CHARACTERISATION OF MMUPUDU (*MIMUSOPS ZEYHERI)* LEAF RUST IN LIMPOPO PROVINCE

by

Shadrack Monyela

MINI-DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture (Plant Protection)**

in the

FACULTY OF SCIENCE AND AGRICULTURE

(School of Agricultural and Environmental Sciences)

at the

UNIVERSITY OF LIMPOPO

SUPERVISOR: DR Z.P. DUBE

CO-SUPERVISOR: PROF M.A. KENA

2021

DECLARATION

I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree Master of Science in Agriculture (Plant Protection) has not been submitted previously by me for a degree at this or any other University; that it is my work in design and in execution, and that all material contained herein had been duly acknowledged.

Monyela. S	21 April 2021
Surname, Initials (Mr)	**Date**

DEDICATION

To my beloved parent, Mapula Dorah Monyela and my nephew, Mosa Monyela.

ACKNOWLEDGEMENTS

My deepest sincere gratitude to the Almighty God, for giving me strength and ability to carry this study successfully. I would not have made it without you, my Father Who art in Heaven. Indeed your steadfast love never ceases and your mercies never come to an end. To my beloved parents Mapula Dorah Monyela and the late Brown Monyela, words will never be sufficient to express my gratitude. Thank you for your prayers, emotional and moral support from the day I enrolled for this degree, for that I am endlessly grateful. Because of your unfailing love and guidance; I am what I am today. I would also like to appreciate my brothers Thomas, Mpho and my sister Sarah for their support and patience throughout my study life, may the God Almighty continue to bless you in all areas of your lives. My special gratitude and unconditional love go to my family and friends, especially my niece, Winny Rachemula, thank you for all your encouragements, your love, support and patience. My deep and sincere gratitude to my hardworking supervisors Dr Z.P Dube and Professor M.A Kena, for their nonpareil support and guidance. Their excellent supervision, logical thinking, constructive criticism and support have been of great value for me. Thank you for believing in me, you sacrificed your time and energy to ensure I reach this far. I also appreciate assistance received from Ms MTP Hlokwe, Plant Pathology laboratory technician for her meaningful contribution in my research experiments and her suggestions. I was blessed with a great opportunity of working with her and learning different aspects of plant pathology. Thanks to the University of Limpopo for giving me an opportunity to study for my Master of Science degree. I am deeply indebted to Inqaba Biotechnology (Hatfield), Pretoria for assisting with DNA extraction and sequencing, Agriculture Research Council for leaf-phytochemical characterisation. I would also like to appreciate my fellow postgraduate students in the School of Agricultural and Environmental Sciences for all the assistance I had received, one way or the other, during the execution of this study.

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Mimusops zeyheri tree groves made up of seventeen trees collected from communities in Southern Africa were used in this study. The trees had high morphological variations in terms of growth rate, fruit (size and taste) and leaf (shapes and sizes) and their identification was made by communal people from where the trees were collected using their morphological characteristics. Generally, this evergreen tree is drought–tolerant, salt–tolerant and highly resistant to root-knot (*Meloidogyne spp*.) nematodes, along with various other pests. This could probably be attributed to high concentration of latex in aboveground organs. Some typical fungal rust symptoms have been observed believed to be the cause of high leaf abscission in certain groves. Currently, there is no report of leaf rust disease on *M. zeyheri* plants and the mechanism of resistance to other pests is not documented. The objective of this study was to (i) identify the pathogen associated with *M. zeyheri* leaf rust symptoms using morphological technique and to (ii) determine levels and types of potential defence chemicals and endophytes in *M. zeyheri*. Samples of *M. zeyheri* leaves showing rust like symptoms were collected from University of Limpopo, South Africa (23°53”10’S, 29°44”15’E) during summer in September 2018. Light compound microscope and electron microscope were used in the identification of the leaf rust spores. The species identity of the seventeen *M. zeyheri* trees that form a grove collection at University of Limpopo was confirmed using internal transcribed spacer (ITS) of ribosomal nuclear DNA. DNA extraction and sequencing was done with the help of Inqaba Biotechnologies. Obtained DNA sequences were aligned using CLUSTALX (2.0), with the phylogenetic tree constructed through the neighbour-joining method (NJM) in MEGA v. 5.1 programme. Evolutionary distances were computed using the Juke–Cantor method. Phytochemicals in leaves were identified and quantified using Liquid chromatography–mass spectrometry (LC–MS) at ARC-VOP. The pustules on the collected leaves contained reddish brown spores. The uredospores were oval and ellipsoidal under a light microscope. The size of spores ranged between 35-37 × 24-26 µm. The cell walls showed bilaminate structures with the outer layer hyaline. The warts were rod shaped with one subequatorial germ pore. The most common identified endophytic fungi observed in all *M. zeyheri* leaves were *Teratosphaeria* species,

Zeloasperium species, Pezizomycotina. In addition, endophytes such as *Cladosporium* species, *Aspergillus* species, *Phyllosticta* species and *Epicoccum* species were also identified to be associated with some *M. zeyheri* trees. There were significant differences on the level of tannins, flavonoids, proteins and phenolics among the *M. zeyheri* trees. The highest level of tannins was 7.2151 mg/g and the lowest being 2.7232 mg/g. The highest level of flavonoids was 1.1537 mg/g the lowest being 0.0123 mg/g. The level of phenolics among the trees ranged from 2.4749 mg/g to 1.5788 mg/g. Protein content ranged from 5.3100% to 2.7967% among the trees. Very high levels of tannins, flavonoids, phenolics among the trees when compared with others studies indicate the potential role of these metabolites in previously reported resistance of *M. zeyheri* to a number of pests. The morphological characteristics of the identified leaf rust pathogen causing rust symptoms on *M. zeyheri* in South Africa is more similar to *Maravalia species* previously isolated from *M. caffra*. And as such, this finding is paramount, as it is the first report of association between the pathogen and the plant. *Teratosphaeria* species has been associated with stem cancer in *Eucalyptus* trees, its presence in *M. zeyheri* species in asymptomatic plants is a major find since the tree is common in Limpopo and Mpumalanga, Provinces well known for forestry production making *M. zeyheri* a potential host for pathogens of commercial forestry production. Other endophytes observed such as *Aspergillus* species, *Phyllosticta* species and *Cladosporium* species have been shown to protect plants against some pests and pathogenic organism. Further studies to determine the direct role of identified phytochemicals and endophytes in the resistance of *M. zeyheri* to pests needs to be conducted.

TABLE OF CONTENTS

CHAPTER 1

RESEARCH PROBLEM

1.1 Background

1.1.1 Description of the research problem

Transvaal red milkwood, (*Mimusops zeyheri* Sond (Family: Sapotaceae Juss.), is a fruit tree indigenous to the northern and eastern regions of South Africa (Coates, 2002). *Mimusops zeyheri* fruit is exceptionally high in vitamin C enabling it to have a high potential to serve in economic and nutritional projects in arid and semi-arid regions (Venter and Venter, 1996). Most of these indigenous fruit trees are associated with treatment of or protection from medical conditions such as malnutrition, heart diseases, cancer and diabetes (Neudeck *et al*., 2012). *Mimusops zeyheri* is highly recommended for scurvy as it reduces its incidences, which are common within poor communities of southern Africa (Baliga *et al*., 2001). According to Mashela and Mollel (2001), in Limpopo Province, South Africa, local communities identified *M. zeyheri* as an indigenous fruit tree with the potential for domestication in their arid areas. Generally, this evergreen tree is drought–tolerant, salt–tolerant and highly resistant to root-knot (*Meloidogyne* species) nematodes, along with various other pests. This has been attributed without any empirical data to high concentration of latex in aboveground organs and thick leathery leaves (Mashela and Mollel, 2001; Pofu *et al.*, 2012).

In a locally established *M. zeyheri* plantation consisting of trees collected from different parts of southern Africa, some typical fungal rust symptoms have been observed believed to be the cause of high leaf abscission in certain groves. Currently, there is no report of leaf rust disease on *M. zeyheri* plants. Globally, plant diseases account for over 16% of food production losses resulting in cost of approximately US$ 220 billion annually, representing a threat to food security (Agrios, 2004; Strange and Scott, 2005). Besides direct disease losses, the use of chemicals in the management of crop diseases has influenced negatively on the environment, chemical residues have found their way into food. With alarming increase in the introduction of new diseases on new host in new

geographic areas and increased incidences and severity of once minor diseases, frequent and continuous global monitoring and national surveillance of diseases is of paramount importance.

Rust diseases are among the most widespread and economically important diseases of crops worldwide, with wheat and coffee rusts being by far among the most important diseases (Cressey, 2013). The frequency, extent and impact of rust diseases has increased significantly in the last two decades causing global concerns (Thomson *et al*., 2010). Their high capacity of developing new races makes them even more difficult to manage.

Besides the two most studied fungal rusts, wheat and coffee rust, rust fungi are far from being fully known at the species level with a considerable species diversity in this group believed to be unknown, mainly due to the fact that plants of many parts of the world, especially in the tropics and subtropics that host rust fungi have received less research attention (Newcombe, 2004).

Limpopo Province being deemed to be the tropical and subtropical fruit-producing region of South Africa, and the area where most of the fruiting vegetables are produced (Ntombela and Moobi, 2013); the appearance of any disease with potential to cause losses is of major concern. The objective of this study was to (i) identify the pathogen associated with *M. zeyheri* leaf rust symptoms using morphological technique and to (ii) determine levels and types of potential defence chemicals and endophytes in *M. zeyheri*.

1.1.2 Impact of the research problem

According to Von Maydell (1986), worldwide rural communities depend on various indigenous plants for enhancement of food security, nutrition and income needs. For instance, income and employment can be obtained through selling of their fruits and leaves, which also contain medicinal benefits. Fruits of *M. zeyheri* have been confirmed to contain the highest edible vitamin C per unit when compared to other locally available fruits, both endemic and exotic (Venter and Venter, 1996). Despite the tree being resistant to other biotic and abiotic stresses such as nematodes, drought and salt, high susceptible

to rust fungi has been observed in some trees. Trees showing rust symptoms also displayed severe leave defoliation and, reduced plant growth.

1.1.2 Possible causes of the research problem

Pest and disease outbreaks have been identified as some of the limiting factors in crop production especially under current global change conditions (Logan *et al*., 2003; Thomson *et al*., 2010; Macfadyen *et al*., 2018). Reports of Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) (Dube *et al*., 2016) and mealybug (*Pseudococcus citr*i Risso) (Mokhoelele *et al*., 2018) on *M. zeyheri*, a plant previously viewed as non-host to identified insect pests (Mashela and Mollel, 2001), could easily serve as indicators of this phenomenon. In the history of plant disease, fungi causing rust have become the most important pathogens co-evolving with different host plants. They have the capacity to cause severe damage to plants that may possibly reduce yield and reproduction in various plants (Kolmer *et al*., 2009). In addition, the presence of rust on *M. zeyheri* may result in poor fruit yield quality and quantity. Furthermore, there is a lack of existing knowledge on the type of rust affecting this tree and impacts negatively on the available management strategies. According to Brown and Hovmøller (2002), with their abundant and typically wind-dispersed spores, rust fungi are also among the most mobile plant pathogens globally.

1.1.3 Proposed solution

Proper identification of this pathogen is of paramount importance, for any conducive management strategy to be developed on pests such as *M. zeyheri* leaf rust on a crop of which most of the communal farmers are dependent on.

1.1.4 General focus of the study

The study is primarily focused on identifying pathogen that cause damage to *M. zeyheri* tree based on the observed leaf-rust like signs and symptoms on the leaves. Since the presence of some chemicals and endophytes in the plant are believed to enhance resistance, this study also focuses on establishing the defence chemicals found on the *M. zeyheri* and endophytes associated with tree.

1.2 Problem statement

Mimusops zeyheri is an indigenous fruit tree in Limpopo Province known for its potential for domestication due to its medicinal and nutritional aspects (Mashela and Mollel, 2001). However, the presence of pests and diseases such as leaf rust on this plant is expected to affect various physiological functions, more especially photosynthesis, which will further affect plant growth and fruit formation. There are currently no reports on rust disease on *M. zeyheri* tree and this can negatively affect domestication of this plant and disease management. For any appropriate and effective disease management strategy to be developed, proper and conclusive identification of the pathogen causing the disease needs to be done. Confirmation of the causal organism is of paramount importance in the development of resistant cultivars for disease management purposes.

1.3 Rationale of the study

Mimusops zeyheri is one of the most important fruit trees with advantageous socio-economic attributes and highly nutritious. This fruit tree is relatively pest-free owing to its copious concentrations of latex in the aboveground organs. However, recent identification of Mediterranean fruit fly (*Ceratitis capitata*), mealybug (*Pseudococcus citri*) on the fruits of *M. zeyheri* poses a major threat to its domestication. The fungal rust presence on this plant also threatens its economic and neutraceutical contributions to the rural poor who are dependent on it. *Mimusops zeyheri*, being evergreen and indigenous to the region that is a major producer of tropical and subtropical commercial fruit and fruit vegetables, pose an additional threat of acting as a source of insect pests and diseases overwintering site. The identification of pests of *M. zeyheri* is not only important for the local rural

community communities of Limpopo Province but for the fruit producing industry, that contributes 2.1% to the gross domestic product (GDP) (Dube *et al*., 2016).

1.4 Purpose of the study

1.4.1 Aim

Identification of pathogen associated with rust on *M. zeyheri* and determination of potential defence chemicals and endophytes.

1.4.2. Objectives

1. To identify the pathogen associated with *M. zeyheri* leaf rust symptoms using morphological technique

2. To determine levels and types of potential defence chemicals and endophytes in *M. zeyheri*

1.5 Hypotheses

1. The pathogen associated with leaf rust-like symptoms on *M. zeyheri* will be identified using morphological technique.
2. *Mimusops zeyheri* has chemicals and endophytes with potential for pest resistance.

1.6 Reliability, validity and objectivity

Reliability of data was based on statistical analysis of data at the probability level of 5%. Validity was achieved by involving experts and state of the art equipment in the field, Inqaba Biotechnologies, leaders in molecular work were engaged in DNA extraction and sequencing used in the identification of endophytes, ARC-VOP was used for extraction of phytochemicals in *M. zeyheri* leaves. Objectivity was achieved by ensuring that the results were discussed on the basis of empirical evidence, thereby, eliminating all attributes of subjectivity (Leedy and Ormrod, 2005).

1.7 Bias

Bias was minimized by ensuring that experimental error in each experiment was contained through sufficient replications (Leedy and Ormrod, 2005).

