.

Bessadat *et al*. (2014) and Naik *et al.* (2010) reported that sampling areas especially with varying climatic conditions, can affect the appearance of the *A. solani* conidia. In their studies, the variations in beak length, the number of transverse and longitudinal septa was also observed. Variations in the total number of septa have also been related to the substrate where *A. solani* was isolated (Marak *et al*., 2014). *Alternaria solani* isolates from different solanaceous plants in India were found by Marak *et al*. (2014) to differ in horizontal (4-6) and transverse (1-2) septa.

In our study, morphological variation colony pigmentation, growth, beak length and conidial septation can be attributed to factors such as temperature, humidity and rainfall occurring in the sampled areas. Furthermore farming practices such as continuous application of synthetic fungicides and monoculture also contribute to the morphological changes and virulence of the pathogen. Many scholarly studies indicated that all these factors affects the genetic structure (DNA) of the pathogen which then affects the morphological traits of *A. solani* isolates (Chaerani *et al*., 2007; Kumar *et al*., 2008; Shahbazi *et al*., 2010). The results on morphological characterisation are presented in the current study, however for the development of appropriate management strategies further research work should focus on the genetic make-up of the isolates.

References

ABADA, K.A., MOSTAFA, S.H. and R. MERVAT. 2008. Effect of some chemical salts on suppressing the infection by early blight disease of tomato. *Egyptian Journal of Applied Science* 23:47-58.

ALHUSSAEN, K. M. 2012. Morphological and physiological characterisation of *Alternaria solani* isolated from tomato in Jordhan valley. *Research Journal of Biological Sciences* 7:316-319.

BESSADAT, N., BENICHOU, S., KIHAL, M. and D.E. HENNI. 2014. Aggressiveness and morphological variability of small spore *Alternaria species* isolated from Algeria. *Journal of Experimental Biology and Agricultural Sciences* 2:2320 8694.

CHAERANI, R., GROENWOLD, R., STAM, P. and R.E. VOORRIPS. 2007. Assessment of early blight (*Alternaria solani*) resistance in tomato using a droplet inoculation method. *Journal of General Plant Pathology* 73:96-103.

JOHNSON, A., GRABOWSKI, M. and A. ORSHINSKY. 2015. Early blight of tomato. University of Minnesota Extension.

KAMBLE, S.B., SANKESHWARI, S.B. and J.S. AREKAR. 2009. Survey on early blight of tomato caused by *Alternaria solani*. *International Journal of Agricultural Sciences* 5:317-319.

KUMAR, V., HALDAR, S., PANDEY, K.K., SINGH, R.P., SINGH, A.K. and P.C. SINGH. 2008. Cultural, morphological, pathogenic and molecular variability amongst tomato isolates of *Alternaria solani* in India. *Journal of Microbiology and Biotechnology* 24:1003-1009.

MARAK, T.R., AMBESH, B.S. and S. DAS. 2014. Cultural, morphological and biochemical variations of *Alternaria solani* causing diseases on *solanaceous* crops. *Journal of Crop Science* 9:1295-1300.

NAIK, M.K., PRASAD, Y., BHAT, K.V. and G.S. DEVIKA RANI. 2010. Morphological, physiological, pathogenic and molecular variability among isolates of *Alternaria solani* from tomato. *Indian Phytopathology* 63:168-173.

NIKAM, P.S., SURYAWANSHI, A.P. and A.A. CHAVAN. 2015. Pathogenic, cultural, morphological and molecular variability among eight isolates of *Alternaria* causing early blight of tomato. *African Journal of Biotechnology* 14:872-877.

PACHORI, A., SHARMA, O., SASODE, R, and R.N. SHARMA. 2016. Collection of different isolates of *Alternaria solani* in Bhind, Morena and Gwalior districts of Madhya Pradesh. *International Journal of Applied Research* 2:217-219.

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

SHAHBAZI, H., AMINIAN, H., SAHEBANI, N. and D.A. HALTERMAN. 2010. Biochemical evaluation of resistance responses of potato to different isolates of *Alternaria solani*. *Journal of Phytopathology* 100:454-459.

SIMMONS, E.G. 2007. *Alternaria* identification manual. CBS Fungal Biodiversity Center, Netherlands.

SINGH, A., SINGH, V. and S.M. YADAV. 2014. Cultural, morphological and pathogenic Variability of *Alternaria solani* causing early blight in tomato. *Plant Pathology Journal* 13:167-172.

SODLAUSKIENE, A., RASINSKIENE, A. and E. SURVILIENE. 2003. Influence of environmental conditions upon the development of *Alternaria* genus fungi *in vitro*. *Sodininkyste Darzininkyste* 22:160-166.

VAN DER WAALS, J.E., KORSTEN, L. and B. SLIPPERS. 2004. Genetic diversity among *Alternaria solani* isolates from potatoes in South Africa. *Plant Disease* 88:959-964.

CHAPTER 4

THE EFFICACY OF VARIOUS FUNGICIDES AGAINST *ALTERNARIA SOLANI* ISOLATES *IN-VITRO*

4.1 Introduction

Current strategies used to manage early blight disease in tomato fields are mainly through the application of protective foliar fungicides (Holm *et al.,* 2003; Van der Waals *et al*., 2005). In South Africa, the most commonly used fungicides in the management of early blight include chlorothalonil, maneb, copper oxychloride and mancozeb (Van der Waals *et al*., 2005).

However, the continued and indiscriminate application of fungicides especially in higher than the recommended dosages have led to environmental contamination and induced mutants of new *A. solani* population strains that are resistant to fungicides (Rosenzweig *et al.*, 2008). The development of fungicide resistance by *A. solani* is one of the major challenges in the management of early blight (Van der Waals *et al*., 2005). The problem is even more prevalent under smallholder farming systems, where farmers lack knowledge on proper application methods (Pasche *et al*., 2005).

Currently in the Limpopo Province, information about the sensitivity of *A. solani* populations on commonly used fungicides is limited. The aim of this study was to evaluate the sensitivity of *A. solani* isolates obtained from different areas in the Limpopo Province to commonly used fungicides mainly chlorothalonil, copper oxychloride and mancozeb in the management of tomato early blight. This will help to understand whether morphological variations observed in Chapter 3 contribute to pathogen resistance against these fungicides.

4.2 Materials and methods

4.2.1 Study location and experimental design

The study was conducted at the University of Limpopo Plant Pathology laboratory, at the Turfloop campus, South Africa (23º 53' 10" S, 29º 44' 15" E). Each experiment was arranged in a complete randomised design (CRD) with six treatments (0; 1; 2 and 3 g/L) replicated four times.

4.2.2 Fungal isolates

Alternaria solani isolates confirmed in Chapter 3 were used to test their sensitivity towards different fungicide groups. From each area four isolates were randomly selected making a total of 16 *A. solani* isolates (Table 4.1).

4.2.3 Fungicide sensitivity bioassay and data collection

Three fungicides namely, chlorothalonil, (Bravo® 720 WP), copper oxychloride (Virikop® 50 WP) and mancozeb (Dithane® WG) obtained from Platinum Pesticide (Pty) Ltd were tested *in-vitro* to evaluate their effectiveness against 16 *A. solani* isolates. Fungicide sensitivity bioassay was carried out following the poisoned food technique (Chohan *et al*., 2015). Thirty nine grams of Potato Dextrose Agar (PDA Baker ®) (as recommended rates) was poured into 1 litter of distilled water, autoclaved and allowed to cool at 50 °C. After autoclaving, cooled PDA was amended with five different concentrations of each fungicide (0; 1; 2 and 3 g/L). Non-amended PDA (0 g/L) was used as control. Both amended and un-amended agar plates were inoculated with a 5 mm diameter plug obtained from seven day old *A. solani* culture using a sterilised cork borer.

Two perpendicular lines were drawn on the underside of the petri-dish and the mycelial plug was placed at the centre of both lines. The plates were then incubated at 25 ± 2 °C for 8 days. After 8 days, the colony diameter was measured using a clear transparent ruler beneath the petri-dish as described by Sallam and Kamal (2012) and Chohan *et al*. (2015).

Table 4.1 Randomly selected *Alternaria solani* isolates tested for sensitivity against different fungicide groups

Sampled areas	Tested isolates			
Blood-river	B1	B2	B7	B9
Mankweng	M1	M5	M8	M9
Tzaneen	T4	T5	T10	T13
Venda	V3	V7	V8	V10

4.2.4 Data analysis

The mycelial growth data were subjected to Analysis of Variance (ANOVA) using the Statistix 10.0 software. Means separation to determine differences in mycelial growth was carried out through least significant difference (LSD) at 5 % probability levels of F-test.

4.3 Results

4.3.1 Sensitivity of *A. solani* isolates towards chlorothalonil

Chlorothalonil significantly (P ≤ 0.05) reduced mycelial growth of all tested *A. solani* isolates in all tested concentrations as compared to un-amended control. However, the level of sensitivity varied in each treatment as shown in Figure 4.1. There was also a significant difference (P ≤ 0.05) among isolates and their sensitivity to different concentrations (Figure 4.1). For example, at the lowest concentration of 1 g/L, isolate M9 displayed the highest mycelial growth (65.75 mm) (Figure 4.1 b) whilst the lowest mycelial growth under the same concentration was recorded on isolate V3 (51.75) (Figure 4.1 d). At 3 g/L concentration, the highest mycelial growth reduction was recorded in isolate T13 (18.00 mm) (Figure 4.1 c) followed by isolates V10 (19.25 mm) and V3 (19.50 mm) (Figure 4.1 d). However, at the same concentration, isolate B1 exhibited the lowest mycelial growth with 35.25 mm (Figure 4.1 a).