1.8 Scientific significance of the study

Findings of this study will expand knowledge on fungal species that are of a major threat to the sustainable cultivation of *M. zeyheri.* Furthermore, this will improve the potential for domestication of this economically valuable fruit tree most importantly in rural communities. This study will also serve as important information for South African national collection of fungi for the future reference. This study will further assist in determining potential phytochemicals and endophytes that improve resistance of this fruit tree against any major pathogens.

1.9 Structure of mini-dissertation

Chapter 1 would focus on description of the research problem, followed by Chapter 2 that addressed work done on the research problem, literature review. Chapters 3 and 4 would focus on addressing Objectives 1 and 2, respectively. In the final Chapter (Chapter 5), findings in all Chapters would be summarized and integrated to provide the significance of the findings and recommendations with respect to future research, followed by conclusions. The citation in text and listing of references adopted the Harvard style as approved by the Senate of the University of Limpopo.

CHAPTER 2

LITERATURE REVIEW

2.1 Work done on the problem statement

2.1.1 Importance of indigenous wild plants

Indigenous plants such as Mmupudu (*Mimusops zeyheri*) have been frequently reported to assume a central role in the lives of people by significantly improving their spiritual and material needs (Chadare *et al*., 2010). Most of indigenous plants have been adopted particularly in deep rural areas for their significant role as traditional medicine and are currently being scientifically researched for their pharmacological properties (McGaw *et al*., 2007). Concurrently, the estimation by the World Health Organization is such that up to 80 percent of people still rely mainly on traditional medicine (WHO, 1999). In Swaziland reports show that a root infusion of *M. zeyheri* is being used in the treatment of candidiasis (Amusan *et al*., 2002). Among the indigenous plants, a related species, *Mimusops elengi* is one of the Sapotaceae family that has been researched in greater details for their medicinal role and has displayed great medicinal value in various parts of the world (Baliga *et al*., 2011). This plant has been explored to play a valuable role in the treatment of dental ailments like bleeding gums, pyorrhea and loose teeth (Prabhat, 2010).

Most research studies have reaffirmed that various parts of *M. elengi* such as unripe, ripe fruits and flowers along with other astringent are reliably used to prepare a lotion for the sores and wounds in many countries (Chaiyan *et al*., 2009). The flowers of *M. elengi* are often dried to prepare a snuff and, this has been reported to cure a disease associated with strong fever, headache, and pain in the neck, shoulders and other parts of the body called ‘Ahwah’, common in Bengal (Siddhuraju, 2002). Powdered flowers of this plant are strongly believed to induce a copious defluxion from the nose and relieve the headache (Kala *et al*., 2011). Several antioxidant activities of plant extracts which are linked with the occurrence of anthocyanins, phenolic acids, flavonoids and tannins are being discovered on various indigenous plants and are firmly believed to be health promoting because of their antioxidant properties (Esam, 2000). According to Huda-Faujan (2009),

currently there is an increased interest in polyphenolic antioxidants because of their elevated capacity in scavenging free radicals associated with various diseases. As a result, this led to higher reliance on indigenous plants as they ensure livelihood security for countless families and communities around the world. In addition, the significant contributions of most indigenous plants such as *Mimusops caffra* in South Africa has led to its protection under the South Africa National Forest Act of 1998 (Government Gazette, 2013). According to Louppe *et al*. (2008), the bark extracts of *M. caffra* are used to treat wounds and sores in Zululand, South Africa. Moreover, a bark maceration of this plant has been reported to be used as an emetic (Neuwinger, 2000) while the root extracts are used in the treatment of sexually transmitted infections such as gonorrhea (De Wet *et al*., 2012). Further studies revealed that the leaf extracts of *M. caffra* contain ursolic acid which has anti-plasmodial activity and has been confirmed to manage malaria (Simelane *et al*., 2013).

Most of indigenous plants continue to play key role especially in rural communities and serve as sources of shelters, clothes, firewood and food. Most food industries use the fruits of *M. caffra* for jelly and it is also used for alcohol production because it is of ethnobotanical significance (Engels *et al*., 2002). Furthermore, another related species, *Mimusops obovata* continue to be exploited by several African countries for traditional medicine and as a source of food (Germishuizen *et al*., 2006). The roots decoction of *M. obovata* have been reported to be used for the treatment of gonorrhea and schistosomiasis (Neuwinger, 2000). In addition, the stem bark has been preferably used as an emetic, where bark maceration is drunk (Germishuizen *et al*., 2006). Concomitantly, inner bark has been reported to be steeped in water and the liquid is drunk for the treatment of stomach pain in various parts in Africa (Neuwinger, 2000). The *M. zeyheri* produce edible fruits for human consumption which were discovered to contain high vitamin C content (90 mg per 100 g) (Lemmens, 2005). Alternatively, the fruits are also used for production of jams, jellies and fermented juice (Lemmens, 2005). Consequently, commercialisation of fruits of edible wild plants such as *M. zeyheri* is increasing because of increased demand for fruit in urban centers and as a result of limited alternative economic options for the rural people. Edible fruits produced by the majority of *Mimusops* species also play a crucial role in poverty alleviation by enhancing ecotourism, access to

improved food, and nutritional security are also well recognized (Makonda *et al*. 2003). The estimation from reports suggest that 15 million people in sub-Saharan African countries earn income from forest-related activities (Oksanen *et al*., 2003). According to Mashela and Mollel (2001), advantages of indigenous plant species are that they are already acclimatised to local conditions, therefore, have a better chance of enhancing more sustainable agro-systems, combating land degradation and conserving the natural plant heritage. *Mimusops zeyheri* has been frequently reported to be prevalently occurring to the regions which are characterised by semi-arid climate with low rainfall, which occurs mainly during hot summers particularly in northern parts of the former Transvaal Province of South Africa (Mashela *et al*., 2013).

2.1.2 Pest and diseases of *Mimusops* species

Although the *Mimusops* species are favorable for commercial forestry and medicinal purposes, speculations are such that they are resistant to various pests and diseases because no close attention has been paid on the biotic stresses that they may experience. And this, may pose a serious limiting factor to the successful expansion of this particular species in South Africa. Mediterranean fruit fly (*Ceratitis capitata*) is a pest of a number of fruit crops that causes economic losses and threatens the sustainability of tropical and subtropical fruits and vegetable industries in many countries (Dube *et al*., 2016). The notoriety of this pest derives from its wide host range, explosive reproduction capacity and unusual resilience to adverse ecological condition. A previous study revealed that *M. zeyheri* is a host of *C. capitata* (Dube *et al*., 2016). Therefore, *C. capitata* from infestation reservoirs of *M. zeyheri* fruit trees could be a major threat to the tropical and subtropical fruit industries in South Africa owing to the fruit-bearing nature of the new host. In recent years, die-back symptoms associated with two Botryosphaeriales species were identified on *M. caffra* in South Africa (Osorio *et al*., 2017). These included the known species *Neofusicoccum mangroviorum* and *Pseudofusicoccum africanum*. According to Crous (2017), this was the first record of Botryosphaeriales on *M. caffra* and the report revealed that *N. mangroviorum* is the most common cause of die-back on these trees. Khatun and Chatterjee (2011), noticed symptoms associated with die-back disease such as thinning of leaves and crown, drying up of the ends of branches, table topped conditions and stag

headness on the plant tree *M. elengi*. The cause of this die-back was identified as *Curvularia lunata* a pathogen associated with enormous economic loss in many countries particularly India (Khatun and Chatterjee, 2011). Lokesh *et al*. (2017), observed the symptoms associated with *Pestalotiopsis clavispora* on the plant *M. elengi* and this was recorded for the first time at the University of Mysore, India. Furthermore, Chaurasia and Suwannarach *et al*. (2013), discovered leaf blight caused by *Pestalotiopsis versicolor* on the *M. elengi* growing on trees at Saugar University, India. This leaf blight disease heavily infected about 95% of *M. elengi* trees. The symptoms of wilt and dieback were frequently observed in the ground of Prince of Songkla University in Thailand and were reported to cause a sudden decline of *M. elengi* (Tarigan *et al*., 2011). And the fungus causing these symptoms was morphologically identified to *Ceratocystis manginecans*, a widely distributed species pathogenic on mango (Ploetz, 2013). Severe leaf spot that first appeared as minute specks with ashy-white centre surrounded by a brownish margin on *M. elengi* leaves which gradually increase in size were reported. These spots showed abundant formation of circular black fruiting bodies at a later stage identified in infection spot the presence of three pycnidial fungi belonging to the *Microdiplodia*, *Phyllosticta* and *Robillarda* species (Keith *et al*., 2006) However, not much has been done in understanding the disease pathogen spectrum on *M. zeyheri* (Suwannarach *et al*., 2013).

Berndt (2008), reported that there are approximately 550 species of rust recorded from southern Africa. According to Roux *et al*. (2016), some introduced taxa have severely impacted agriculture, forestry and endangered native plants such as *Uromycladium acaciae* (Little and Payn 2016). Furthermore, apart from causing diseases to indigenous fruit trees such as *M. zeyheri,* rust fungi have also been intentionally introduced to South Africa as biological control agents, such as *Uromycladium tepperianum*, to control the weedy and exotic *Acacia saligna* (Wood and Morris, 2007). One of the best known pathogen that continues to ravage most of indigenous plants is the fungus *Puccinia psidii* and is more established on *Eucalyptus*. However, recently it has been spotted on *Mimusops laurifolia* suspected to be the one causing severe damage (Glen *et al*., 2007). Recent research has revealed the fungus occurs as a native on native Myrtaceae in southern Africa and it is presumed to have undergone a host shift to infect other indigenous plants (Heath *et al*., 2006).

2.1.3 The importance of endophytic fungi on plants

Endophytes are generally known as microorganisms residing in intercellular spaces or inside cells of host plant causing no apparent damage or disease (Suryanarayana, 2011). Concomitantly, endophytes have been reported to improve plant growth by secreting phytohormones and in nutrition improvement using bidirectional nutrient transfer and enhancement of the health of plants by protecting them against plant pathogens (Andreozzi *et al*., 2019). Furthermore, the considerable contribution of endophytes to the host defences against phytopathogenic organisms generally improves plant growth (Giménez *et al*., 2007). Reports from several studies have reaffirmed that plants infected with endophytes obtain growth promotion (Barka *et al*., 2002). Many research studies demonstrated that an increase in plant growth usually will prevent a variety of abiotic and biotic stresses reflecting plant vigor or persistence and regarded as a potential protection to pathogen challenge (Kuldau and Bacon, 2008). Collectively, many of these endophytes are found in wide range of plants and have become important due to their ability to secrete bioactive metabolites that have capacity to hinder the growth of fungal pathogens (Khan *et al*., 2019). Fungi and bacteria are the most recognised beneficial endophytes that are believed to actively colonise the internal tissues of plants without causing visible damage thus protecting the plants against plant pathogens (Kumar *et al*., 2012). And as a result, endophytic niche sequentially become established. After the endophytic niche is successfully established, endophytes constantly obtain good source of nutrition supplied from fragment, exudates and leachates and protect host against other microorganism (Suryanarayana, 2011). According to Pal and Gardener (2006), fungal endophytes are believed to protect plant by rapid colonisation and thereby exhausting the limited available substrates to ascertain that none would be available for pathogens.

Worldwide, there are at least 1 million species of endophytic fungi in all plants (Kumar *et al*., 2012). Numerous previous research studies have reaffirmed endophyte fungi to have the ability to protect host from disease and limit the damage caused by plant pathogens (Arnold *et al*., 2003). *Cryptosporiopsis cf. quercina* and *Colletotrichum* sp., have been reported to be effective against phytopathogens such as *Rhizoctonia cerealis*, *Phytophthora capsici*, *Pyricularia oryzae* and *Gaeumannomyces graminis* (Lu *et al*.

2000). In addition, *Trichoderma* and *Aspergillus* are used to manage many soil-borne plant pathogens (McLeod, 1995). According to Gunatilaka (2006), plenty of secondary metabolites have been discovered particularly those isolated and characterised from endophytic fungi and bacteria. These secondary metabolites are strongly believed to display antifungal and antibacterial properties which have been proven to be effective in inhibiting the growth of phytopathogenic microorganism. These metabolites include alkaloids, terpenoids, steroids, isocoumarins and chromones, phenolics and volatiles (Kumar, 2012). Alkaloids have been shown to be suppressive towards a number of microbes. Altersetin, an alkaloid isolated from endophytic fungus *Alternaria* spp., showed antibacterial activity against several pathogenic gram-positive bacteria (Hellwig *et al*., 2003). In addition, extensive research on endophytic fungus, *Muscodor albus* of tropical tree species, revealed great potential in producing many volatile organic compounds including tetrohydofuran, 2-methyl furan, 2-butanone and aciphyllene which have antibiotic activities (Atmosukarto *et al*., 2005). Many isolation of endophytes from medicinal plants led to the discovery of potential compound which are currently applied in the health fraternity (Daisy *et al*., 2002). Isolates of endophytic fungi, *Rhizoctonia bataticola* from inner tissues of root and stems of *Coleus forskohlii* has been reported to stably synthesise forskoline and release it into the broth (Mir *et al*., 2015). According to Pateraki *et al*. (2017), the potential application of forskolin has since proved to alleviate glaucoma, treatment of hypertension, body weight control and heart failure and these remarkable outcomes led to its approval. *Penicillium* species and *Aspergillus* species are among the endophytes that have been recognised as rich source of bioactive metabolites (Fill *et al*., 2007). They have been reported to produce a range of medicinally important metabolites including antifungal (Nicoletti *et al*., 2007), insecticidal (Singh, 2003) and taxol which today is largely used to treat human cancer (Stierle *et al*., 2006). They are also popularly considered as soil inhabitant or as contaminant of food, fruits, fibre and other starchy materials, which are believed to play crucial role in plants tolerance to abiotic stress (Visagie *et al*., 2014). A wide range of activities of endophytic fungi has been reported either by induction of host plant resistance or by production of secondary metabolites, which in turn protect the plant (Kusari *et al*., 2012). Most of the fungal endophytes are believed to impart enhanced tolerance to abiotic stresses. For example,

enhanced host plant resistance to insects has been reported in *Acremonium* endophyte host interactions (Schulz *et al*., 2015). According to Faeth and Fagan (2002), an increase in growth rate has been observed in tall fescue plants infected with *Neotyphodium* endophytic fungi, however, beneficial effects of endopohytic fungi on plant growth diminished with increased soil moisture and nutrients. In addition, root infection with endophytic fungi have been constantly reported to produce more phenolics elicits greater plant defense reaction (Schulz *et al*., 2015). In many studies, endophytes and cell-free washings of their culture plates were proved to reduce the density and size of *Puccinia* pustules in a susceptible cultivar of wheat, when inoculated 3, 7, and 14 days prior to invasion of the pathogen (Dingle and McGee, 2003). This interactions between endophytes and *Puccinia* has been suggested to be most probably mediated by defence mechanism (Dingle and McGee, 2003). Most researchers have discovered that fungal endophytes produce large amount of bioactive compound not only useful for plants but also are of economical importance to humans (Rodriguez *et al*., 2009). They have been confirmed to serve as antibiotics, drug or medicines, or the compounds of high relevance in research or as compounds useful to food industry (Rodriguez *et al*., 2009). For example, an endophytic microorganism *Metarhizium anisopliae* isolated from *Taxus chinensis* was recently reported to produce taxol in abundance in vitro which is used to cure breast cancer, ovarian cancer and lung cancer (Liu *et al*., 2009).