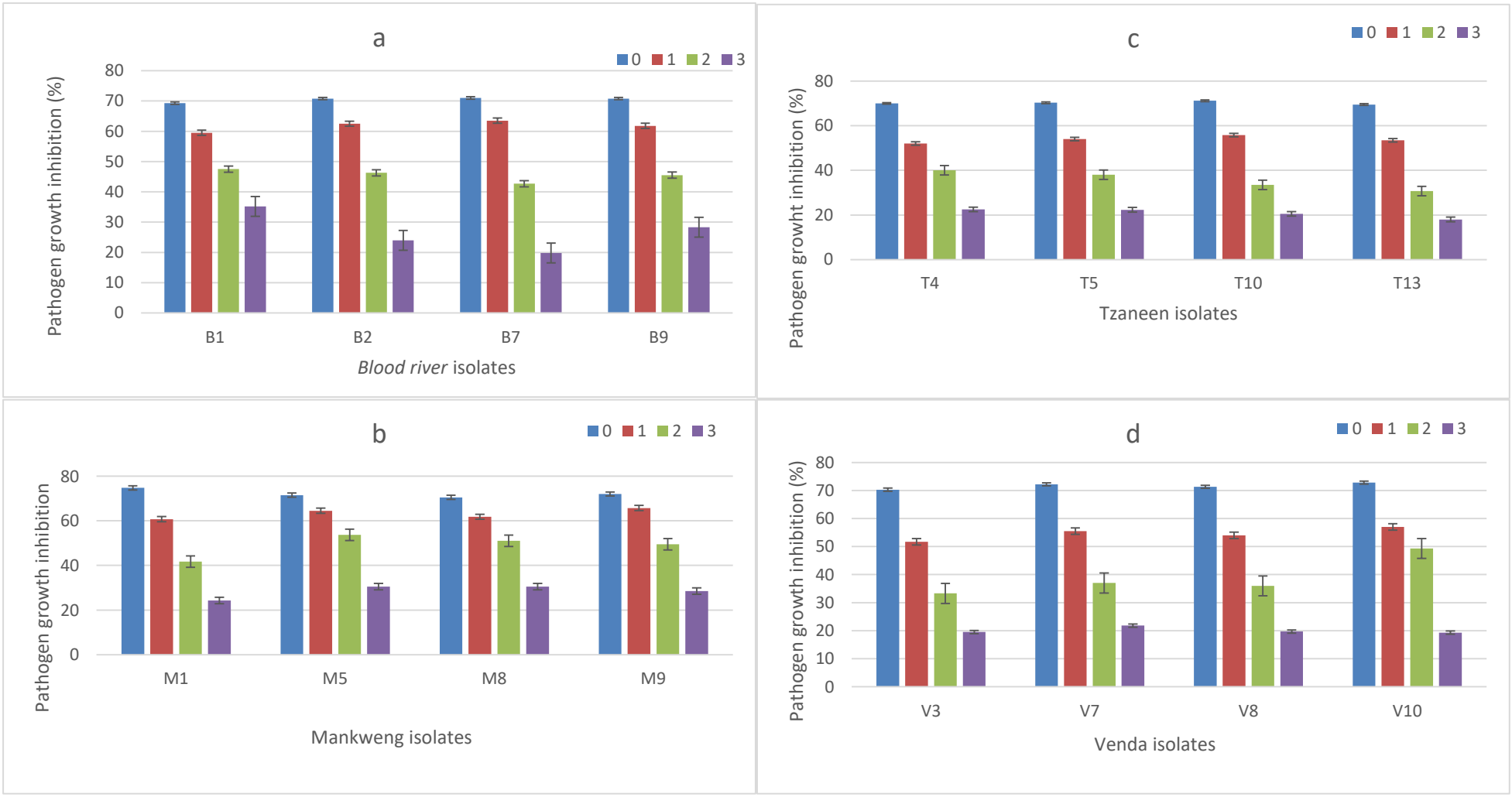


Figure 4.1 Effect of different concentration of chlorothalonil on the mycelial growth of *A. solani* isolates collected from (a) Blood-river, (b) Mankweng, (c) Tzaneen and (d) Venda under laboratory condition

4.3.2 Sensitivity of *A. solani* isolates towards copper oxychloride

All tested *A. solani* isolates showed sensitivity towards the copper oxychloride fungicide regardless of area of collection. The mycelial growth was significantly ($P \leq 0.05$) reduced at all concentration when compared to the control treatment (Figure 4.2). However, the level of sensitivity varied in each treatment. The results show that an increase in fungicide concentration resulted in the decrease in mycelial growth for all isolates. For example, at 1 g/L concentration the highest mycelial growth was observed in isolate, B7 (71.00 mm) (Figure 4.2 a), followed by M1 (48.00 mm) and M8 (44.25) isolates respectively (Figure 4.2 b). At 3 g/L concentration, the lowest mycelial growth was observed on isolates T10 (8.25 mm), T4 and T13 isolates with mycelial growth of 9.25 mm (Figure 4.2 c). Compared to all tested isolates, B7 displayed reduced sensitivity towards this fungicide even at high concentration of 3 g/L (Figure 4.2 a) with the highest mycelial growth of 49 mm under the same concentration.

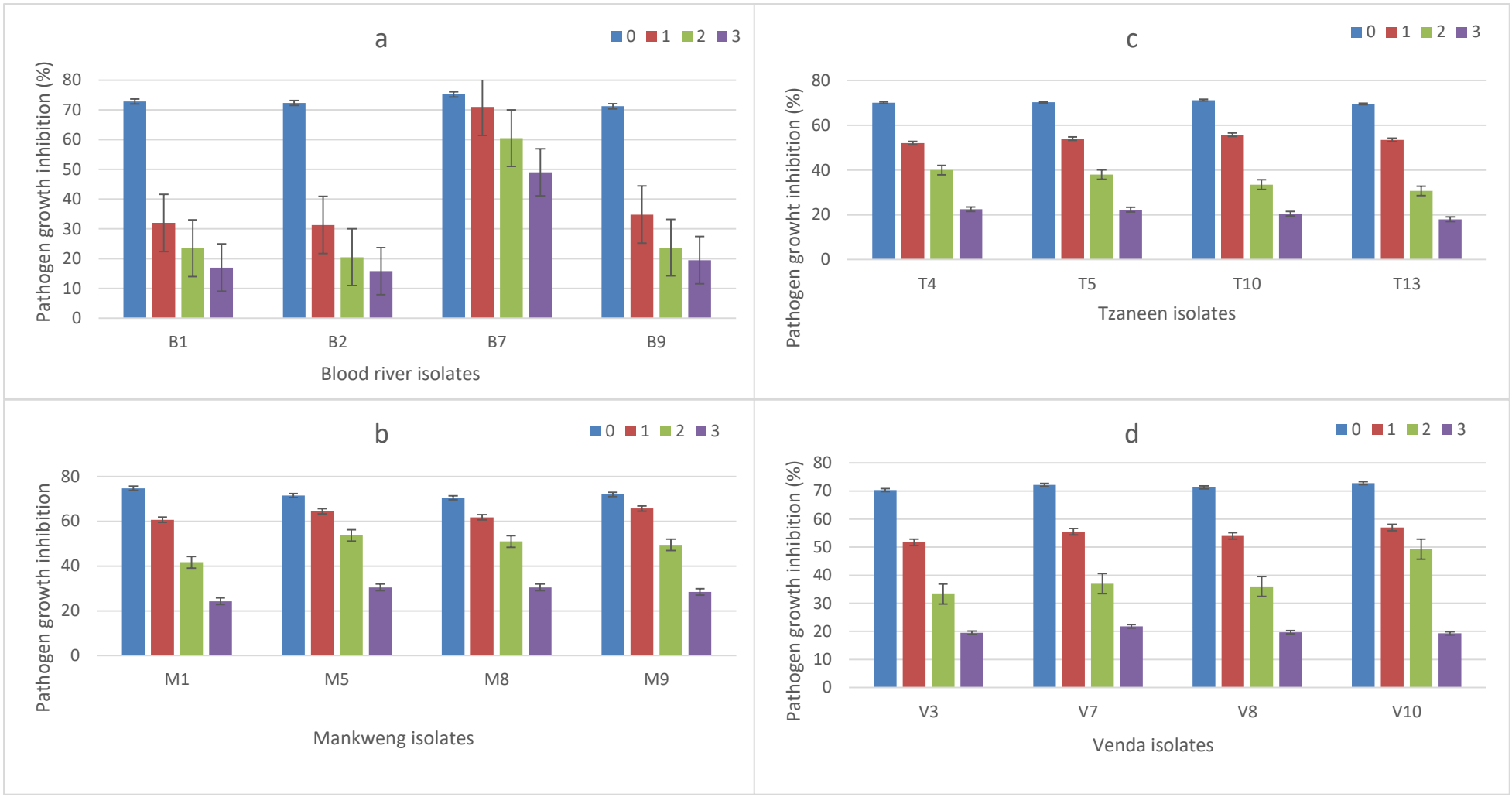


Figure 4.2 Effect of different concentration of copper oxychloride on the mycelial growth of *A. solani* isolates collected from (a) Blood-river, (b) Mankweng, (c) Tzaneen and (d) Venda under laboratory conditions

4.3.3 Sensitivity of *A. solani* isolates towards mancozeb

Different concentrations of mancozeb significantly ($P \leq 0.05$) reduced mycelial growth of all tested *A. solani in-vitro*. At different concentrations of mancozeb there was a significant difference ($P \leq 0.05$) on the mycelial growth reduction (Figure 4.3). For example, at 1 g/L concentration the highest mycelial growth was recorded for isolate B7 (65.50 mm) (Figure 4.3 a) whilst the lowest growth reduction was recorded in isolate V3 (44.5 mm) (Figure 4.3 d). Furthermore at the highest concentration of 3 g/L, the lowest mycelial growth of 18.25 mm was recorded in both B1 and B2 isolates (Figure 4.3 a) followed by 18.75 mm observed on isolate V10 (Figure 4.3 d). Isolate B7 displayed a reduced sensitivity towards mancozeb at all concentrations resulting in growth diameters highest mycelial growth across all the concentrations as compared to other tested isolates (Figure 4.3 a).