2.1.4 Mechanism of plant rust infection

According to Agrios (2009), plant pathogenic fungi are likely to cause most of the diseases occurring in agricultural and horticultural setups. Concomitantly, the fungal pathogens including the rusts have developed mechanisms and ways to attack any plant (Knogge, 1996), primarily to gain entry and source nutrients forcefully for their growth and development (Horbach *et al*., 2011). Generally, these pathogens have the capacity to overcome plant immune defences and this can therefore negatively affect plant heath, plant homeostasis, plant physiology and in some cases causes systemic damage (Agrios, 2005). Rust fungi are prevalently known to have complex life cycles and most of which are foliar pathogens involving two parasitic stages, dikaryotic and monokaryotic stages (Agrios, 2005). Voegele *et al*. (2009), reported that dikaryotic stage in most of rust fungi

is initiated when urediospore germ tube responds to topographical features of the leaf surface so that it grows towards a stoma and recognises its presence by responding to the ridges around the stomatal lips (Terhune *et al*., 1991). Numerous research studies indicated that in response to the stomatal lips, the fungus sequentially forms an appressorium over the stomatal opening, an infection peg that grows between the guard cell and a spherical substomatal vesicle, and an infection hypha that grows intercellularly between mesophyll cells (Loehrer *et al*., 2014). This specialised structure is strongly believed to be a prerequisite for disease initiation. According to Stone *et al*. (2012), this morphological differentiation is accompanied by changes in fungal wall composition and the induction of mitosis changes in protein synthesis (Talhinhas *et al*., 2014). In addition, the initiation of ribosome synthesis and secretion of variety of hydrolases takes place (Cooper *et al*., 2016). The germ tube continues to grow on the leaf surface until its endogenous reserves are depleted and it dies if there is no differentiation taking place (Agrios, 2005). Numerous studies indicated that most rust fungi develop appressoria more frequently over or near the anticlinal wall of the epidermis as their form of infection to their host plants. Most rust fungi, such as rust bean, *Uromyces appendiculatus* has been greatly studied in details and has been reported to be triggered to form numerous appressoria equally well on a variety of substrates, such as leaf stomata or polystyrene replicas of stomata (Cooper *et al*., 2006). Yin *et al*. (2015), reported appressorium formation of *Puccinia graminis* on collodion membrane.

In addition, haustoria is thought to be one of the important specialised infection structure of rust fungi that is responsible for secretion of elicitors. According to Catanzariti *et al*. (2006), this specialised structure usually forms after penetrating a live host cell, expanding on the inner cell wall while invaginating the surrounding plasma membrane. Through haustoria, the pathogen obtains nutrients from the host and secretes virulence proteins that are known as effectors and are believed to be key players in manipulating the physiological immune response of host cells (Voegele *et al.*, 2003) and ultimately causing disease on the plant. Pasturage (2003), reported that other obligate biotrophs, such as the oomycete downy mildew pathogens and the ascomycete powdery mildews, have independently evolved a similar infection process, however, the powdery mildews propagate aerial hyphae on the leaf surface and form haustoria only in epidermal cells.

Melampsora lini indicated similar infection process in most of its host plants including *Linum marginale* found in a broad range of habitats (Burdon, 1999).

2.1.5 Role of phytochemicals in defence mechanism

According to Hammerschmidt (2005), most of the plants have been proven to produce a large range of secondary metabolites, and some of them accumulate in high concentrations in bark, roots and needles. These metabolites are involved in plant response to numerous biotic stress factors, including fungal pathogens (Chong *et al*., 2009). Phenolic metabolites have been confirmed to be frequently linked to defense mechanisms of plants against natural enemies, such as pathogenic fungi (Hammerschmidt, 2005). The evidence confirming the antifungal function of phenolics originates from studies with herbaceous plants, such as tobacco, for which advanced molecular methods have long been available (Maher *et al*., 1994). Other metabolites that are involved in the plant response against the pathogenic fungi may include flavonoids, tannins, terpenes and alkaloids. Flavonoids, such as taxifolin and its glucoside, have been reported to occur constitutively in Scots pine phloem tissues and have been related to reaction efficiency against the bark beetle associated fungi *Ophiostoma brunneo-ciliatum* and *Leptographium wingfieldii* (Six and Wingfield, 2011). Several flavonoids were further confirmed to accumulate locally in the reaction zone after fungal infection and when they are induced systemically in the needle. Production of flavonoids was induced by different challenges such as wounding, indicating that the response was nonspecific (Mouradov and Spangenberg, 2014). Furthermore, tannins also among the most widely occurring secondary metabolites of plants and are functionally known to play a role in plant resistance by exhibiting direct toxicity against a wide variety of microbes (Sivakumaran *et al*., 2004). However, the mechanistic basis for this activity is said to be not well described. One explainable and reasonable mechanism may be associated with the ability of tannins to bind and precipitate proteins (Barbehenn and Constabel, 2011) that are necessary for pathogen growth and survival, such as the *Phytophthora* elicitin protein. In addition, terpenoids are also one of the exceptionally diverse class of secondary metabolites with enzymes which are numerous and highly promiscuous (Pichersky and Raguso, 2018). Many terpenoids have been frequently reported to be

incredibly active against pests and pathogens and an evolutionary arms race with these attackers are presumed to be a major driver behind the increasing terpenoid diversity seen throughout plant evolution (Pichersky and Raguso, 2018). Terpenoid aldehydes are the most group which have been studied extensively particularly in plant nematode interactions and have been proven to be effective in controlling some pests and pathogens. For example, an extract from *Gossypium arboreum*, which is believed to produce only unmethylated terpenoid aldehydes showed significantly lower anti-nematode activity (Kihika *et al*., 2017). Furthermore, Saponins have also been confirmed to be one of the secondary metabolites involved in plant defense against pests and pathogens. A major example of saponins are glycoalkaloids produced by various Solanaceae, including α-tomatine from tomato and α-solanine and α-chaconine from potato (Moses *et al*., 2014). α-Tomatine proved to play a crucial role in tomato resistance to fungal pathogens and insect pests, however, when evaluated against *Meloidogyne incognita* no correlation between α-tomatine production and resistance could be discerned (Moses *et al*., 2014). Moreover, root α-tomatine concentrations were reported to be unchanged by *M. incognita* infection at all examined time points (between 3 and 14 days postinoculation) in both a resistant and a susceptible cultivar (Osbourn, 1996).

2.2 Work not yet done on the problem statement

The evergreen Transvaal Red Milkwood (*Mimusops zeyheri*) had been targeted as an urban- and rural-greening tree in Limpopo Province due to its excellent nutritional, medicinal and aesthetic attributes (Radzuma, 2017). However, this tree tends to be attacked by disease caused by fungus which may eventually cause enormous economic loss in our country and also reduces its potential for domestication. Recent reports suggest that plant species representing grasses, palms and conifers have been studied worldwide for their presence of endophytic fungi (Stone *et al*., 2000). However, it is apparent that to date very few medicinal indigenous tree species such as *M. zeyheri* have been screened for their fungi or endophytic fungi. As much as the symptoms have been observed on *M. zeyheri* believed to be the cause of high leaf abscission, more work needs to be done to identify the fungi causing these symptoms or leaf rust diseases because currently there is no report on such. Furthermore, the ecological roles played by

endophytic fungi are diverse and varied (Saikkonen, 1998). According to Clay (1998), endophytic fungi are best described as mutualists that protect various indigenous plants, grasses and conifers (Carroll, 1991) against pathogenic organism that may eventually cause disease to the plants. Furthermore, many of those fungi produce biologically active secondary metabolites (Ruma *et al*., 2013). Gunatilaka (2006), reported antibacterial or antifungal activity for more than 30% of the endophytic isolates from ericaceous plants, and Wu *et al*. (2013), reported antibiotic activity from isolates of the endophytic *Pleurophomopsis* species and *Cryptosporiopsis* species, as well as from a sterile endophyte from *Abies alba*. In addition, many indigenous plants have been studied from the point of view of potential endophytic interactions, and have shown many benefits, such as production of antibiotics, secondary metabolites of pharmacological interest, biomarkers of vitality, and biological control agents against pests and diseases (Sun *et al*., 2008). Despite the biotechnological potential of *M. zeyheri* to our knowledge no-one has examined the endophytic fungal community of this plant or the biotechnological potential of these microorganisms.

2.3 Addressing the gap

As much as the symptoms assumed to be caused by leaf rust on *M. zeyheri* plant has been observed, there is no sufficient information on the pathogen associated with identified symptoms. And, as such this could make it difficult for many farmers to develop appropriate and effective disease management strategy.

CHAPTER 3

MORPHOLOGICAL IDENTIFICATION OF THE CAUSE OF LEAF RUST SYMPTOMS ON *MIMUSOPS ZEYHERI*

3.1 Introduction

Mimusops zeyheri fruit tree has been thought to be relatively pest free owing to its copious concentrations of latex in the above-ground organs (Mashela and Mollel, 2001). Identification of Mediterranean fruit fly (*Ceratitis capitata*) (Dube *et al*., 2016), mealybug (*Pseudococcus citri*) (Mokhoele *et al*., 2018) on *M. zeyheri* and recently the fungal symptoms resembling rust infection on this plant could threaten the economic and neutraceutical contributions of the plant to rural poor who are dependent on it. According to Berndt (2008), identification of rust is challenging for a number of reasons, including their complex lifecycles, multiple species on one host and the fact that there are few contemporary resources with their information about their biology and morphology. In most cases identification is supported by comparison to images of symptoms and spore stages made from reference specimen. According to Agrios (2004), rust has been frequently witnessed to affect many economically important plant species and in most cases appear as yellow, orange, red, rust and brown or black powdery pustules on leaves and in some cases teliospores germinate *in situ* to produce basidiospores that infect plant (Hansen and Lewis, 1997).

Plant growth and productivity are heavily reduced by rust pathogens and some plants wither and die back. Furthermore, rusts can also cause severe defoliation which result in reduced flowering of the plant and fruit yield (Agrios, 2004). More importantly, they can also significantly impact biodiversity, e.g., myrtle rust species in Australia currently spreading on Myrtaceae on a continental scale threatening many native trees and ecosystems (Berthon *et al.,* 2019). Myrtle rust pathogen has potential to reduce the photosynthetic activities on their host, diverting and mobilizing photosynthates to their own biomass, therefore reducing carbon sequestration of their host plants (Dawnson *et al*., 2005). According to Dawnson *et al*. (2005), in energy plantations, for example, dry

matter yield losses in excess of 40% have been recorded following rust infection. Most rust pathogens survive through continuous urediniospore cycle on the plant. According to Agrios (2004), urediniospore penetrate through the stomata on the abaxial surface of the leaves and yellow-orange coloured lesion develop later. New maturing urediniospores emerge through the stomata, rather than breaking through the epidermis of the leaves to form pustules, which are characteristic of most rust fungi. The lesions can coalesce and form large necrotic spots as they age, and the leaves drop off prematurely resulting in the death of the entire plant (Agrios, 2004). In some cases the fungus can produce teliospore that germinate and give rise to basidia and basidiospores, example of such includes *Maravalia mimusops* (Countinho *et al*., 1995). *Maravalia mimusops* is one of the most ancient rust genera (Wingfield, 2004) that has proven to be a great threat to indigenous plants such as *Mimusops caffra* resulting in extensive damage leading to enormous economic losses in South Africa. *Maravalia mimusops* has been linked mostly with plants belonging to Sapotaceae family. This rust fungi is a genus belonging to Chaconiaceae family and is characterized by pale, single-celled, thin walled teliospore that elongate to form external basidia (Ono, 1984). Consequently, understanding mechanisms of virulence in rust fungi and devising innovative ways to protect crops against them is essential. *Mimusops zeyheri*, being evergreen and indigenous to the region, which is a major producer of tropical and subtropical commercial fruit and fruit vegetables, pose an additional threat of acting as a source of insect pests and diseases overwintering site. The identification of pests of *M. zeyheri* is not only important for the local rural communities of Limpopo Province but for national fruit producing industry, that contributes 2.1% to the South Africa's gross domestic product (GDP). The main objective of this study was therefore to characterize the fungus causing leaf rust and plant response to infection.

3.2 Materials and methods

3.2.1 Description of study area

Samples of *M. zeyheri* leaves showing rust like symptoms were collected randomly from 17 trees at the University of Limpopo, South Africa (23°53”10’S, 29°44”15’E) during summer, September 2018.

3.2.2 Fungal spore collection

Samples of *M. zeyheri* leaves showing reddish brown pustules with brownish spores underneath were collected, placed in paper bags and transferred to the laboratory for further study. Spores were then removed from the pustules using a sterile needle and glass rod. Spores were examined to determine their appearance based on shape, colour and ornamentation. For identification purpose, collected spores were compared with *Maravalia mimusops* type specimen which were previously isolated from *Mimusops caffra* (Ono, 1984).

3.2.3 Microscope

Light compound microscope (Nikon digital microscopes, South Africa) and electron microscope (Zeiss DSM962, Carl Zeiss, South Africa) were used in the identification of spores. Light microscopy was done at University of Limpopo whereas scanning electron microscopy was done at the Electron Microscopy Unit (EMU), Sefako Makgatho University. For light microscopy, rust spores were dispersed from the infected leaf tissue and mounted on microscope slide and observed at a magnification of x 60 before a picture was captured using triple camera 24MP 1.18/27 ASPH. Scanning electron microscopy (SEM) specimens were mounted on aluminium stubs using adhesive carbon tabs, sputter coated with gold/palladium for two minutes resulting in a deposition of ca 12 nm, and examined in a Zeiss DSM962 scanning electron microscope. Specimens were then identified using existing description keys (Oyo, 2006).

3.2.4 Data analysis

Data for plant variables were subjected to analysis of variance using statistix 10. The least significant difference (LSD) was used to separate means which were significant at 5% level of probability.