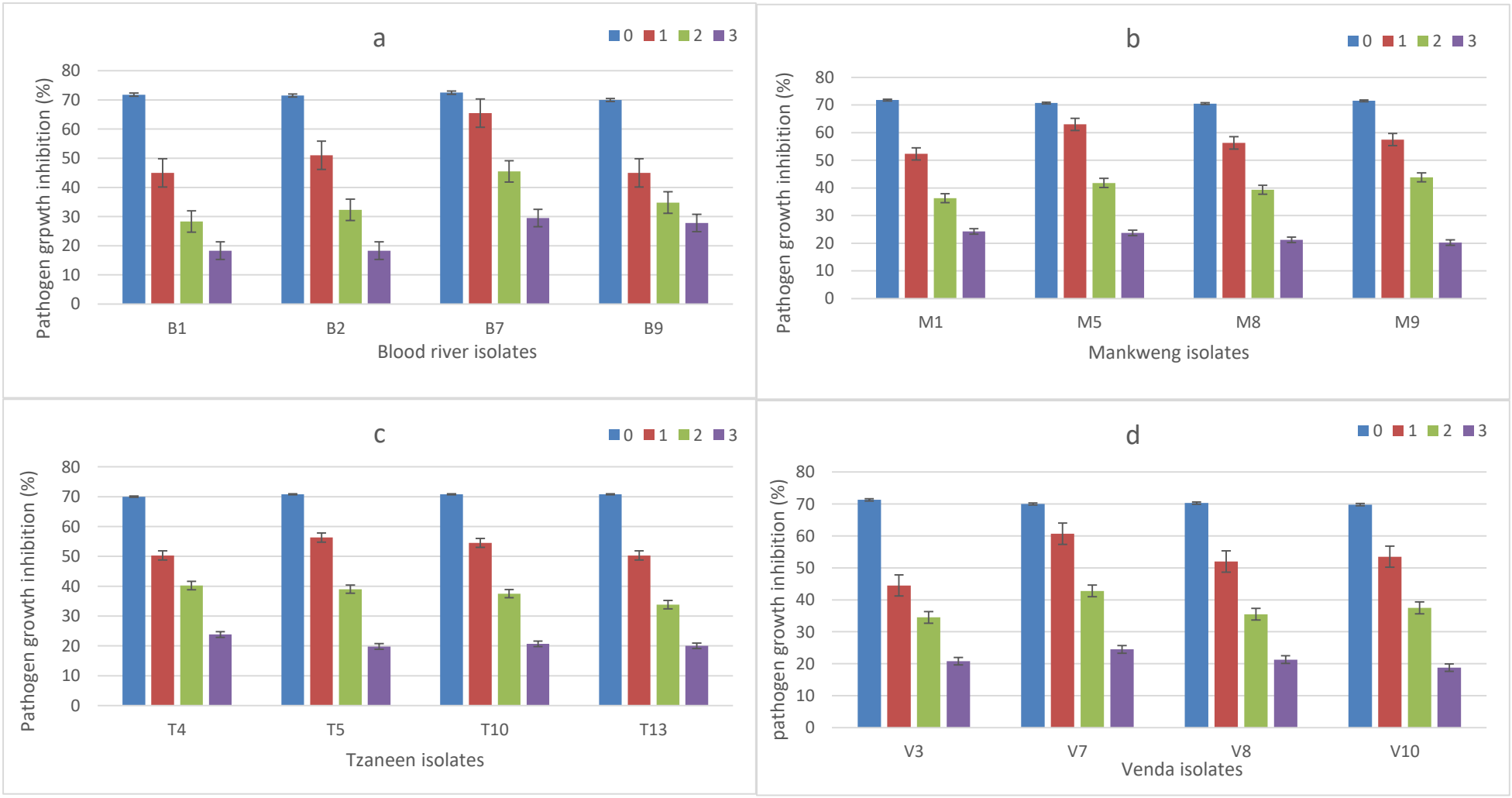


Figure 4.3 Effect of different concentration of mancozeb on the mycelial growth of *A. solani* isolates collected from (a) Blood-river, (b) Mankweng, (c) Tzaneen and (d) Venda under laboratory condition

4.4 Discussion

Fungicide application is the main control measure used to manage tomato early blight worldwide (Holm *et al*., 2003; Pasche and Gudmestad, 2008). Pathogen sensitivity to various fungicide concentrations is an important measure used in determining the development of resistance in isolates and populations (Kumar *et al*., 2017). In the current study, most of the tested *A. solani* isolates showed some level of sensitivity to the used fungicides across different concentrations. However, the level of sensitivity varied among the isolates, these resulting in some of the isolates showing reduced sensitivity towards the tested fungicides. For example, isolates B7 and V3 were observed to have the reduced sensitivity towards all the tested fungicides across all the concentrations. These finding were in agreement with those of Abu-El Samen *et al*. (2016) who reported a reduced sensitivity in some of the *A. solani* isolates towards mancozeb and chlorothalonil. According to Ganie *et al*. (2013), Ghazanfar *et al*. (2016) and Kumar *et al*. (2017), an increase in fungicide concentration, resulted in the decrease in pathogen growth and vice-versa, the same phenomenon was also observed in our study, however some isolates exhibited a reduced level of sensitivity on some of the concentrations.

According to Stepanović *et al*. (2015) area of isolate collection as determined by environmental factors and farming practices can play a major role in *A. solani* sensitivity towards fungicides used in its control. This phenomenon was also observed in our study where isolates B7 and V3 collected from Blood-river and Venda showed a reduced sensitivity towards the used fungicides. Varying conditions and farming practices employed by small-holder farmers in these areas, especially indiscriminate application of fungicides might be the reasons for this reduction in sensitivity.

Kumar *et al*. (2017) also reported that the climatic conditions in different sampled areas might play a role in the reduced sensitivity of isolates towards the used fungicides. Relative humidity and high temperatures were shown by different authors to promote pathogen selection resulting in reduced sensitivity (Pasche and Gudmestad, 2008; Rosenzweig *et al*., 2008).

There also numerous reports on a reduction in efficacy of various fungicides used to control early blight, due to development of fungicide resistance by *A. solani* populations (Pasche and Gudmestad, 2008; Rosenzweig *et al*., 2008; Fairchild *et al.,* 2013). In our study, isolate B7 collected from Blood-river showed less sensitivity to copper oxychloride fungicide. These finding are in agreement with those of Stepanović *et al*. (2015) who reported a reduced sensitivity of some of the *A. solani* isolated towards the commercially used fungicides. Van der Waals *et al*. (2004) and Rani *et al*. (2017) suggested that farming practices such as continued application of synthetic fungicides and improper dosage of fungicide result in the reduced sensitivity of the pathogen over time and these can also contribute towards our findings in this study.

Among the three fungicides tested in this study, copper oxychloride was found to be the most effective fungicide in reducing the mycelial growth of *A. solani*, followed by chlorothalonil and mancozeb, except in a situation where the isolate developed resistance to copper oxychloride fungicide. In another study, Stepanović *et al*. (2015) reported that copper oxychloride fungicides exhibited the highest toxicity to *A. solani* isolates as compared to chlorothalonil and mancozeb fungicides. However, these disagree with the findings of Ghazanfar *et al*. (2016) who reported that mancozeb (Dithane® M-45), was the most effective fungicide on reducing the average linear mycelial growth of *A. solani* as compared to other fungicides such as chlorothalonil

(Kavach® 75 WP) and copper oxychloride (Blitox® 50 WP) *in-vitro*. Although Babu *et al*. (2000) reported that, copper oxychloride and mancozeb fungicide were superior in controlling early blight; the reduced sensitivity of *A. solani* isolates from Limpopo Province toward these fungicides was detected. These might attributed to the continuous application of these fungicides in areas where these isolates were collected.

Our findings in this study highlight the possibility of the development of new *A. solani* strains that are resistant to the commonly used fungicides. However, further collection of more *A. solani* isolates from multiple regions across numeral years is needed to confirm the frequency and the future potential fungicide resistance within the Limpopo Province.

References

ABU-EL SAMEN, F., SABA, J.G., AL-SHUDIFAT, A. and I. MAKHADMEH. 2016. Reduced sensitivity of tomato early blight pathogen (*Alternaria solani*) isolates to protectant fungicides, and implication on disease control. *Archives of Phytopathology and Plant Protection* 49:5-6.

BABU, S., SEETHARAMAN, K., NANDAKUMAR, R. and I. JOHANSON. 2000. Efficacy of fungal antagonists against leaf blight of tomato caused by *Alternaria solani*. *Journal of Biological Control* 14:79-81.

CHAERANI, R., GROENWOLD, R., STAM, P. and R.E. VOORRIPS. 2007. Assessment of early blight (*Alternaria solani*) resistance in tomato using a droplet inoculation method. *Journal of General Plant Pathology* 73:96-103.

CHOHAN, S., PERVEEN, R., MEHMOOD, M.A., NAZ, S. and N. AKRAM. 2015. Morpho-physiological studies, management and screening of tomato germplasm against *Alternaria solani*, the causal agent of tomato early blight. *International Journal of Agriculture and Biology* 17:111-118.

FAIRCHILD, K.L., MILES, T.D and P.S. WHARTON. 2013. Assessing fungicide resistance in populations of *Alternaria* in Idaho potato fields. *Crop Protection* 49:31-9.

GANIE, S.A., GHANI, M.Y., NISSAR, Q., JABEEN, N., ANJUM, Q., AHANGER, F.A. and A. AYAZ. 2013. Status and symptomatology of early blight (*Alternaria solani*) of potato (*Solanum tuberosum L*.) in Kashmir valley. *African Journal of Agricultural Research* 8:5104-5115.

GHAZANFAR, M.U., RAZA, W., AHMED, K.S., QAMAR, J., HAIDER, N. and M.H. RASHEED. 2016. Evaluation of different fungicides against *Alternaria solani* (Ellis & Martin) Sorauer cause of early blight of tomato under laboratory conditions. *International Journal of Agricultural Studies* 5:8-12.

HOLM, A.L., RIVERA, V.V., SECOR, G.A. and N.C. GUDMESTAD. 2003. Temporal sensitivity of *Alternaria solani* to foliar fungicides. *American Journal of Potato Research* 80:33-40.

KUMAR, V., G, SINGH and A, TYAGI. 2017. Evaluation of different fungicides against Alternaria leaf blight of tomato (*Alternaria solani*). *International Journal of Current Microbiology and Applied Sciences* 6:2343-2350.

PASCHE, J.S. and N.C. GUDMESTAD. 2008. Prevalence, competitive fitness and impact of the F129L mutation in *Alternaria solani* from the United States. *Crop Protection* 27:427-435.

PASCHE, J.S., PICHE, L.M. and N.C. GUDMESTAD. 2005. Effect of the F129L mutation in *Alternaria solani* on fungicides affecting mitochondrial respiration. *Plant Disease* 89:269-278.

RANI S., SINGH, R and S, GUPTA. 2017. Development of integrated disease management module for early blight of tomato in Jammu. *Journal of Phytochemistry* 6: 268-273.

ROSENZWEIG, N., ATALLAH, Z.K., OLAYA, G. and W.R. STEVENSON. 2008. Evaluation of QoI fungicide application strategies for managing fungicide resistance and potato early blight epidemics in Wisconsin. *Plant Disease* 92:561-568.

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

STEPANOVIĆ, M., JEVREMOVIĆ, S., REKANOVIĆ, E., MIHAJLOVIĆ, M., MILIJAŠEVIĆ-MARČIĆ, S., POTOČNIK, I. and B. TODOROVIĆ. 2015. *In-vitro* sensitivity of *Alternaria solani* to conventional fungicides and a biofungicide based on tea tree essential oil. *Journal of Pesticides and Phyto-medicines* 30:25-33.

VAN DER WAALS, J.E., KORSTEN, L. and B. SLIPPERS. 2004. Genetic diversity among *Alternaria solani* isolates from potatoes in South Africa. *Plant Disease* 88:959-964.

VAN DER WAALS, J.E., KORSTEN, L. and F.D.N. DENNER. 2005. Early blight in South Africa: knowledge, attitudes and control practice of potato growers. *Potato Research* 46:27-37.

CHAPTER 5

AGGRESSIVENESS OF *ALTERNARIA* SOLANI LIMPOPO ISOLATES ON TWO SELECTED CULTIVARS OF TOMATO

5.1 Introduction

Alternaria solani is one of the most aggressive pathogens of tomato causing serious yield losses (Chaerani and Voorrips, 2006). The aggressiveness of the pathogen is influenced mainly by environmental conditions such as moisture and temperature stress (Tymon *et al*., 2016), farming practices including mono-cropping and nutrient stress (Tsedaley, 2014) and continuous application of synthetic fungicides (Gannibal *et al*., 2014) these resulting in the development of new aggressive fungal strains (Chaerani *et al*., 2007). Other factors such as plant vigour, cultivar susceptibility and plant age can also influence the aggressiveness of the *A. solani* pathogen to tomato plants (Warton and Kirk, 2012).

One of the main control measures used in management of early blight disease is mainly based on the use of tomato resistant cultivars (Kemmitt, 2002; Duarte *et al.*, 2014). However, world-wide including, South Africa there is no cultivar available with complete levels of resistance to early blight (Van der Waals *et al*., 2001). Therefore, the objective of this study was to assess the aggressiveness of *A. solani* isolates collected from different areas across Limpopo Province on two selected susceptible tomato cultivars namely Money-maker and Rodade.

5.2 Materials and methods

5.2.1 Screening of tomato cultivars under laboratory condition

Study location and experimental design

The study was conducted at the University of Limpopo Plant Pathology Laboratory, at the Turfloop campus, South Africa (23º 53' 10" S, 29º 44' 15" E). Each experiment was arranged in complete randomised design (CRD) with eight different isolates from each area as the treatments (Table 5.1), replicated four times on each tested cultivar.

Leaf disc experiment under laboratory condition

For the leaf disc experiment*, A. solani* isolates confirmed in Chapter 3 of this study were tested for aggressiveness on two susceptible tomato cultivars (Money-maker and Rodade). From each area, eight isolates were selected making a total of 32 tested isolates (Table 5.1).

Table 5.1 *Alternaria solani* isolates from different areas across Limpopo Province

Areas	Isolates							
Blood-river	B1	B2	B6	B7	B8	B9	B10	B20
Mankweng	M1	M2	M4	M5	M8	M9	M10	M14
Tzaneen	T5	T8	T9	T10	T11	T13	T22	T24
Venda	V1	V3	V7	V8	V9	V10	V11	V12

Healthy leaves were detached from ten week old Money-maker and Rodade cultivars. Circular discs were cut from the leaves using a flame sterilised 10 mm cork- borer and disinfected with 1% sodium hypochlorite solution for five minutes and rinsed three times in sterilised distilled water for one minute. Six leaf discs were placed inside 90 mm petri dishes on a wet filter paper (Figure 5.1).

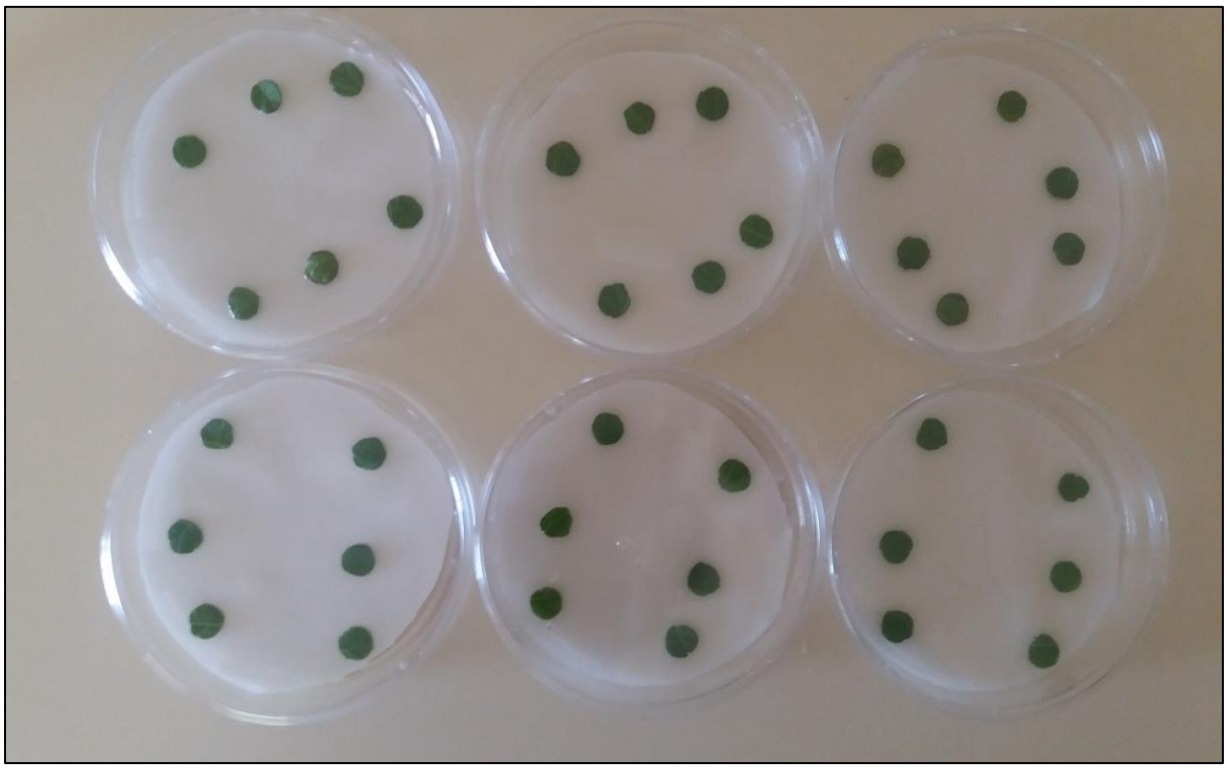


Figure 5.1 Leaf discs cultured on the filter paper before incubation

Pathogen inoculum preparation and inoculation

Alternaria solani inoculum was prepared by growing pure cultures of each isolate on PDA for seven days incubated at 25 ± 2°C. Spores of fresh grown early blight pathogens were harvested by adding 10 ml of sterile distilled water to each plate and colonies were carefully scraped with a sterile needle. The resulting conidial suspensions from each isolate were then added into sterilised bottles.

Following this, 10 µl droplets of a conidial suspension (10^6 ml^{-1}) of each isolate were applied by micro-pipette on the leaf discs and allowed to run-off the disc surface. The inoculated leaf discs were then incubated at 25 ± 2°C for seven days. Control treatments were inoculated with sterile distilled water.

Data collection

For assessment of *A. solani* aggressiveness, each leaf disc was visually rated for early blight symptoms using modified 0-4 rating scale (Table 5.2) as described by Nuppenau *et al*. (2005) seven days after inoculation.

Table 5.2 Disease severity assessment scale (0-4)

Scale	Description
0	Healthy disc (no disease symptom)
1	Only single and small necrotic spots, less than half of the droplet area is necrotic
2	More than half of the droplet area is necrotic
3	The droplet area is fully necrotic
4	The disease has spread over the whole leaf disc

The disease severity (%) for each isolate was determined using the formula by Pandey *et al*. (2003) where: Disease severity (%) = [(Number of disc scored for each rating x the rating value) / Total disc scored] x 100.

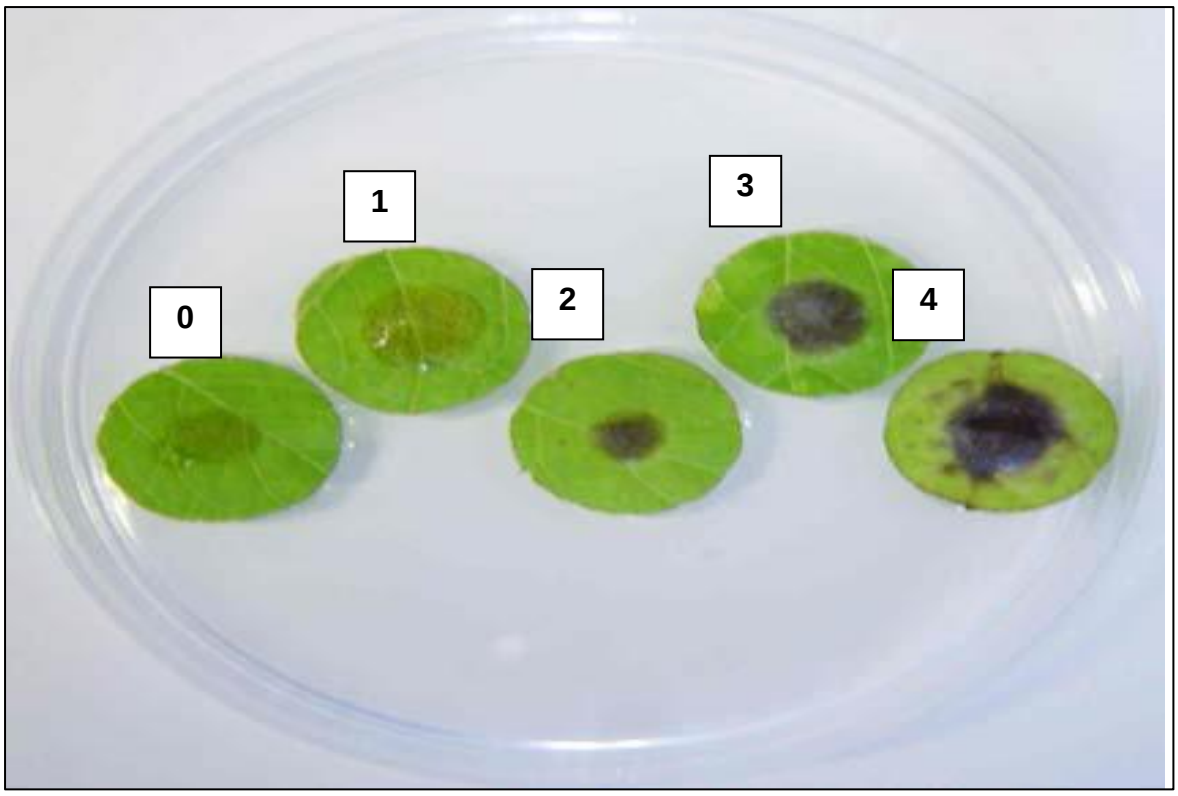


Figure 5.2 Leaf disc disease severity scale with grades 0-4

Data analysis

Disease severity data were arcsine-square root transformed prior to analysis in order to homogenise the variances, however untransformed data were reported (Gomez and Gomez, 1984; Chaerani *et al*., 2007). Data were subjected to analysis of variance using Statistix 10.0 software. Tukey's HSD at $P \leq 0.05$ was used to test the differences between treatment means.

5.2.2 Screening of tomato cultivars for susceptibility under greenhouse conditions

Study location and experimental design

The study was conducted under greenhouse conditions at the University of Limpopo, (23º 53' 10" S, 29º 44' 15" E), South Africa. Two cultivars (Money-maker and Rodade) were planted in the greenhouse. The experiment was laid out in a Randomised Complete Block Design (RCBD), with 12 isolates (treatments) per cultivar (Table 5.3) replicated four times.

Experimental procedures and cultural practices

Uniform, four-week old tomato seedlings of Money-maker and Rodade were transplanted inside 25 cm diameter plastic pots, at 0.3 m inter-row spacing and 0.25 m intra-row spacing. Prior to planting, the pots were filled with steam-pasteurised sand and Hygromix at 3:1 ratio. Plants were irrigated with 500 ml tap water every other day until the termination of the experiment. Pests and diseases were also monitored and when necessary, control measures were applied.

Pathogen inoculum preparation and inoculation

Greenhouse experiments were carried out to confirm the aggressiveness of different *Alternaria solani* isolates that displayed varied levels of virulence under laboratory conditions (Leaf disc). Based on leaf disc experiment observations three isolates per area, which displaying low, moderate and high aggressiveness were used for this greenhouse experiment. This making the total of 12 tested isolates on each cultivar (Table 5.3). *Alternaria solani* inoculum was prepared as described under Section 5.2.1.