3.3 Results

The pustules on the collected leaves started as brownish blister-like structures that burst into reddish-brown pustules (Figure 3.4A). The pustules dislodged reddish-brown spores. The spores from the leaves had characteristic oval and ellipsoidal shape when viewed under a microscope (Table 3.1; Figure 3.1-3.3). The size of spores ranged between 35-37 × 24-26 µm (Figure 3.1-3.3). The cell walls exhibited bilaminate structures with the outer layer hyaline. The warts were rod shaped with one subequatorial germ pore (Figure 3.2).

Table 3.1. Morphological characteristics of spores from *Mimusops zeyheri* leaf

Urediniospores
➢ ellipsoidal, ➢ oval, (Figure 3.1; 3.3) ➢ reddish brown, (Figure 3.1) ➢ 37 × 26 µm; (Figure 3.3) wall bilaminate (Figure 3.1-3.3) • outer wall hyaline, o rod shaped warts, o one subequatorial germ pore.
Teliospores: Not observed from the samples collected.

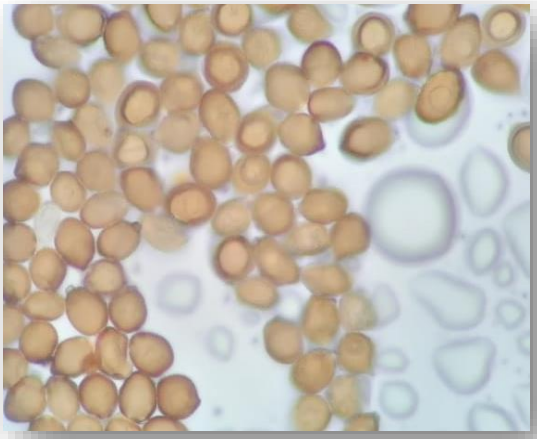

Figure 3.1. Light microscope of *Mimusops zeyheri* leaf rust fungus showing the detailed outer wall surface characters of urediniospores (magnification x60)

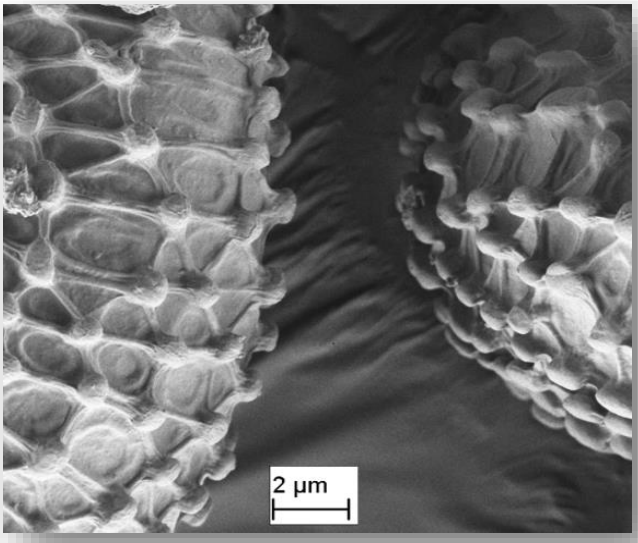

Figure 3.2. Electron microscope of rust spores on *Mimusops zeyheri* leaf showing the ornamentation of urediniopores.

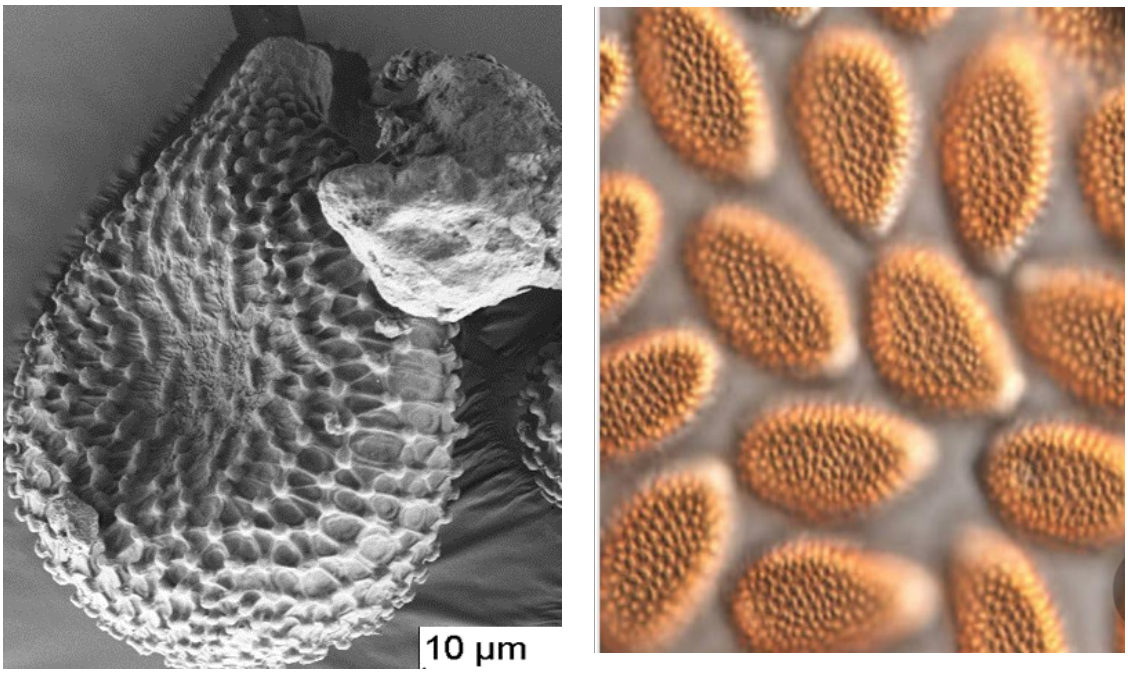

Figure 3.3. Electron microscope rust spores on *Mimusops zeyheri* (left) and *Maravalia mimusops* (right) leaf showing shape and spore surface characters.

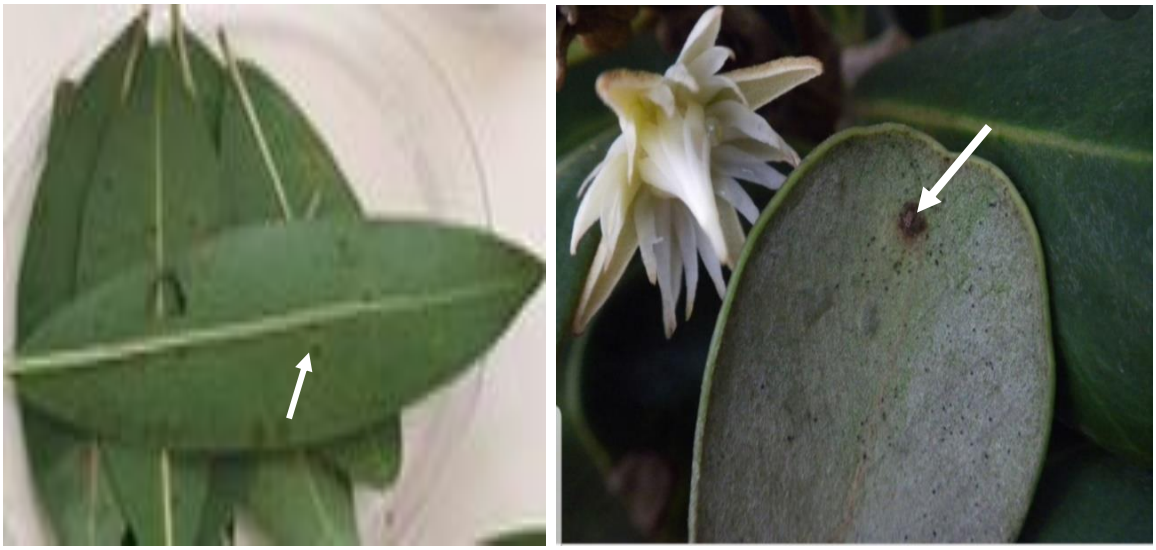

Figure 3.4. *Mimusops zeyheri* (left) and *Mimusops caffra* (right) showing rust symptoms.

The complete classification of the *M. zeyheri* leaf rust then could be as given below (Table 2).

Table 3.2. Possible classification of the *Mimusops zeyheri* leaf rust fungus

Taxa	*Mimusops zeyheri* leaf rust fungus
Kingdom	Fungi
Division	Basidiomycota
Class	Pucciniomycetes
Order	Pucciniales
Family	Chaconiaceae
Genus	*Maravalia*

3.4 Discussions

The morphological characteristics of spores found on *M. zeyheri* resembles that of genus *Maravalia* type specimen in terms of size, colour and shape (Arthur, 1922). Over thirty-five species of *Maravalia* have been described across the world. The genus was first described in 1922 (Arthur, 1922) and it is usually associated with Sapotaceae plants of which *M. zeyheri* is a member. In South Africa, *Maravalia mimusops* was first described in 1984 as the cause of rust diseases in *Mimusops caffra* (Ono, 1984). The samples collected from *M. zeyheri* had uredospores only and no teliospore hence the fungus could only be described to genus level as *Maravalia* species. Teliospores of this fungus have been found in other plants of Sapotaceae family, but none were isolated from *M. zeyheri* yet. Widening sampling of *M. zeyheri* plants across the country over a wider geographical conditions and seasons, which was beyond the scope of this study, could help generate more information on the fungus. This could be the first plant-disease association of the *Maravalia* species rust on *M. zeyheri*.

Identification of the fungus on *M. zeyheri* does not offer only the opportunity for reducing its impact on the plant important to some rural economies but also its use as biocontrol of alien invasive plants of Sapotaceae family. Over 900 species of Sapotaceae family are

alien species worldwide (Richardson and Rejmanek, 2011) and invasive species in South Africa (Mack *et al*., 2000). Isolate of *Maravalia cryptostegiae*, from Northern Madagascar, released in Australia in 1993 for control of rubber vine was proved to be selectively to the *Cryptostegia* variants present attacking the target rubber-vine weed only in mildly (Evans and Tomley, 1994). Moreover, rust fungus *Prospodium tuberculatum* was also released as biocontrol of *Lantana camara* and has proven to be effective attacking mostly the leaves of its host (*Ellison et al.,* 2000).

3.5 Conclusions

The leaf rust pathogen identified on the basis of described morphological characteristics causing rust symptoms on *M. zeyheri* in South Africa is similar to *Maravalia species* isolated from *M. caffra* (Ono, 1984). This finding is important, as it the first report of association between the pathogen and the plant. The study on the teliospores, genetic diversity of the fungus on *M. zeyheri* and potential management strategies could be critical for communal farmers who are dependent on the crop.

CHAPTER 4

PHYTOCHEMICALS AND ENDOPHYTES IN *MIMOSOPS ZEYHERI* WITH POTENTIAL FOR PLANT DISEASE RESISTANCE

4.1 Introduction

Transvaal red milkwood (*Mimusops zeyheri* Sond (Family: Sapotaceae Juss.) has been described as highly resistant to pests (Mashela *et al*., 2013). Pofu *et al*. (2012), observed that the tree is resistant to the southern root-knot (*Meloidogyne incognita*) nematode, a major economic pest of horticultural crops. The evergreen tree has also been reported to be drought-tolerant and salt-tolerant making it ideal for commercialisation in drought-prone area of South Africa (Mashela *et al*., 2013). Furthermore, the dark, glossy foliage is reported to be attractive and young leaves are rust-coloured with a velvety under surface (Schmidt, 2002). This tree produce edible fruits which are oval and ripen to a bright yellow-orange colour and the roots of this tree are fairly compact (Coates Palgrave, 2002). In addition, this tree is believed to have the potential for alleviating vitamin C deficiencies and the bark decoction is used to treat ulcers (Coates Palgrave, 2002). Moreover, the fruits can be processed into a traditional dried pulp called “sesema” which has a long shelf life and serves as a tasty food for during winter (Schmidt, 2002).The ability of *M. zeyheri*, to resist pests has been linked without any empirical evidence to their ability to profusely produce large quantities of latex on their above ground organs (DAFF, 2012). The plant protective chemicals associated with these above ground parts has not been investigated. In other plants chemicals such as phenolic, flavonoids and tannins have been found to exhibit pest protective properties (Bodeker, 2000; Kim *et al*., 2003). Of these defence-related plant chemicals, phenolic compounds have been confirmed to have fungicide properties and can be involved in resistance mechanisms as precursors of defence-related compounds or polymers, and can modulate the activity of other phytochemicals (Schultz and Nicolas, 2000). Moreover, they have been reported to have the capacity to be incorporated into the cell wall and form mechanical barriers of pests (Cvikrová *et al*., 2008). Previous research studies have also demonstrated phenolics as having a role in resistance to *Phytophthora ramorum (*Ockels *et al*., 2007). Several group of protein-binding poly-phenolic compounds known as tannins are

commonly found in plant cell walls and vacuoles and they are used by plants as structural components and for defence against insects and microbial attack (Dixon *et al*., 2005). Flavonoids are also among plant chemicals that play essential role in plant resistance against pathogenic fungi (Treutter, 2005). These compounds are generally transported to the site of infection and induce the hypersensitivity reaction, which is the earliest defence mechanism employed by the infected plants and programme cell death (Buer *et al*., 2010).

Land plants especially indigenous forestry plants are known to live in commensal relationships with large numbers of endophytes (Sieber, 2007). These endophytic organisms tend to reside within the plant without causing any harm or disease to the plant (Saikkonen *et al*., 1998), while in some cases they influence the host plant's physiology which enhances its resistance to a range of both biotic and abiotic stress factors (Lewis, 2004). Fungal endophytes are documented to protect various plants by producing secondary metabolites and some of these compounds have been reported to contain antifungal and antibacterial activities which strongly inhibit the growth of other microorganisms including plant pathogens (Gunatilaka, 2006). Most research studies have confirmed that plant pathogens are extremely sensitive to multiple kinds of antibiotics including terpenoids, flavonoids, alkaloids, aromatic compounds and polypeptides produced by endophytic fungi (Mousa and Raizada, 2013). Generally, the collective presence of numerous microbial species in the plant propels the secretion of metabolites by the endophytes or the host has been associated with inhibition of growth of pathogenic microbes (Kusari *et al*., 2012). Consequently, the potential diversity of plant defense chemicals and endophytes with potential to increasing plant resistance in *M. zeyheri* has never been explored. The objectives of this study were to (i) determine the plant defence chemicals contained in the leaves of *M. zeyheri* and (ii) to determine the plant endophytes on the leaves of *M. zeyheri* with potential for defensive characteristics.