The conidial suspension (10^6 ml^{-1}) of each isolate was added to a sterilised hand sprayer and ten weeks old tomato plants were inoculated with the *A. solani* pathogen. To enhance natural infection and to obtain uniform disease pressure, leaves were injured prior to inoculation, by rubbing the thumb and forefingers which were sterilised with 90% ethanol. Fifty-two plants of each cultivar were inoculated with different tested isolates; ten leaves were selected, tagged and inoculated from each plant. In control plants, leaves were sprayed with sterile distilled water. After inoculation, plants were covered with polyethylene bags for 48 hours to increase humidity and accelerate infection. The test plants were then uncovered and kept in the greenhouse at 27 ±1°C.

Table 5.3 Selected isolates with different aggressive status

Location	Isolates (Treatments)		
	Low aggressive	Moderately aggressive	Highly aggressive
Blood-river	B2	B6	B20
Mankweng	M10	M2	M8
Tzaneen	T8	T22	T24
Venda	V9	V10	V11

Data collection

Early blight severity was evaluated 21 days after inoculation using a modified scale of 0-5 according to Pandey *et al*. (2003) (Table 5.4). Final disease severity was calculated using the formula: Disease severity (%) = [(Number of leaves scored for each rating x the rating value) / Total plants scored] x 100. The lesion sizes (mm) on the inoculated leaves were measured using a 30 cm transparent ruler.

Table 5.4 Disease severity rating scale (0-5)

Scale	Description
0	No symptoms on the leaf area
1	≤ 2 mm-3 mm on the main lesion with one to few spots on the leaf area
2	3 mm- 5 mm on the main lesion with few spots on the leaf area
3	5 mm-7 mm on the main lesion with few to many spots on the leaf area
4	7 mm-10 mm on the main lesion with many spots on the leaf area leave
5	≥ 10 mm on the main lesion on leaf area being fully necrotic

Data analysis

Disease severity data were arcsine-square root transformed in order to homogenize the variances; however untransformed data were reported (Gomez and Gomez, 1984; Chaerani *et al*., 2007). Disease severity data were subjected to Analysis of variance (ANOVA) using Statistix 10.0 software. Tukey's HSD at $P \leq 0.05$ was used to determine the difference among the treatment means.

5.3 Results

5.3.1 The leaf disc experiment under laboratory condition

All the tested isolates displayed a certain level of aggressiveness to both tomato cultivars (Figures 5.3 and 5.4). There was a significant difference ($P \leq 0.05$) (Figures 5.3 and 5.4) and variations in the ability of isolates to the cause disease and levels of early blight severity differed based on the area of collection and inoculated cultivars (Figures 5.3 and 5.4). For example, both cultivars displayed high susceptibility to isolate V11 from Venda, resulting in 95% disease severity on Rodade (Figure 5.4 d) and 100% on the Money-maker cultivar (Figure 5.3 d). On the other hand, isolate B2 showed less aggressiveness towards both cultivars with the least disease severity of 20.01% on Money-maker (Figures 5.3 a) and 17.96% (Figure 5.4 a) on the Rodade. Although both cultivars were susceptible to all tested isolates, Money-maker was most susceptible to all the isolates as compared to Rodade which showed varied level of susceptible based on tested isolate.

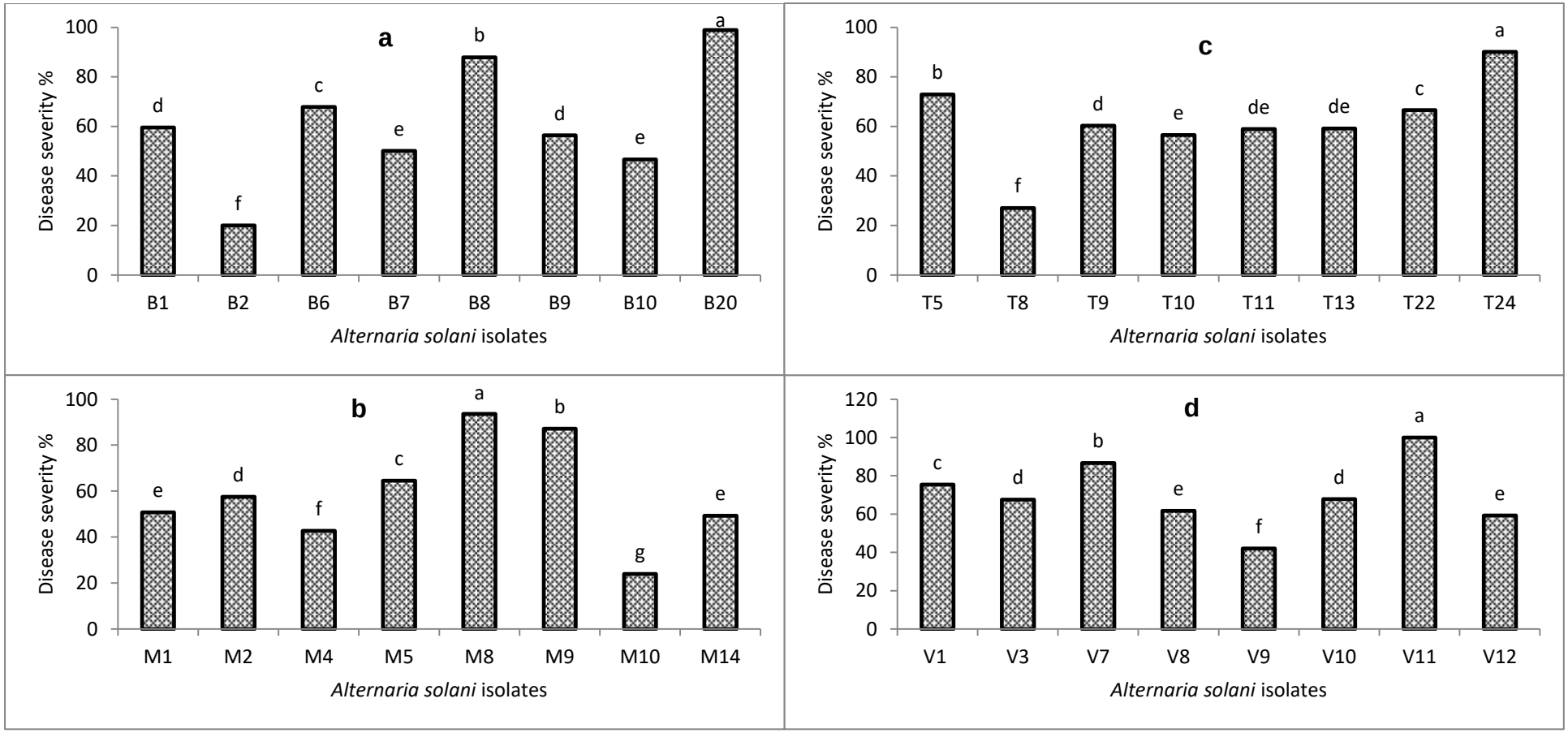


Figure 5.3 Disease severity of *A. solani* isolates collected from (a) Blood-river, (b) Mankweng, (c) Tzaneen and (d) Venda on Money maker cultivar under laboratory conditions. The bars with the same letters within a cultivar were not significantly different according to Tukey's HSD at $P \leq 0.05$

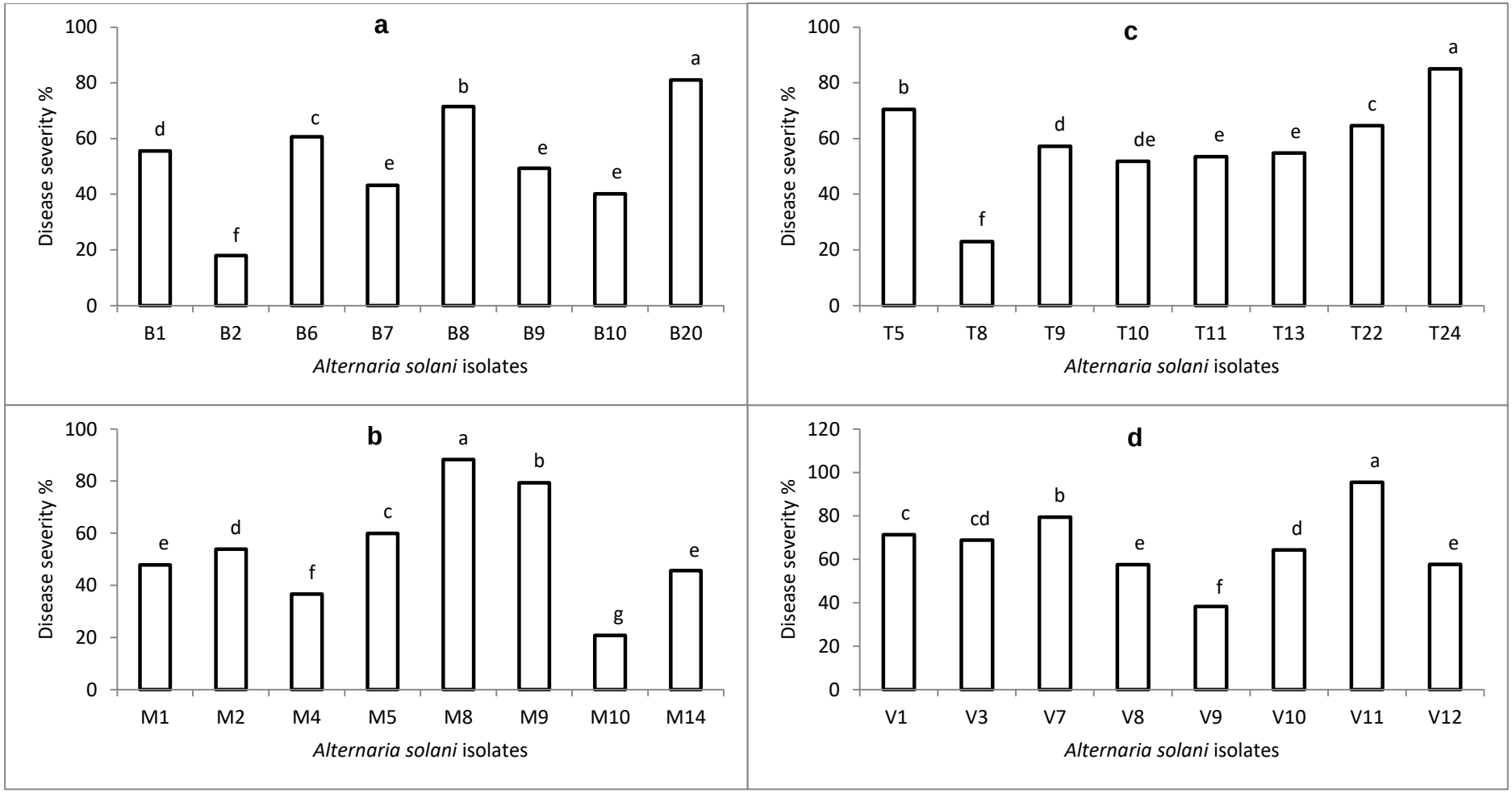


Figure 5.4 Disease severity of *A. solani* isolates collected from (a) Blood-river, (b) Mankweng, (c) Tzaneen and (d) Venda on Rodade cultivar under laboratory conditions. The bars with the same letters within a cultivar were not significantly different according to Tukey's HSD at $P \leq 0.05$

5.3.2 The greenhouse experiment

The control treatment inoculated with sterile distilled water did not show any early blight symptoms. However, the disease severity exhibited by different isolates collected from these four areas differed significantly ($P \leq 0.05$) at 21 days after inoculation (Figure 5.5). Tested *A. solani* isolates differed in their level of aggressiveness on both cultivars. These resulted in variations in cultivar susceptibility against tested isolates (Figure 5.5). For example inoculation of Money-maker and Rodade with isolate V11 resulted in high disease severity with 92.66% (Figure 5.5 a) and 83.94% (Figure 5.5 b) respectively, whilst the least disease severity was recorded in isolate B2 with 56.70% severity on Money-maker (Figure 5.5 a) and 35.08% on Rodade (Figure 5.5 b). Although none of the cultivars were resistant to all the tested isolates, Money-maker cultivar was highly susceptible to isolates M8, M10, T8 and susceptibility was reduced in isolates B2, B6, B20, M2, V9, V10 and V11 (Figure 5.5).