4.2 Materials and Methods

4.2.1 Presence of defence chemicals in *M. zeyheri* leaves

Extraction for characterization of plant defence chemicals in leaves of *M. zeyheri* as an indicator of their ability to resist pests was conducted at ARC-Roodeplaat, Pretoria. *Mimusops zeyheri* leaves were collected from the University of Limpopo plant grove and air-dried in an oven at 60 ℃ for 72 h. The dried leaves were ground into powder and sieved through 500 µm sieve. Phenols were determined using previously described method (Dewanto *et al*., 2002). Briefly, dried plant materials were ground into powder and extracted non-sequentially (1:20w/v) with 70% aqueous acetone (aq.), water, 80% aqueous methanol and 50% aqueous ethanol in an ultra sound bath for 1 h. The extracts were filtered under vacuum through Whatman's No. 1 filter paper. The 80% aqueous methanol, 50% aqueous ethanol and acetone (aq.) extracts were concentrated under vacuum using a rotary evaporator at 35 ℃ and completely dried under a stream of air while water extracts were freeze-dried. Freshly prepared 80% aqueous methanol extracts were used in the characterization of phenolic acids. Phenols were expressed as milligrams of gallic acid equivalents per gram of dry leaf mass (mg GAE/g DW). All samples were analysed in three replicates.

Flavonoids were measured by the aluminium chloride ($AlCl_3$) reagent using quercetin as standard (Zhishen *et al*., 1999). The results were expressed in milligrams of quercetin equivalents per gram of dry leaf mass (mg QE/g DW).

Tannins were determined according to the previously described method (Sun *et al*., 1998). The results were expressed as mg of catechin equivalents per gram of dry leaf mass (mgCAE/g DW). All samples were analysed in three replicates.

4.2.2 Presence of endophytes on leaves

Plant leaf collection: Fresh leaves were collected from twenty *M. zeyheri* plant grove established at University of Limpopo. Leaves were surface sterilized to remove all epiphytes by first soaking them in 70% ethanol for a minute, then in 1% sodium hypochlorite for 3 minutes before soaking them again in 70 % ethanol for 1 minute. The surface sterilized leaves were then rinsed in distilled water twice for a period of 1 minute

to remove excess alcohol. The leaves were then cut into 1-cm pieces and prepared for endophyte DNA extraction.

DNA extraction, amplification, cloning and sequencing: Extraction of DNA from endophytic fungi in the leaves was done at Inqaba Biotechnical Industries (Pty) Ltd, Pretoria. DNA extraction protocol was as previously described (Neubert *et al*., 2006) except that the nuclear ribosomal internal transcribed spacer region (ITS) were used. Briefly, liquid nitrogen was used to ground each leaf into fine powder. A 50 mg leaf powder was then put in 1.5 ml centrifugal tube, followed by homogenisation in 50 µL of extraction buffer comprising 100mM of Tris, 50mM of NaCl, 50mM of EDTA, 100 µg mL^{-1} of proteinase K and 1% SDS. The centrifuge tubes were heated at 55°C for 30 min, with 10 µL of RNase A added to the solution prior to incubation at 37°C for 2 h. Incubated solutions were extracted twice using 100 µL of buffer-saturated phenol-chloroform. Thereafter, DNA was precipitated in a mixture of 10 µL of sodium acetate, 10 µL of linear polyacrylamide carriers and 250 µL of absolute ethanol prior to washing with 70% ethanol. Finally, DNA was dissolved in 50 µL of TE buffer (10mM of Tris + 0.1mM of EDTA).

An ITS ~600 base pair region frequently used in fungal systematics at the species level, used for amplification and sequencing were ITS1F (5′-GGTCAACAAATCATAAAGATA TTG-3′) and HCO-2198 (5′-TAAACTTCAGGGTGACCAAAAAAT CA-3′). Nineteen polymerase chain reactions (PCRs) were done in a final volume of 25 µL with 2µL of DNA solution, 0.5µL of Mytaq polymerase, 5 µL of MyTaq buffer (Bioline, London, UK) and 16.5 µL of double-distilled water. The amplification programme consisted of initial denaturation at 94°C for 1min, annealing at 50°C for 1min and 1 min elongation step at 72°C, which were followed by a 10 min elongation at 72°C. PCR products of expected size (~600 bp) were extracted from agarose gel using GeneJetTM Gel Extraction kit (Thermo Fisher Scientific, Waltham, MA, USA) and cloned using CloneJetTM PCR Cloning kit (Fermentas). Fragments were sequenced using the same primers through ABI Prism® Big DyeTM Terminator 3.0 Ready Reaction Cycle sequencing kit (Applied Biosystems, Foster City, CA). Sequences were established using the ABI PRISMTM 3100 Genetic Analyser (Applied Biosystems), with DNA of opposite strands being edited

and consensus sequences obtained using CLC Main Workbench v.6.1 (CLC Bio, www.clcbio.com) and MEGA v.5.20.

Phylogenetic analysis: The obtained sequences were submitted to the National Centre for Biotechnology Information (NCBI) database (www.ncbi.nlm.nih.gov) for species identification. The identification results were verified by conducting a phylogenetic analysis. The generated DNA sequences and those from the NCBI database (with the highest bit values (E-value $<10^{-5}$) were aligned using CLUSTALX (2.0), with the phylogenetic tree constructed by the neighbour-joining (NJ) method using MEGA v.5.1.20. Evolutionary distances were computed, with the model of nucleotide substitution and the rate variation modelled using the gamma distribution (shape parameter = 8). Branch support was evaluated using the bootstrap method with 1000 replications. Confidence levels of the NJ tree were assessed by calculating the confidence probability (CP) of each branch to test the reliability of the tree. All positions containing gaps and missing data were eliminated. Gaps were treated by pair wise deletion and the estimated transition or transversion bias.

4.3 Results

4.3.1 Presence of defence chemicals in *M. zeyheri* leaves

The level of phytochemical composition among the trees of *M. zeyheri* were found to be significantly different ($P \leq 0.05$) (Table 4.1; Appendix 3.1–3.4). The levels of tannins, flavonoids and phenolics varied from 7.2151 – 3.2547, 1.1537 – 0.0123 and 2.4749 – 1.5788 mg/g, respectively. Whereas levels of proteins in the tree leaves varied from 5.3100 – 2.7967%. Tree number 7 had consistently the highest level of tannins, flavonoids and phenolics compared to all other trees in the grove (Table 4.1).

Table 4.1. Phytochemical composition of *Mimusops zeyheri* leaves

Tree	Tannins mg/g	Flavonoids mg/g	Phenolics mg/g	Protein (%)
1	3.4661^{cd}	0.3460^{de}	1.7676^{de}	3.0433^{c}
2	5.1039^{abc}	0.0330^{h}	2.1739^{abc}	3.9867^{abc}

3	5.3838^{abc}	0.0403^{h}	2.1001^{abcd}	3.6567^{bc}
4	5.0845^{abc}	0.0406^{h}	2.2004^{ab}	3.1233^{c}
5	5.4508^{abc}	0.0567^{h}	1.5980^{e}	3.4967^{bc}
6	5.5222^{abc}	0.0123^{h}	2.3751^{ab}	3.7333^{bc}
7	7.2151^{a}	1.1537^{a}	2.3683^{ab}	3.5500^{bc}
8	5.0845^{abc}	0.5730^{b}	2.0139^{bcd}	3.3333^{bc}
9	7.1392^{ab}	0.3750^{d}	1.8005^{cde}	2.7967^{c}
10	4.8880^{bcd}	0.3267^{e}	1.5788^{e}	3.7333^{bc}
11	3.5450^{cd}	0.3703^{de}	2.1767^{ab}	3.6767^{bc}
12	2.7232^{d}	0.3397^{de}	2.2711^{ab}	3.9500^{abc}
13	3.2547^{cd}	0.4427^{c}	2.2355^{ab}	3.6700^{bc}
14	5.0823^{cd}	0.3650^{de}	2.4749^{a}	3.4967^{bc}
15	4.0823^{cd}	0.3463^{de}	2.1261^{abcd}	3.5067^{bc}
16	5.3436^{abc}	0.2607^{f}	2.4444^{a}	5.3100^{a}
17	5.2751^{abc}	0.1797^{f}	2.2957^{ab}	4.5300^{ab}
$LSD_{0.05}$	0.6244	0.0235	0.2014	0.3733
P-value	0.0000	0.0227	0.1409	0.0001
F-value	7.73	2.31	1.51	4.75

Column means followed by the same letters are not significant (P≤0.05) different according to Fisher's least Significant Difference test.

The dominant metabolites in the chromatogram spectrums were the ions at 551, 549, 479 and 463 m/z. The mass spectrometry accurate data analysis reveal that the ions at 551 m/z and those appearing at 549 m/z differs with only 2 atomic mass unit, an indication

that they only differ with a double bond. Their elemental composition reveal that they have the following elemental composition 551 ($C_{25}H_{44}O_{13}$) and 549 ($C_{25}H_{42}O_{13}$) the two molecules are different with only two H atoms which support the presence of double bond (Figure 4.1, 4.2).

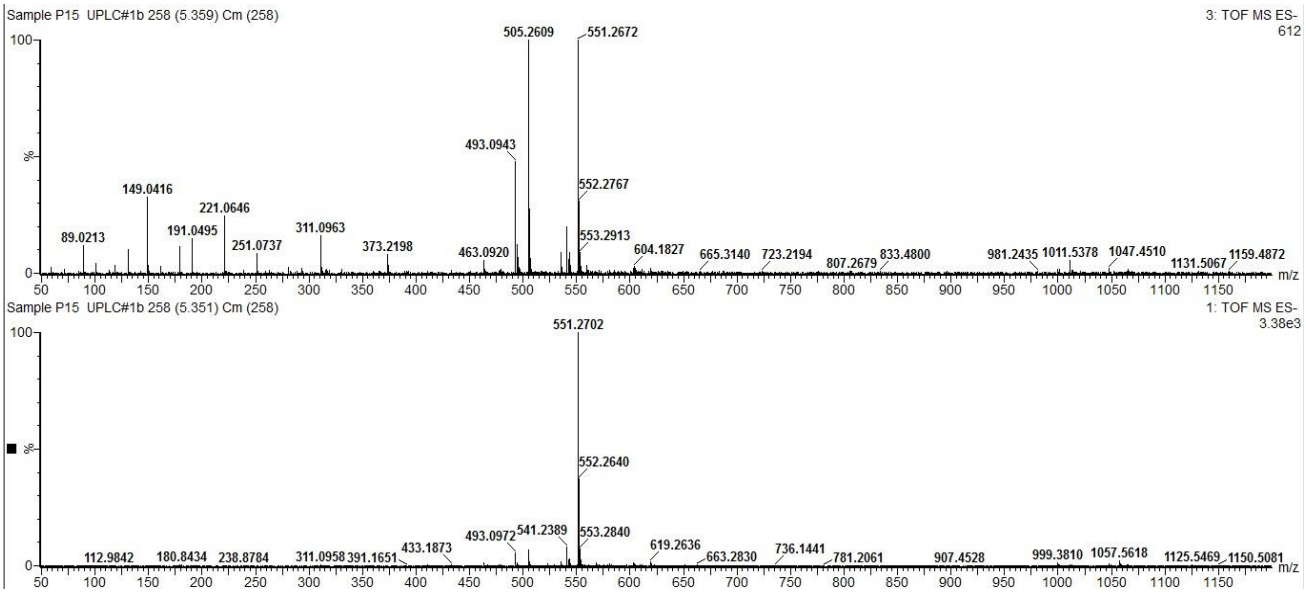

Figure 4.1. High energy spectra for 551 ions

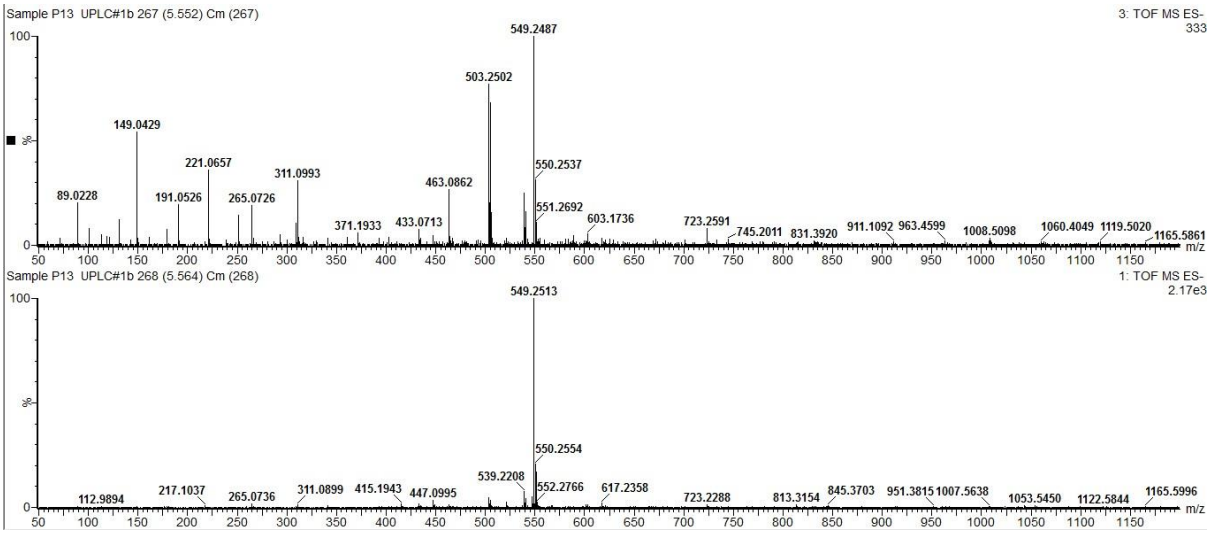

Figure 4.2. High energy spectra for 549 ions

Other dominate metabolite molecules were 479, 463 (the most abundant) and 449, the first two only differed with a mass unit of 16 (oxygen) and they differed from 449 by 2 hydrogen ions (Figure 4.3, 4.4, 4.5). Their elemental compositions are $C_{21}H_{20}O_{13}$ for 479, $C_{21}H_{20}O_{12}$ for 463 and $C_{21}H_{18}O_{12}$, for 449. The other interesting thing is that they have a similar aglycone moiety, with the first molecule showing the loss of a glucosyl (-162 Da) and the later showing a loss of the rhamnosyl (-143 Da).

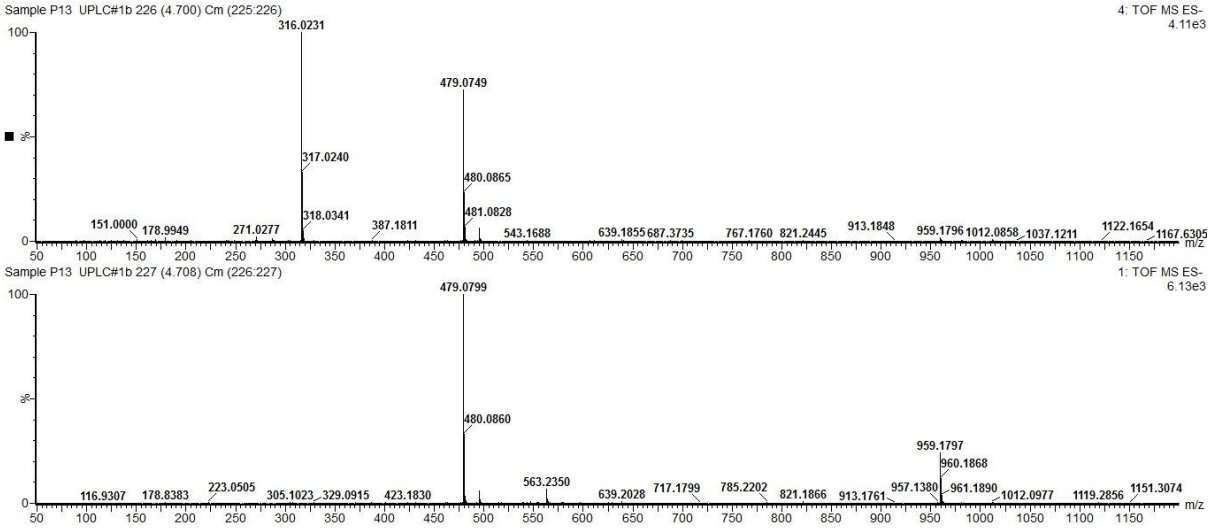

Figure 4.3. High energy spectra for 479 ions

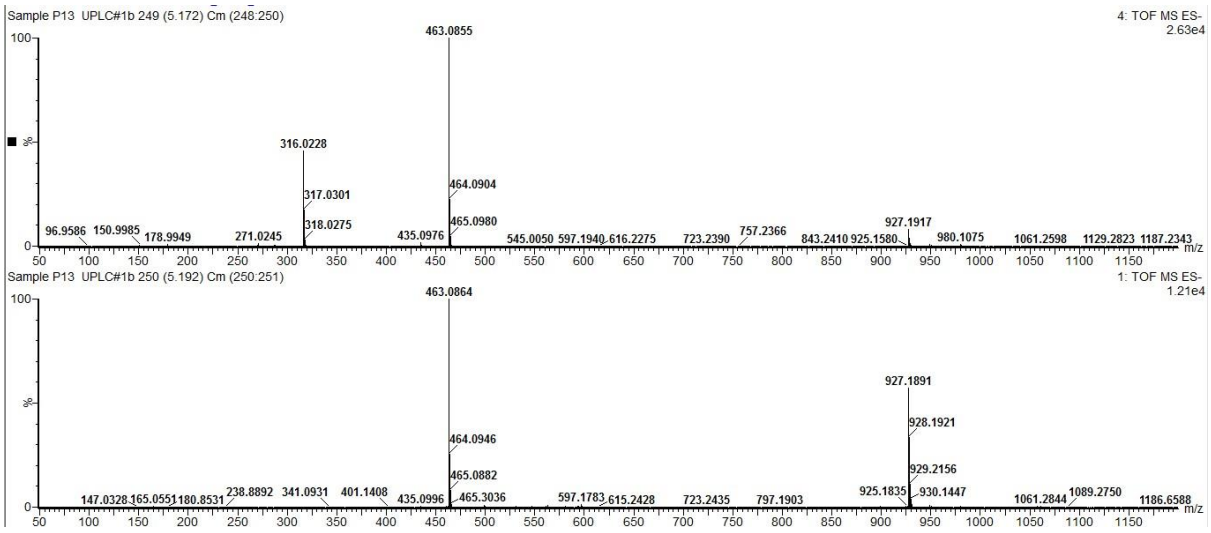

Figure 4.4. High energy spectra for 463 ions

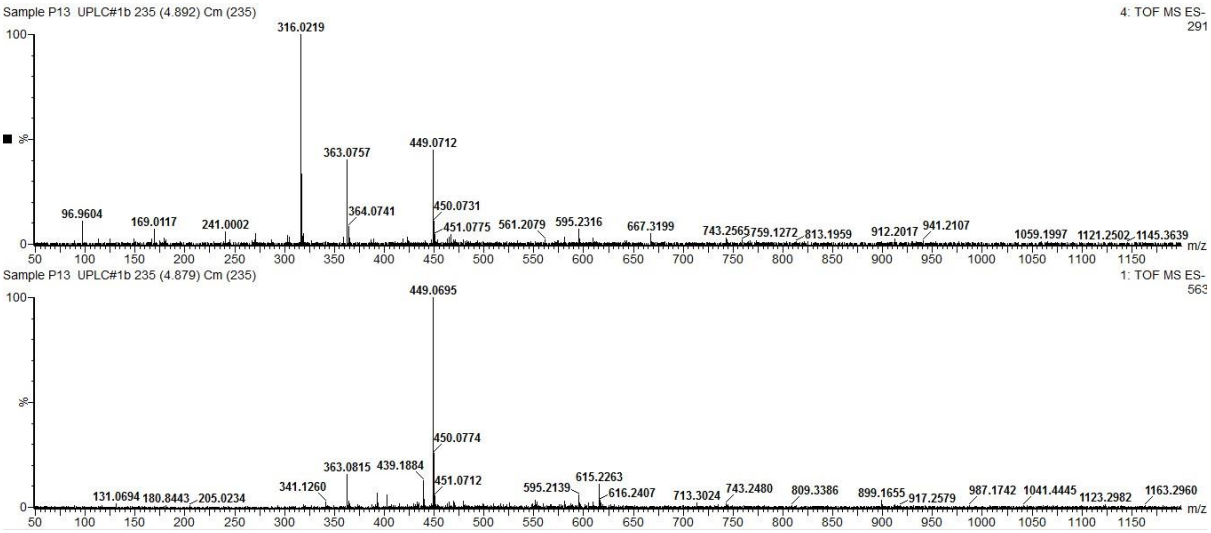

Figure 4.5. High energy spectra for 449 ions

The presence of the agkycone moiety is evident of the presence of Myrecitin, thus the peak at 479 m/z is identified as Isomyricitrin (Myricetin 3-O-beta-D-glucopyranoside, Myricetin 3-O-beta-D-glucoside, Myricetin 3-glucoside) (Figure 4.6)

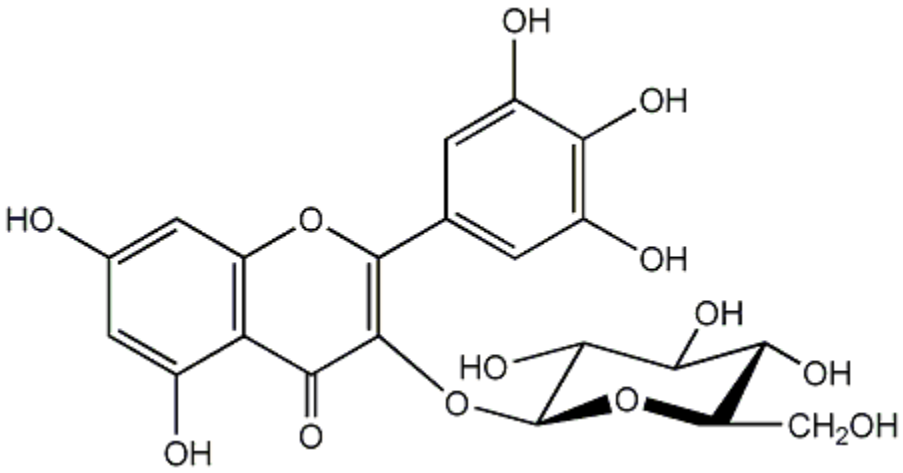

Figure 4.6. Molecular structure of Isomyricitrin.

The peak at 463 m/z has been identified as Myricitrin (Myricetin 3-O-alpha-L-rhamnopyranoside, Myricetin 3-O-alpha-L-rhamnoside, Myricetin 3-O-rhamnoside) (Figure 4.7)

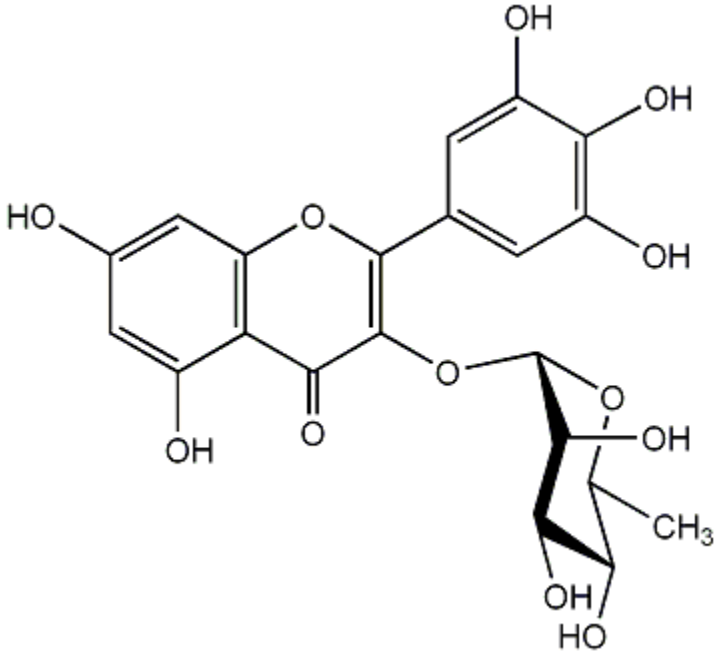

Figure 4.7. Molecular structure of myricitrin.

The peak at 449 m/z has been identified as Myricetin 3-alphaL-arabinopyranoside (Figure 4.8)

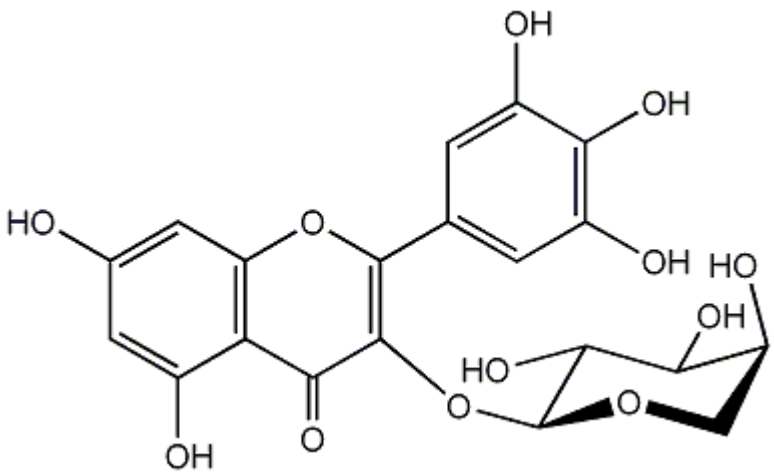

Figure 4.8. Molecular structure of Myricetin 3-alphaL-arabinopyranoside

4.3.3 Presence of endopytes in *M. zeyheri* leaves

Twenty three sequences where obtained from the *M. zeyheri* leaves. A Basic Local Alignment Search Tool (BLAST) to query these sequences with the biological sequences within the NCBI database gave at least seven homologous sequences with E-values of lower than 10^{-6}. The twenty three sequences obtained from *M. zeyheri* leaves were homologous to NCBI database sequences of *Teratosphaeria* species, *Zeloasperium* species, Pezizomycotina, Cladosporium species, *Aspergillus* species, *Phyllosticta* species and *Epicoccum* species with some indicating an unculturable endophyte fungi (Appendix 4.1). The endophytes from *M. zeyheri* did not group closely at species taxa level of individual homologue but showed close evolutional relationship at genus taxa level (Figure 4.9; Figure 4.10).

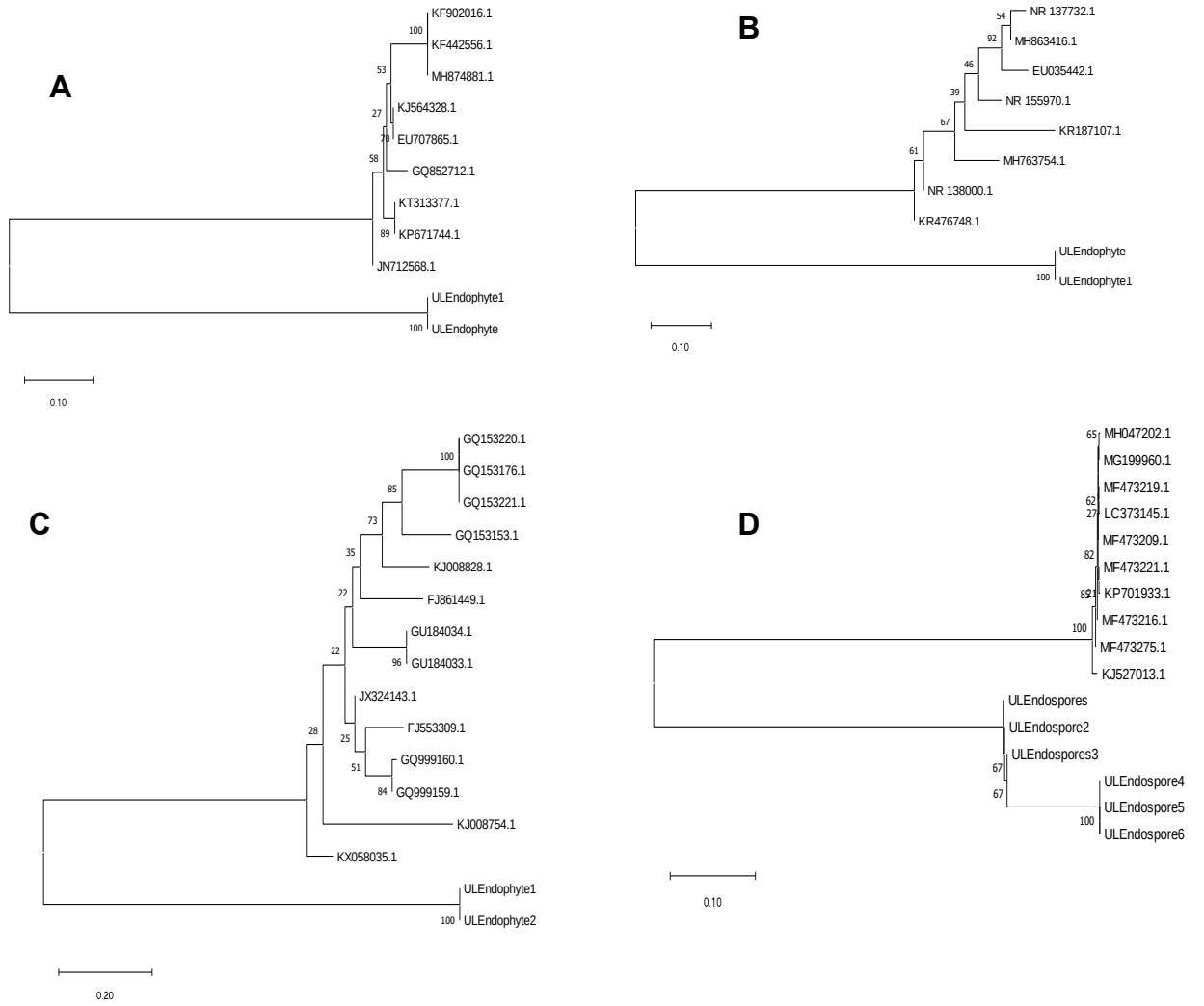

Figure 4.9. Phylogenetic trees showing isolates of *Teratosphaeria* species (A), *Zeloasperium* species (B), Pezizomycotina (C) and *Cladosporium* species (D) obtained from NCBI database (ID codes) with those homologous to the groups from *Mimusops zeyheri* leaves (ULEndophytes). The numbers below the branches indicate bootstrap support values. The scale distance is shown under the tree.