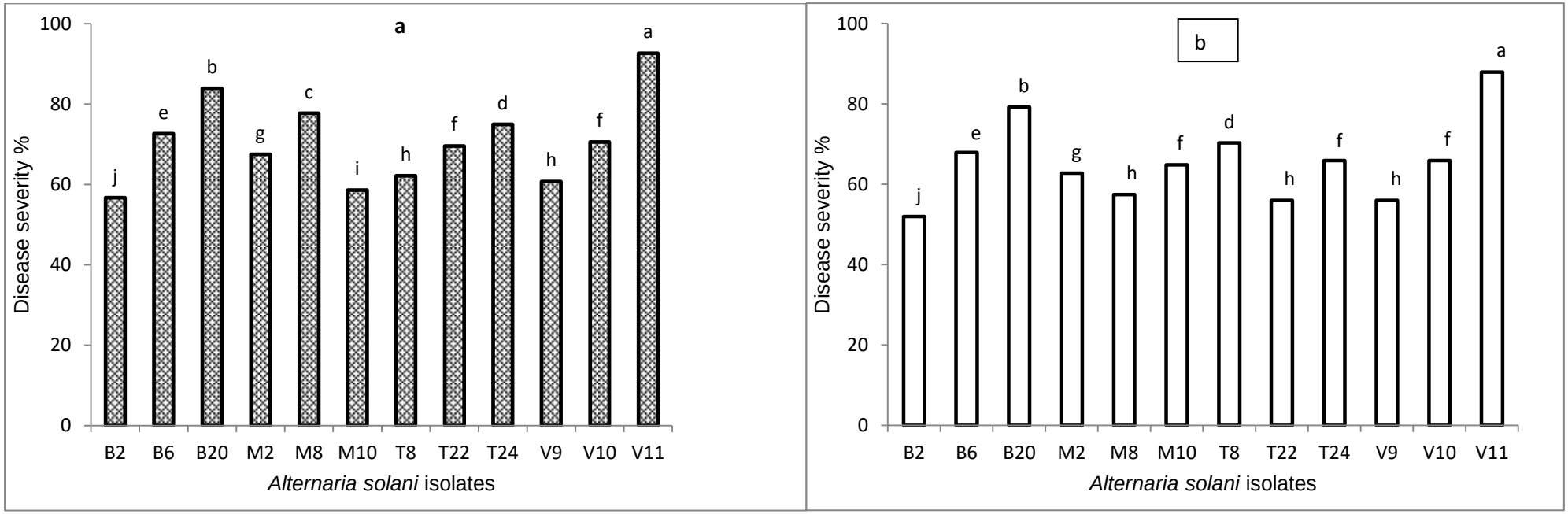


Figure 5.5 Disease severity of *A. solani* isolates on (a) Money-maker and (b) Rodade cultivar, under greenhouse conditions. The bars with the same letters within a cultivar were not significantly different according to Tukey's HSD at $P \leq 0.05$

5.4 Discussion

Numerous studies have reported on the susceptibility of different tomato cultivars to *A. solani* isolates and it was noted that cultivars respond differently to different isolates based on the area of collection (Van der waals *et al*., 2004; Shahbazi *et al*., 2010; Bessadat *et al*., 2014). Kemmitt (2002) also reported the lack of complete resistance to early blight in commercial tomato cultivars with most commercially produced tomato cultivars showing reduced levels of tolerance towards this disease. In our study, both tested cultivars were also found to be susceptible to different *A. solani* isolates both under laboratory and greenhouse condition. These results are also in agreement with those of Bessadat *et al*. (2014) who reported high susceptibility of early blight on other two tomato cultivars namely Cherry and Saint Pierreto. Area of isolate collection was found to significantly increase pathogen aggressiveness and cultivar susceptibility (Chohan et al., 2015), however this was not confirmed in our study. Although in the current study both Money-maker and Rodade were found to be susceptible to *A. solani*, according to Fontem (2003) both cultivars have reduced susceptibility towards early blight.

Assessing the level of aggressiveness of different isolates collected from different areas is an important component of evaluating the resulting disease damage that each isolate causes on the plant (Bessadat *et al*., 2014). In our study, a number of *A. solani* isolates displayed a specific level of aggressiveness which resulted in notable disease symptoms on both tested cultivar under both laboratory and greenhouse conditions. However, different isolates varied with the level of aggressiveness. For example, isolates B20 and V11 were found to be highly aggressive towards the two cultivars resulting in a significant increase in disease severity under both conditions.

These results are in agreement with those of Shahbazi *et al*. (2010) who reported that different *A. solani* isolates from different geographical areas exhibited variations in aggressiveness. Other authors also reported similar results (Chaerani *et al*., 2007; Bessadat *et al*., 2014; Chohan *et al*., 2015).

Many studies have reported on the effect of farming practices such as continuous application of chemical fungicides and monoculture on the aggressiveness of the pathogen (Bessadat *et al*., 2014; Tymon *et al*., 2016). Such conditions might influence the development of new fungal strains which over time can become more aggressive to the cultivated tomato cultivars than pre-existing strains (Tsedaley, 2014). In our study, isolate V11 from Venda was the most aggressive and these practices were commonly practice in this area and these could be the reason for our findings. Furthermore Chaerani *et al*. (2007) and Warton and Kirk (2012) reported that the prevailing temperature and relative humidity in the area influence the genetic make-up of the pathogen, resulting in the development of new fungal strains that are more aggressive and these could be the reason for our findings.

Our findings demonstrate the possibility of the development of new *A. solani* strains that are more aggressive and virulent toward the commercial cultivars. However, further collection of more *A. solani* isolates from multiple regions across multiple years is needed to confirm the aggressiveness and virulence of *A. solani* isolates within the Limpopo Province.

References

BESSADAT, N., BENICHOU, S., KIHAL, M. and D.E. HENNI. 2014. Aggressiveness and morphological variability of small spore *Alternaria species* isolated from Algeria. *Journal of Experimental Biology and Agricultural Sciences* 2:2320 8694.

CHAERANI, R. and R.E. VOORRIPS. 2006. Tomato early blight (*Alternaria solani*): the pathogen, genetics, and breeding for resistance. *Journal of General Plant Pathology* 72:335-347.

CHAERANI, R., GROENWOLD, R., STAM, P. and R.E. VOORRIPS. 2007. Assessment of early blight (*Alternaria solani*) resistance in tomato using a droplet inoculation method. J*ournal of General Plant Pathology* 73:96-103.

CHOHAN, S., PERVEEN, R., MEHMOOD, M.A., NAZ, S. and N. AKRAM. 2015. Morpho-physiological studies, management and screening of tomato germplasm against *Alternaria solani*, the causal agent of tomato early blight. *International Journal of Agriculture and Biology* 17:111-118.

DUARTE, H.S.S., ZAMBOLIM, L., RODRIGUES, F.A., PAUL, P.A., PADUA, J.G., RIBEIRO, J.I., JUNIOR, A.F.N. and A.W.C. ROSADO. 2014. Field resistance of potato cultivars to foliar early blight and its relationship with foliage maturity and tuber skin types. *Tropical Plant Pathology* 39:294-306.

FONTEM, D.A. 2003. Quantitative effects of early and late blights on tomato yields in cameroon. *Tropiculture* 21:36-41.

GANNIBAL, P.B., ORINA, A.S., MIRONENKO, N.V. and M.M. LEVITIN. 2014. Differentiation of the closely related species, *Alternaria solani* and *Alternaria tomatophila*, by molecular and morphological features and aggressiveness. *European Journal of Plant Pathology* 24:345-368.

GOMEZ, K.A. and A.A. GOMEZ. 1984. Statistical Procedures for Agricultural Research. Wiley: New York.

KEMMITT, G. 2002. Early Blight of potato and tomato. *The American Phytopathological Society*, St. Paul, MN, USA.

NUPPENAU, W., KÖHLER, K.H., KOGEL, S., SCHNELL and J. SAUERBORN. 2005. Resistance induction in the pathosystem tomato *Alternaria solani*. PhD. Thesis Submitted to University of Giessen-Hesse, Germany.

PANDEY, K.K., PANDEY, P.K., KALLO, G. and M.K. BANERJEE. 2003. Resistance to early blight of tomato with respect to various parameters of disease epidemics. *Journal of General Plant Pathology* 69:364-371.

SHAHBAZI, H., AMINIAN, H., SAHEBANI, N. and D. HALTERMAN. 2010. Biochemical evaluation of resistance responses of potato to different isolates of *Alternaria solani*. *Phytopathology* 100:454-459.

TSEDALEY, B. 2014. Review on early blight (*Alternaria spp.)* of potato disease and its management options. *Journal of Biology, Agriculture and Healthcare* 4: 2224-3208.

TYMON, L. S., CUMMINGS, T. F. and D. A. JOHNSON. 2016. Pathogenicity and aggressiveness of three *Alternaria* species on potato foliage. *Journal of Phytopathology* 25:451-462.

VAN DER WALLS, J.E., KORSEN, L. and T.A.S. AVELING. 2001. A review of early blight of potato. *African Plant Protection* 70:91-102.

VAN DER WAALS, J.E., KORSTEN, L. and B. SLIPPERS. 2004. Genetic diversity among *Alternaria solani* isolates from potatoes in South Africa. *Plant Disease* 88:959-964.

WARTON, P. and W. KIRK. 2012. Early Blight. Potato Disease, Michigan State University.

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATIONS

Early blight is one of the most destructive tomato diseases, which causes severe defoliation and yield reduction under favourable climatic conditions (Abada *et al*., 2008). This study evaluated the morphological variation *A. solani* isolates collected from various areas across Limpopo Province. The results obtained in this study show that *A. solani* isolates exhibit high variations in mycelial pigmentation, number of septa, beak length and colony diameter. This variation is mainly due to changing climatic conditions, continued application of synthetic fungicides and natural mutation (Van der Waals *et al*., 2004; Chaerani and Voorrips, 2006).