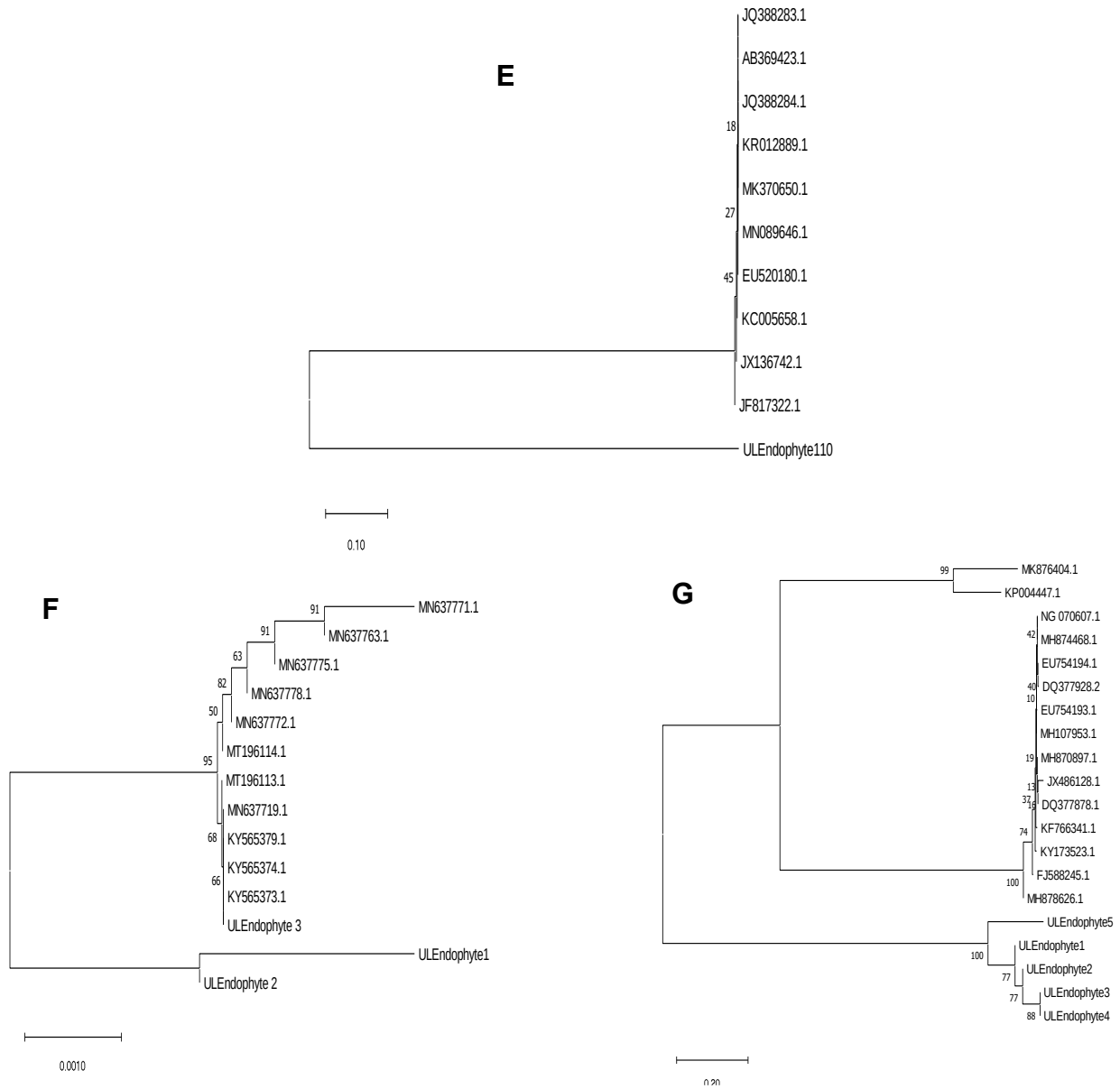

Figure 4.10. Phylogenetic trees showing isolates of Epicoccum species (E), *Aspergillus* species (F) and *Phyllosticta* species (G) obtained from NCBI database (ID codes) with those homologous to the groups from *Mimusops zeyheri* leaves (ULEndophytes). The numbers below the branches indicate bootstrap support values. The scale distance is shown under the tree.

4.4 Discussions

4.4.1 Presence of defence chemicals in *M. zeyheri* leaves

High levels of phytochemical were observed in levels of *M. zeyheri* groves at University of Limpopo, possible a factor in the reported resistance of the plants to *Meloidogyne species*, drought tolerant and salt–tolerant (Mashela and Mollel, 2001; Pofu *et al.*, 2012). Differences in the concentrations of these phytochemicals varied with individual trees in the grove explaining the possible observed differences in response to leaf rust disease. Consequently, obtained findings are in agreement with obtained findings of Prats *et al*. (2006), who reported that in *Pisum sativum* L. inhibition of the early stages of fungal development has been associated with the excretion of specific antifungal plant metabolites, including phenolics, to the leaf surface that interfere with *Uromyces pisi-sativi* fungal development. Moreover, Haering (2008), indicated that different elicitor proteins released by rust fungi induced tannins in oak trees. Additionally, *M. elengi* has demonstrated to contain antibacterial (Nair and Chanda, 2007) and antifungal activity (Satish *et al*., 2007). According to Theerthagiri *et al*. (2007), higher levels of total phenols following infection with the pathogens have been frequently reported where phenols may play an important role as post-infection factors in the disease resistance. This was therefore confirmed with the die-back disease of *M. elengi*, where the greater activity of peroxidase and polyphenol, along with higher amount of total phenols where discovered to enhance host resistance (Khathun *et al*., 2011).

Cowan (1999), reported that plants have the ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, lavones, flavonoids, flavonols, tannins, and coumarins which could effectively suppress spectrum of plant pathogens. However, in many previous studies the underlying mechanisms of this disease suppression is not clearly understood, but the involvement of induced resistance is considered (Fokkema, 1993). This phenomenon of inducing resistance in plants are highly recommended because they are more environment-friendly approach to crop protection against disease infections (Morsy *et al*., 2011). Most indigenous plants have become the reservoirs of valuable phytochemicals and have since been investigated worldwide for their antimicrobial activities. Recently, *Adiantum capillus veneris* has been

investigated for its resistance to fungal pathogens and different extracts obtained from the plant had shown potential antibacterial activities against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Escherichia coli*, and antifungal activity against *Candida albicans* (Shaliniand and Sampathkumar, 2012). Furthermore, phytochemicals screening of extracts of all leaves, stems, and roots of *Adiantum capillus veneris* showed the presence of alkaloids, flavonoides, tannins, saponins, terpenoids, steroids, glycosides, and reducing sugars which is in line with many other studies conducted worldwide (Kumar and Nagarajan 2012).

4.4.2 Presence of endophytes in *M. zeyheri* leaves

The endophytic communities associated with the *M. zeyheri* groves showed high species diversity. And the fungal species composition were distinct in different leaves of the trees. These endophytes were identified as *Teratosphaeria, Zeloasperium* species, *Pezizomycotina*. In addition, endophytes such as *Cladosporium* species, *Aspergillus* species, *Phyllosticta* species and *Epicoccum* species were also identified to be associated with *M. zeyheri* tree. Most endophytes are generally ubiquitous and have shown the potential to produce various bioactive compounds which continuously prove to inhibit the growth of some plant pathogen (Suryanarayanan, 2013). Endophyte isolated from *Cassia spectabilis*, named *Phomopis cassiae* was reported to synthesize five secondary metabolites with antifungal activities against *Cladosporium cladosporioid*es and *Cladosporium sphaeros*permum (Silva *et al*., 2006). This study pioneered in most endophyte studies linked to crop protection resulting in many bioactive metabolites such as lavonoids, peptides, quinones, alkaloids, phenols, steroids, terpenoids, and polyketides being isolated from endophytic fungi (Lugtenberg *et al*., 2016). However, many of these endophytes identified on *M. zeyheri* are reported to be major pathogens in other plants. *Teratosphaeria* species have been frequently reported to cause Kirramyces leaf disease (Crous *et al*., 2009) and stem canker particularly in South Africa (Wingfield *et al*., 1996) and result in significant losses to the forestry industry. Recently, leaf spot revealing the necrotic spots on the upper leaf surface has been reported for the first and has been confirmed to be caused by *Epicoccum* on *Lablab purpureus* resulting in 20 to 35% estimated yield loss in India (Bruten, 1993). Moreover, *Cladosporium* exist as an

important pathogen which has a potential to cause scab disease in cucumber all over plant the world over and water-soaked spots generally occur on leaves and runners of the infected plant (Lee, 1997). In many plants, several *Phyllosticta* species are found to be existing as endophytes, however, they are prevalently known to cause citrus black spot disease in fruits of citrus plants (Kotzé, 2000). Citrus black spot disease is usually identified by presence of hard spot often characterised by sunken, pale brown necrotic lesions with a dark reddish brown border (Baldassari *et al*., 2008). Bioactivity of endophytic fungi has proven to be effective in controlling disease affecting valuable indigenous plants and are normally isolated from different parts of the plants. For example, when a new strain of fungus *Cladosporium sphaerospermum* was isolated from the roots of *Glycine max* (L) Merr the presence of higher amounts of bioactive GA3, GA4, and GA7, which induced maximum plant growth in both rice and soybean varieties was observed (Hamayun *et al*., 2009). Furthermore, an endophytic fungi like *Acremonium terricola*, *Aspergillus niger* and *Cladosporium sphaerospermum* have indicated their promising potential for deployment in biotechnological processes involving production of pectinases, cellulases, xylanases, and proteases. An endophyte, *Aspergillus niger* isolated from *Taxus baccata* has proven to produce lovastatin treat high blood cholesterol and reduce the risk of cardiovascular disease (Raghunath *et al*., 2012).

4.5 Conclusions

High concentrations of tannins, flavonoids and terpenes were extracted from the *M. zeyheri* plant leaves and these varied among trees. Three major phytochemicals were dominant in the trees, isomyricitin, myricitrin and myricetin-3-alpha L-arabinopyranosine. Many different endophytes were observed with most found to be closely related to *Teratosphaeria* species, *Zeloasperium* species and Pezizomycotina. Other endophytes closely related to *Cladosporium* species, *Aspergillus* species, *Phyllosticta* species and *Epicoccum* species. *Teratosphaeria* species has been associated with stem cancer in *Eucalyptus* trees, its presence in asymptotical *M. zeyheri* species is an interesting find.

CHAPTER 5

SUMMARY, SIGNIFICANCE OF FINDING, RECOMMENDATION AND CONCLUSIONS

6.1 Summary

Consistent occurrence of pests and fungal symptoms on *Mimusops zeyheri* are suspected to severely affect physiological functions and fruit formation. The leaves of *M. zeyheri* made up of seventeen trees displaying reddish brownish blister-like structures that burst into reddish-brown pustules were collected. The spores were viewed under microscope and revealed characteristic oval and ellipsoidal shape. In addition, the size of spores were observed to range between 35-37 × 24-26 µm. Furthermore, the cell wall showed bilaminate structures with the outer layer hyaline. The warts were rod shaped with one subequatorial germ pore. The leave rust causing fungus was identified as a *Maravalia* species. The levels of tannins, flavonoids, protein and phenolics showed significant difference among the *M. zeyheri* tree. The level of tannins ranged from 7.2151 mg/g to 2.7232 mg/g. And the highest level of flavonoids was 1.1537 mg/g with the lowest being 0.0123 mg/g. Moreover, the highest level protein was 5.3100% with the lowest being 2.7967% among the trees. Phenolic level among the trees ranged from 2.4749 mg/g to 1.5788 mg/g. The NCBI BLAST showed that the endophytic fungi from *M. zeyheri* tree were homologous to *Teratosphaeria* species*, Zeloasperium* species, *Pezizomycotina*. Other endophytes associated with *M. zeyeheri* tree were homologous to *Cladosporium* species, *Aspergillus* species, *Phyllosticta* species and *Epicoccum* species found in the NCBI database.

6.2 Significance of findings

The finding in this study will help to device management strategy of fungal pathogens that may have been long neglected thus improving food security especially in rural communities. And also provide insight on the factors that are likely to contribute towards the enhancement of the resistance of *M. zeyheri* tree against pathogens. Moreover, to

reduce threats to plant productivity and food security. The study is the first emperical report of endophytes and phytochemicals associated with *M. zeyheri* trees in the quest to identify the source of reported resistance of the trees to nematodes and other environmental stresses.

6.3 Recommendations

Prevention is always the best strategy to protect plants from disease. Therefore, fast and reliable detection of the presence of *M. mimusops* and other plant pathogens at early stage of disease development before yield losses occur in the host is highly recommended. In this way, their distribution and severity could be attenuated. Additionally, an effective framework to mitigate the impact of biological invasion of plant pathogens should be employed in different environment because generally, the emergence of fungal leaf disease is closely connected to environmental speciation worldwide. Also the presence of pathogens associated with serious diseases of commercial forestry plants in asymptomatic plant is a worrisome discovery hence presence of this evergreen tree next to the commercial forestry plantations could be considered in pest management under this cropping system. Furthermore, determination of phytochemicals on *Mimusops* species is very necessary as they have proven to contribute significantly to protection against degenerative disease. Additionally, endophytic fungi are highly recommended on plants of economic importance as they have proven to offer great potential in plant protection, imparting tolerance against several biotic and abiotic stress factors.