The control of tomato early blight disease is based mainly on the application of synthetic fungicides. However due to the ability of *A. solani* to maintain high morphological and genetic variability, high incidences of fungicide resistance has been reported (Van der Waals *et al*., 2004; Rosenzweig *et al.*, 2008). The study was conducted to evaluate the sensitivity of *A. solani* isolates obtained from different areas in the Limpopo Province to commonly used fungicides in the management of tomato early blight. All the tested fungicides (chlorothalonil, copper oxychloride and mancozeb) reduced the mycelial growth of *A. solani* isolates collected from different areas around the Limpopo Province, even at lower concentrations. However copper oxychloride fungicide provides better inhibition of mycelial growth as compared to other tested fungicides.

Furthermore, the level of aggressiveness of the different isolates collected from various areas in the Province was investigated using Money-maker and Rodade tomato cultivars. The results obtained in our, study revealed that isolates from different areas differed in terms of their level of aggressiveness on both cultivars. However, all the tested isolates were aggressive in their ability to cause early blight in both cultivars. There was a difference on how both cultivars responded to isolates from different areas with the Money-maker being highly susceptible to all the tested isolates as than the Rodade. Factors such as varying environmental conditions, farming practices continuous application of chemical fungicides and monoculture might influence the evolution of new fungal strains which over time can become more aggressive than the pre-existing strains.

It is recommended that more isolates are collected across numerous areas in the Province and further studies need to investigate the genetic variation; sensitivity of isolates to the commonly used fungicides and aggressiveness of the isolates to other tomato cultivars commonly cultivated in the province. This is important as it will help to validate the existing morphological variations, fungicide sensitivity and aggressiveness of the isolates in order to identify the less susceptible tomato cultivar to early blight disease.

References

ABADA, K.A., MOSTAFA, S.H. and R. MERVAT. 2008. Effect of some chemical salts on suppressing the infection by early blight disease of tomato. *Egyptian Journal of Applied Science* 23:47-58.

CHAERANI, R. and R.E. VOORRIPS. 2006. Tomato early blight (*Alternaria solani*): the pathogen, genetics, and breeding for resistance. *Journal of General Plant Pathology* 72:335-347.

ROSENZWEIG, N., ATALLAH, Z.K., OLAYA, G. and W.R. STEVENSON. 2008. Evaluation of QoI fungicide application strategies for managing fungicide resistance and potato early blight epidemics in Wisconsin. *Plant Disease* 92:561-568.

VAN DER WAALS, J.E., KORSTEN, L. and B. SLIPPERS. 2004. Genetic diversity among *Alternaria solani* isolates from potatoes in South Africa. *Plant Disease* 88:959-964.

APPENDICIES

Appendix 3.1 Lists of isolates collected and confirmed as *A. solani* isolates from different areas around Limpopo Province

Blood-river		
Sample number	Isolate code	*A. solani* confirmation
1	B1	+
2	B2	+
3	B3	-
4	B4	-
5	B5	+
6	B6	+
7	B7	+
8	B8	+
9	B9	+
10	B10	+
11	B11	-
12	B12	-
13	B13	+
14	B14	-
15	B15	-
16	B16	-
17	B17	-

18	B18	+

Mankweng

Sample number	Isolate code	*A. solani* confirmation
1	M1	+
2	M2	+
3	M3	-
4	M4	+
5	M5	+
6	M6	-
7	M7	-
8	M8	+
9	M9	+
10	M10	+
11	M11	-
12	M12	+
13	M13	-
14	M14	-

Tzaneen

Sample number	Isolate code	*A. solani* confirmation
1	T1	+
2	T2	-
3	T3	-
4	T4	+
5	T5	+

6	T6	-
7	T7	-
8	T8	+
9	T9	+
10	T10	+
11	T11	-
12	T12	+
13	T13	+
14	T14	-
15	T15	-

Venda		
Sample number	Isolate code	*A. solani* confirmation
1	V1	+
2	V2	+
3	V3	+
4	V4	-
5	V5	-
6	V6	-
7	V7	+
8	V8	+
9	V9	+
10	V10	+
11	V11	-

12	V12	**+**
13	V13	**-**
14	V14	**-**
15	V15	**-**

(**+**) confirmed as *A. solani* and (**-**) not *A. solani.*

Appendix 3.2 Analysis of variance for mycelial growth for different *A. solani* isolates from different areas across Limpopo Province

SOURCE	DF	SS	MS	F	P
		Blood-river			
Treatment (Isolates)	9	253.53	28.1694	4.16	0.0015
Error	30	203.25	6.7750		
Total	39	456.775			
		Mankweng			
Treatment (Isolates)	8	497.50	62.19	9.69	0.0000
Error	27	173.250	6.42		
Total	35	670.75			
		Tzaneen			
Treatment (Isolates)	7	228.22	32.60	5.11	0.0012

Error	24	153.25	6.39		
Total	31	381.47			
		Venda			
Treatment (Isolates)	7	110.47	15.78	4.81	0.0017
Error	24	78.75	3.28		
Total	31	189.23			

Appendix 4.1 Analysis of variance for mycelial growth for different *A. solani* isolates at different concentrations of chlorothalonil

SOURCE	DF	SS	MS	F	P
		B1 isolate			
Treatment (Isolates)	5	2904.83	580.97	615.14	0.00
Error	18	17.00	0.944		
Total	23	2921.83			
		B2 isolate			
Treatment (Isolates)	5	5881.71	1176.34	345.70	0.00
Error	18	61.25	3.40		
Total	23	5942.96			
		B7 isolate			
Treatment (Isolates)	5	6896.50	1379.30	354.68	0.00
Error	18	70.00	3.89		
Total	23	6966.50			

Appendix 4.1 Analysis of variance for mycelial growth of different *A. solani* isolates at different concentrations of chlorothalonil

SOURCE	DF	SS	MS	F	P
		B9 isolate			
Treatment (Isolates)	5	4862.33	972.46	357.23	0.00
Error	18	49.00	2.72		
Total	23	4911.33			
		M1 isolates			
Treatment (Isolates)	5	6875.88	1375.18	529.48	0.00
Error	18	46.75	2.60		
Total	23	6922.63			
		M5 isolates			
Treatment (Isolates)	5	4299.83	859.97	524.73	0.00
Error	18	29.50	1.64		
Total	23	4329.33			

Appendix 4.1 Analysis of variance for mycelial growth of different *A. solani* isolates at different concentrations of chlorothalonil

SOURCE	DF	SS	MS	F	P
		M8 isolates			
Treatment (Isolates)	5	3644.88	728.98	121.78	0.00
Error	18	107.75	5.99		
Total	23	3752.63			

Appendix 4.1 Analysis of variance for mycelial growth of different *A. solani* isolates at different concentrations of chlorothalonil

SOURCE	DF	SS	MS	F	P
		T5 isolates			
Treatment (Isolates)	5	6002.33	1200.47	436.53	0.00
Error	18	49.50	2.75		
Total	23	6051.83			
		T10 isolates			
Treatment (Isolates)	5	6960.50	1392.10	177.09	0.00
Error	18	141.50	7.86		
Total	23	7102.00			

T13 isolates					
Treatment (Isolates)	5	7340.83	1468.17	593.87	0.00
Error	18	44.50	2.47		
Total	23	7385.33			
M9 isolates					
Treatment (Isolates)	5	4952.38	990.48	105.96	0.00
Error	18	168.25	9.35		
Total	23	5120.63			
T4 isolates					
Treatment (Isolates)	5	5226.83	1045.37	545.41	0.00
Error	18	34.50	1.92		
Total	23	5261.33			

Appendix 4.1 Analysis of variance for mycelial growth of different *A. solani* isolates at different concentrations of chlorothalonil

SOURCE	DF	SS	MS	F	P
		V3 isolates			
Treatment (Isolates)	5	6704.71	1340.94	353.65	0.00
Error	18	68.25	3.79		
Total	23	6772.96			
		V7 isolates			
Treatment (Isolates)	5	6811.33	1362.27	255.43	0.00
Error	18	96.00	5.33		
Total	23	6907.33			
		V8 isolates			
Treatment (Isolates)	5	7221.71	1444.34	237.97	0.00
Error	18	109.25	6.07		
Total	23	7330.96			
		V10 isolates			
Treatment (Isolates)	5	7289.88	1457.98	69.47	0.00
Error	18	377.75	20.99		
Total	23	7667.63			

Appendix 4.2 Analysis of variance for mycelial growth of different *A. solani* isolates at concentrations of copper oxychloride fungicide

SOURCE	DF	SS	MS	F	P
		B1 isolate			
Treatment (Isolates)	5	8539.00	1707.80	1336.54	0.00
Error	18	23.00	1.28		
Total	23	8562.00			
		B2 isolates			
Treatment (Isolates)	5	9056.88	1811.38	762.68	0.00
Error	18	42.75	2.38		
Total	23	9099.63			
		B7 isolates			
Treatment (Isolates)	5	2170.33	434.08	179.61	0.00
Error	18	43.50	2.43		
Total	23	2213.83			

Appendix 4.2 Analysis of variance for mycelial growth of different *A. solani* isolates at different of copper oxychloride fungicide

SOURCE	DF	SS	MS	F	P
		B9 isolate			
Treatment (Isolates)	5	7622.00	1524.40	685.98	0.00
Error	18	40.00	2.22		
Total	23	7662.00			
		M1 isolates			
Treatment (Isolates)	5	8119.00	1623.80	129.90	0.00
Error	18	225.00	12.50		
Total	23	8344.00			
		M5 isolates			
Treatment (Isolates)	5	6929.71	1385.94	53.51	0.00
Error	18	466.25	25.90		
Total	23	7395.96			

Appendix 4.2 Analysis of variance for mycelial growth of different *A. solani* isolates at concentrations of copper oxychloride fungicide

SOURCE	DF	SS	MS	F	P
		M8 isolates			
Treatment (Isolates)	5	6770.21	1354.04	47.81	0.00
Error	18	509.75	28.32		
Total	23	7279.96			
		M9 isolates			
Treatment (Isolates)	5	6184.33	1236.87	56.94	0.00
Error	18	391.00	21.72		
Total	23	6575.33			
		T4 isolates			
Treatment (Isolates)	5	8154.33	1630.87	793.39	0.00
Error	18	37.00	2.06		
Total	23	8191.33			

Appendix 4.2 Analysis of variance for mycelial growth of different *A. solani* isolates at different concentrations of copper oxychloride

SOURCE	DF	SS	MS	F	P
		T5 isolates			
Treatment (Isolates)	5	8862.50	1772.50	725.11	0.00
Error	18	44.00	2.44		
Total	23	8906.50			
		T10 isolates			
Treatment (Isolates)	5	8862.50	1772.50	590.83	0.00
Error	18	54.00	3.00		
Total	23	8916.50			
		T13 isolates			
Treatment (Isolates)	5	8154.50	1630.90	326.18	0.00
Error	18	90.00	5.00		
Total	23	8244.50			

Appendix 4.2 Analysis of variance for mycelial growth of different *A. solani* isolates at different concentrations of copper oxychloride

SOURCE	DF	SS	MS	F	P
		V3 isolate			
Treatment (Isolates)	5	7084.33	1416.87	22.43	0.00
Error	18	1137.00	63.17		
Total	23	8221.33			
		V7 isolates			
Treatment (Isolates)	5	8153.33	1630.67	1334.18	0.00
Error	18	22.00	1.22		
Total	23	8175.33			
		V8 isolates			
Treatment (Isolates)	5	8067.71	1613.54	452.04	0.00
Error	18	64.25	3.57		
Total	23	8131.96			
		V10 isolates			
Treatment (Isolates)	5	7860.50	1572.10		
Error	18	114.00	6.33	248.23	0.00
Total	23	7974.50			

Appendix 4.3 Analysis of variance for mycelial growth of different *A. solani* isolates at different concentrations of mancozeb

SOURCE	DF	SS	MS	F	P
		B1 isolates			
Treatment (Isolates)	5	7822.71	1564.54	346.61	0.00
Error	18	81.25	4.51		
Total	23	7903.96			
		B2 isolates			
Treatment (Isolates)	5	7149.00	1429.80	405.30	0.00
Error	18	63.50	3.53		
Total	23	7212.50			
		B7 isolates			
Treatment (Isolates)	5	4835.21	967.04	69.70	0.00
Error	18	249.75	13.87		
Total	23	5084.96			

Appendix 4.3 Analysis of variance for mycelial growth of different *A. solani* isolates at different concentrations of mancozeb

SOURCE	DF	SS	MS	F	P
		B9 isolate			
Treatment (Isolates)	5	4713.71	942.74	93.62	0.00
Error	18	181.25	10.07		
Total	23	4894.96			
		M1 isolate			
Treatment (Isolates)	5	5862.21	1172.44	237.79	0.00
Error	18	88.75	4.93		
Total	23	5950.96			
		M5 isolate			
Treatment (Isolates)	5	6431.71	1286.34	105.61	0.00
Error	18	219.25	12.18		
Total	23	6650.96			

Appendix 4.3 Analysis of variance for mycelial growth of different *A. solani* isolates at different concentrations of mancozeb

SOURCE	DF	SS	MS	F	P
		M8 isolate			
Treatment (Isolates)	5	6447.83	1289.57	50.90	0.00
Error	18	456.00	25.33		
Total	23	6903.83			
		M9 isolate			
Treatment (Isolates)	5	6362.33	1272.47	77.64	0.00
Error	18	295.00	16.39		
Total	23	6657.33			
		T4 isolate			
Treatment (Isolates)	5	5119.21	1023.84	298.45	0.00
Error	18	61.75	3.43		
Total	23	5180.96			

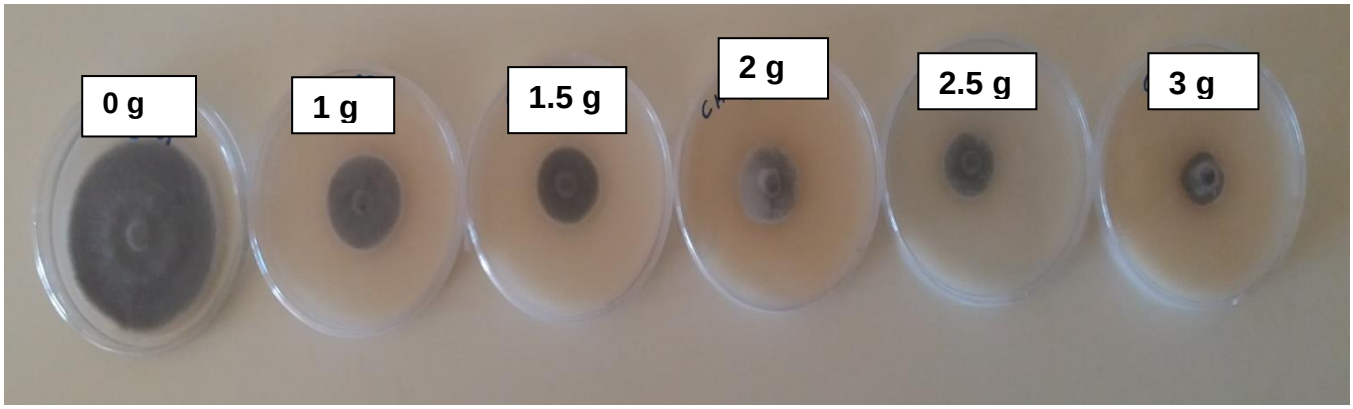
Appendix 4.3 Analysis of variance for mycelial growth for different *A. solani* isolates at different concentrations of mancozeb

SOURCE	DF	SS	MS	F	P
		T5 isolate			
Treatment (Isolates)	5	6484.50	1296.90	69.07	0.00
Error	18	338.00	18.78		
Total	23	6822.50			
		T10 isolate			
Treatment (Isolates)	5	6436.38	1287.28	220.15	0.00
Error	18	105.25	5.85		
Total	23	6541.63			
		T13 isolate			
Treatment (Isolates)	5	6455.33	1291.07	123.61	0.00
Error	18	188.00	10.44		
Total	23	6643.33			

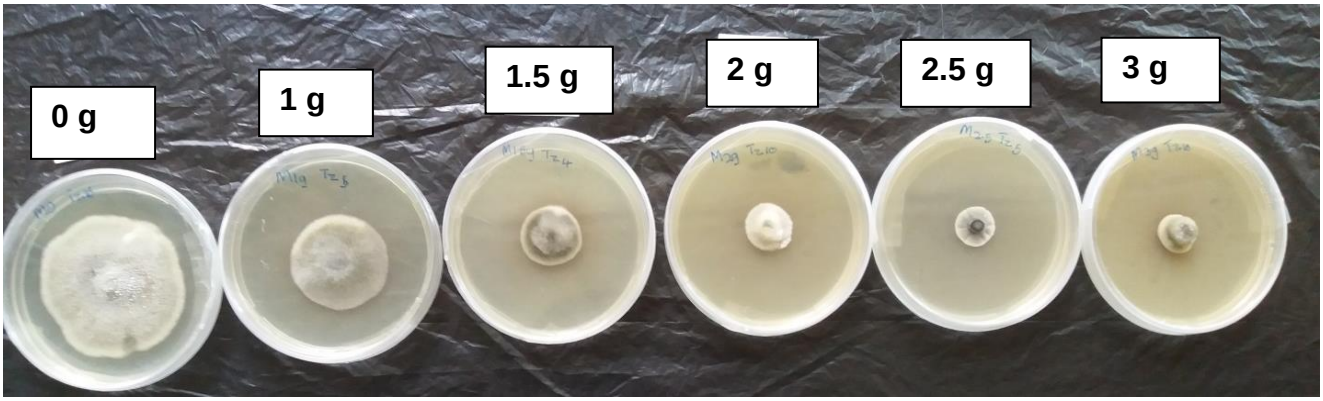
Appendix 4.3 Analysis of variance for mycelial growth of different *A. solani* isolates at different concentrations of mancozeb

SOURCE	DF	SS	MS	F	P
		V3 isolate			
Treatment (Isolates)	5	6119.33	1223.87	171.44	0.00
Error	18	128.50	7.14		
Total	23	6247.83			
		V7 isolate			
Treatment (Isolates)	5	5542.83	1108.57	118.42	0.00
Error	18	168.50	9.36		
Total	23	5711.33			
		V8 isolate			
Treatment (Isolates)	5	6315.38	1263.08	63.82	0.00
Error	18	356.25	19.79		
Total	23	6671.63			
		V10 isolate			
Treatment (Isolates)	5	6489.38	1297.88	114.94	0.00
Error	18	203.25	11.29		
Total	23	6692.63			

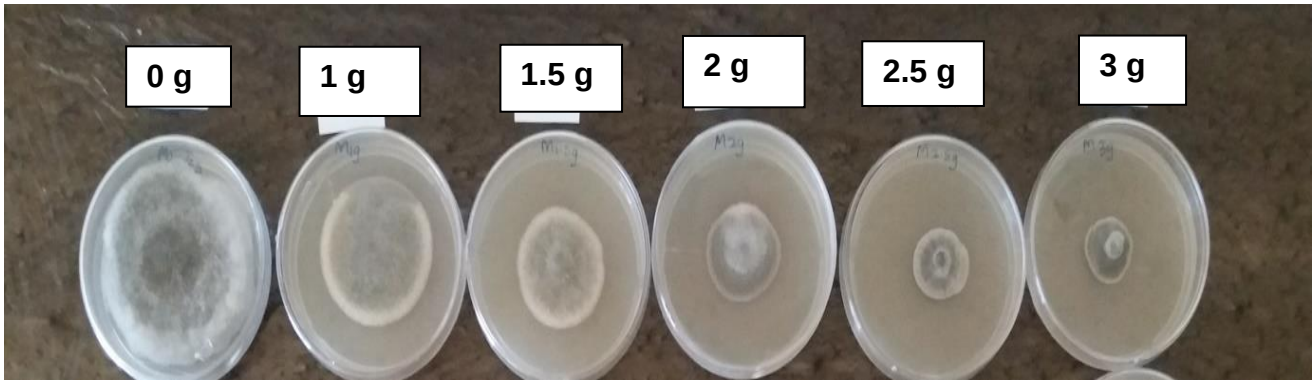
Appendix 4.4 Sensitivity of chlorothalonil fungicide at different concentrations against *A. solani* isolates seven days after incubation



Appendix 4.5 Sensitivity of copper oxychloride fungicide at different concentrations against *A. solani* isolates seven days after incubation



Appendix 4.6 Sensitivity of mancozeb fungicide at different concentrations against *A. solani* isolates seven days after incubation



Appendix 5.1 Tomato plants in the greenhouse covered with clear plastic create conducive environment for pathogen growth

Appendix 5.2 Partitioning of the treatment sum of square derived from the analysis of variance (ANOVA) for disease severity of Blood-river isolates (BI), Mankweng isolates (MI), Tzaneen isolates (TI) and Venda isolates (VI) of Money-maker and Rodade under laboratory conditions

		Money-maker cultivar								Rodade cultivar							
		BI		MI		TI		VI		BI		MI		TI		VI	
SOURCE	DF	SS	%	SS	%	SS	%	SS	%	SS	%	SS	%	SS	%	SS	%
Isolates	8	30148.3	99**	26977.2	99**	22208.7	99**	26195.8	99**	20533,6	99**	23831,9	99**	20674,8	99***	23724,9	99**
Error	27	169.0	1	83.9	0.31	133.3	1	142.0	1	156.0	1	44,9	1	294.3	1	125.7	1
Total	35	30317.3	100	27061.1	100	22342.0	100	26337.8	100	20689.7	100	23876.8	100	20969.0	100	23850.6	100

** = Significant at P ≤ 0.05

Appendix 5.3 Partitioning of the treatment sum of square derived from the analysis of variance (ANOVA) for disease severity of *A. solani* isolates on Money-maker and Rodade under greenhouse conditions

		Money-maker cultivar		Rodade cultivar	
SOURCE	DF	SS	%	SS	%
Replication	3	8.3	1	56.8	0
Isolates	12	23451.3	99**	21056.4	99**
Error	36	58.1	0	62.2	1
Total	51	23517.7	100	21175.4	100

** = Significant at P ≤ 0.05.