6.4 Conclusions

The identification of rust leaf fungus as the cause for sudden reddish brown symptoms in *M. zeyheri* is vital to the rural communities who depend on it as an alternative crop for food and medicinal purpose. In addition, this would help to improve the sustainability of this valuable plant tree. Moreover, the derived information would allow for the initiation of efficacy tests for various products on management strategies of this rust resembling *Maravalia mimusops* on *M. zeyheri* tree and thereby, allowing for the registration of the synthetic fungicides.

REFERENCES

Agrios, G.N. 2004. Plant Pathology, 5th end. San Diego: Academic Press.

Agrios, G.N. 2004. Plant Pathology. Elsevier Academic Press, San Diego.

Agrios, G.N. 2005. Plant pathology. Fifth edition. Elsevier Academic Press, Amsterdam.

Agrios, G.N. 2009. Plant pathology and disease: general introduction. Elsevier Inc, University of Florida; Gainesville, FL USA.

Amusan, O.O.G., Dlamini, P.S., Msonthi, J.D and L.P. Mkhubu. 2002. Some herbal remedies from the Manzini region of Swaziland. *Journal of Ethnopharmacology* 79: 109-112.

Andreozzi, A., Prieto, P., Mercado-Blanco, J., Monaco, S., Zampieri, E and S. Romano 2019. Efficient colonization of the endophytes Herbaspirillum huttiense RCA24 and Enterobacter cloacae RCA25 influences the physiological parameters of Oryza sativa L. cv. Baldo rice. *Environmental Microbiology* 1: 3489-504.

Arnold, A. E., Mejía, L. C., Kyllo, D., Rojas, E. I., Maynard, Z and N. Robbins. 2003. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences*, U.S.A 100: 15649-15654.

Arthur, J.C. 1922. Uredinales collected by R. Thaxter and J. B. Rorer in Trinidad. Botanical Gazette Crawfordsville 73: 58-69

Atmosukarto, I., Castillo, U., Hess, W. M., Sears, J and G. Strobel. 2005. Isolation and characterization of *Muscodor albus* I-41.3 s, a volatile antibiotic producing fungus. *Plant Science* 169: 854-861.

Baldassari, R.B., Wickert, E and A. de Goes. 2008. Pathogenicity, colony morphology and diversity of isolates of Guignardia citricarpa and G. mangiferae isolated from *Citrus* spp. *European Journal of Plant Pathology* 120: 3-110.

Baliga, M.S., Pai, R.J., Bhat, H.P., Palatty, P.L and R. Boloor. 2001. Chemistry and medicinal properties of the Bakul (*Mimusops elengi* L). A review. *Food Research International* 44: 1823-1829.

Barbehenn, R.V and C.P. Constabel. 2011. Tannins in plant–herbivore interactions. *Phytochemistry* 72(13): 1551-1565.

Barka, E.A., Gognies, S., Nowak, J., Audran, J.C and A. Belarbi. 2002. Inhibitory effect of endophyte bacteria on *Botrytis cinerea* and its influence to promote the grapevine growth. *Biological Control* 24: 135-142.

Berndt, R. 2008. The rust mycobiota of Southern Africa: species richness, compositionand affinities. *Mycological Research* 99: 407-419.

Berthon, K.A., Winzer, L.F., Sandhu, K., Cuddy, W., Manea, A., Carnegie, A.J. and M.R. Leishman. 2019. Endangered species face an extra threat: susceptibility to the invasive pathogen *Austropuccinia psidii* (myrtle rust) in Australia, *Australasian Plant Pathology* 48: 385-393.

Bodeker, G. 2002. Traditional health system: valuing biodiversity for human health and well-being. In cultural and spiritual values in biodiversity. In: Posey, D.A (edn). Practical Action, Nairobi 261-284.

Brown, J.K.M. and M.S. Hovmøller. 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* 297: 537-541.

Buer, C.S., Imin, N and M.A. Djordjevic. 2010. Flavonoids: New roles for old molecules. *Journal of Integrative Plant Biology* 52: 98-111.

Burdon, J.J., Thrall, P.H and A.H.D. Brown. 1999. Resistance and virulence structure in two *Linum marginale-Melampsora lini* host-pathogen metapopulations with different mating systems. *Evolution* 53: 704-716.

Carroll, G. 1988. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. *Ecology* 69: 2-9.

Catazanriti, A.M., Dodds, P.N., Lawrence, G.J., Ayliffe, M.A and J.G. Ellis. 2006. Haustorially expressed secreted proteins from flax rust are highly enriched for avirulence elicitors. *Plant Cell* 18: 243-256.

Chadare, F.J., D.P. Gayet, P. Azokpota, M.J.R. Nout, A.R. Linneman, J.D. Hounhouigan, and M.A.J.S. Van Boekel. 2010. Three traditional fermented baobab foods from Benin, mutchayan, dikouanyouri, and tayohounta: preparation, properties and consumption. *Ecology of Food and Nutrition* 49: 279-297.

Chaiyan, B., Sunanta, W., Oranart, S and C. Rasamee. 2009. Antioxidant capacity and phenolic content of *Mimusops elengi* fruit extract. *Kasetsart Journal-Natural Sciences* 43: 21-27

Chandra, S. 2012. Endophytic fungi: novel sources of anticancer lead molecules. *Applied Microbiology and Biotechnology* 95: 47-59.

Chong, J., Poutaraud, A and P. Hugueney. 2009. Metabolism and roles of stilbenes in plants. *Plant Science* 177: 143-155.

Clay, K.1988. Fungal endophytes of grasses: a defensive mutualism between plants and fungi. *Ecology* (69): 10-16.

Coates Palgrave, M. 2002. *Keith Coates Palgrave Trees of Southern Africa*, edn 3. Struik, Cape Town.

Coates, M.K. 2002. Trees of Southern Africa, 3rd edition. Struik, Cape Town.

Cooper, B., Campbell, K. B., Beard, H. S., Garrett, W. M and N. Islam. 2016. Putative rust fungal effector proteins in infected bean and soybean leaves. *Phytopathology* 106: 491-499.

Cooper, B., Garrett, W. M and K.B. Campbell. 2006. Shotgun identification of proteins from uredospores of the bean rust *Uromyces appendiculatus*. *Proteomics* 6: 2477-2484.

Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Review* 12(4): 564-582.

Cressey, D. 2013. Coffee rust regains foothold. *Nature* 50: 493-587

Crous, P.W, Wingfield M.J and T.I. Burgess. 2017. Fungal planet description sheets: 469-557. *Persoonia* 37: 218.

Cvikrová, M., Malá, J., Hrubcová, M., Eder, J and S. Foretová. 2008. Induced changes in phenolic acids and stilbenes in embryogenic cell cultures of Norway spruce by culture filtrate of Ascocalyx abietina. *Journal of Plant Diseases and Protection* 115: 57-62.

Daisy, B. H., G. A., Strobel, U., Castillo, D., Ezra, J and F. Sears. 2002. Naphthalene, an insect repellent, is produced by *Muscodor vitigenus*, a novel endophytic fungus. *Microbiology* 148: 3737-3741.

Dawson, W.M., McCracken, A.R and D. Carlisle. 2005. Short-rotation coppice willow mixtures and yield. In:Pei MH, McCracken A.R, eds. *Rust disease of wallow and poplar.* Wallingford, UK: CABI 195-208.

De Wet, H., Nzama, V and S. van Vuuren. 2012. Medicinal plants used for the treatment of sexually transmitted infections by lay people in northern Maputaland, KwaZulu-Natal Province, South Africa. *South African Journal of Botany* 78: 12-20.

Department of Agriculture, Forestry and Fisheries (DAFF). 2012. Red milkwood. Pretoria, South Africa.

Dewanto, V., Wu, X., Adom, K.K and R.H. Liu. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry* 50: 3010-3014.

Dingle, J and P.A. McGee. 2003. Some endophytic fungi reduce the density of pustules of *Puccinia recondita* f. sp*. tritici* in wheat. *Mycological Research* 107: 310-316.

Dixon, R.A., Xie, D.Y and S.B. Sharma. 2005. Proanthocyanidins - a final frontier in flavonoid research? *New Phytolologist* 165: 9-28.

Dube, Z.P, Mashela P.W and R.V. Mathabatha. 2016. Mediterranean fruit fly on *Mimusops zeyheri* indigenous to South Africa: a threat to the horticulture industry. *Pest Management Science* 72(8): 1517-20.

Engels, J.M.M., Rao, V.R., Brown, A.H.D and M.T. Jackson. 2002. Managing Plant Genetic Diversity. CABI Publishing, New York, USA 373.

Esam, H.M and H.K. Ali. 2000. Evaluation of antioxidant activity of some plant extracts and their application to ground beef patties. *Food Chemistry* 69: 135-141.

Evans, H.C and A.J. Tomley. 1994. Studies on the rust, *Maravalia Cryptostegia,* potential biological control agent of rubber-vine weed, Cryptostegia in Australia: host range. *Mycopathologia* 126(2): 93-103.

Faeth, S. H and W.F. Fagan. 2002. Fungal endophytes: common host plant symbionts but uncommon mutualists. *Integrative and Comparative Biology* 42: 360-368.

Fill, T.P., Pereira, G.K., Santos, R.M.G and E. Rodrigues-Filho. 2007. Four additional meroterpenes produced by *Penicillium* sp., found in association with *Melia azedarach*. possible biosynthetic intermediates to Austin. *Zeitschrift fuer Naturforschung* 62: 1035-1044.

Fokkema, N.J.1993. Opportunities and problems of control of foliar pathogens with microorganisms. *Pesticide Science* 37:411-416.

Germishuizen, G., Meyer, N.L., Steenkamp, Y and M. Keith. 2006. A checklist of South African plants. Southern African Botanical Diversity Network Report No. 41. SABONET, Pretoria.

Gimenez, C., Cabrera, R., Reina, M and A. Gonzalez-Coloma. 2007. Fungal endophytes and their role in plant protection. *Current Organic Chemistry* 11: 707-720.

Glen, M., Alfenas, A.C., Zauza, E.A.V., Wingfield, M.J and C. Mohammed. 2007. *Puccinia psidii*: a threat to the Australian environment and economy. *Australasian Plant Pathology* 36: 1-16.

Gunatilaka, A.L. 2006. Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. *Journal of Natural Products* 69: 509-526.

Haering, D.A., Huber, M.J., Suter, D., Edwards, P.J and A. Luescher. 2008. Plant Enemy-derived Elicitors Increase the Foliar Tannin Concentration of Onobrychis viciifolia Without a Trade-off to Growth. *Annals of Botany* 102: 979-987.

Hamayun, M., Afzal Khan, S and N. Ahmad. 2009. "*Cladosporium sphaerospermum* as a new plant growth-promoting endophyte from the roots of *Glycine max* (L.) Merr," *World Journal of Microbiology and Biotechnology* 25(4): 627-632.

Hammerschmidt, R. 2005. Phenols and plant–pathogen interactions: the saga continues. *Physiological and Molecular Plant Pathology* 66: 77-78.

Hansen, M and K.J. Lewis. 1997. *Compendium of Conifer Diseases*. St. Paul, MN: American Phytopathological Society.

Heath, R.N., Gryzenhout, M., Roux, J and M.J. Wingfield. 2006. Discovery of the *Cryphonectria* canker pathogen on native *Syzygium* species in South Africa. *Plant Disease* 90: 433-438.

Hellwig, V., Grothe, T., Mayer-Bartschmid, A., Endermann, R., Geschke, F. U and T. Henkel. 2002. Altersetin, a new antibiotic from cultures of endophytic Alternaria. *Journal of Antibiotics* 55: 881-892.

Horbach, R., Navarro-Quesada, A.R., Knogge, W and H.B. Deising. 2011. When and how to kill a plant cell: infection strategies of plants pathogenic Fungi. *Journal of Plant Physiology* 168: 51-62.

Huda-Faujan, N., Noriham, A., Norrakiah, A.S and A.S. Babji. 2009. Antioxidant activity of plants methanolic extracts containing phenolic compounds. *African Journal of Biotechnology* 8(3): 484-489.

Kala, S., Johnson, M., Iyan, R., Dorin, B., Jeeva, S and N. Janakiraman. 2011. Preliminary

phytochemical analysis of some selected medicinal plants of south India. *Journal of Natura Conscientia* 2(5): 478-481.

Keith, L.M., Velasquez, M.E and FT. Zee. 2006. Identification and characterization of Pestalotiopsis spp. causing scab disease of *Guava,Psidium guajava*, in Hawaii . *Plant Disease.*

Khan, M. A., Asaf, S., Khan, A. L., Ullah, I., Ali, S and S.M. Kang. 2019. Alleviation of salt stress response in soybean plants with the endophytic bacterial isolate *Curtobacterium* sp. SAK1. *Annals of Microbiology* 69: 797-800.

Khatun, S and N.C. Chatterjee. 2011. Biochemical defense against die-back disease of a traditional medical plant *Mimusops elengi* (Linn.). *European Journal of Medicinal Plants* 1(3):40-49*.*

Kihika, R., Murungi, L. K., Coyne, D., Ng'ang'a, M., Hassanali, A., Teal, P. E. A., et *al*. 2017. Parasitic nematode *Meloidogyne incognita* interactions with different *Capsicum annum* cultivars reveal the chemical constituents modulating root herbivory. *Scientific Reports* 7:2903.

Kim, D., Jeond S and C. Lee. 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry* 81: 321-326.

Kim, D., Jeond S. and C. Lee. 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry* 81: 321-326.

Knogge, W. 1996. Fungal infection of plants. *Plant Cell* 8: 1711-1722.

Kolmer, J.A., Dyek, P.L and A.P. Roelfs.1991. An appraisal of stem and leaf rust resistance in North American hard red spring wheats and probability of multiple mutations in populations of cereal rust fungi. *Phytopathology* 81: 237- 239.

Kolmer, J.A., Liu, J.Q and M. Sies. 1995. Virulence and molecular polymorphism in *Puccinia recondita* f sp. *tritici* in Canada. *Phytopathology* 85: 276-285.

Kolmer, J.A., Ordonez, M.E and J.V. Groth. 2009. Encyclopedia of Life Sciences. Chichester: John Wiley and Sons, Ltd. The Rust Fungi. DOI: 10.1002/9780470015902.a0021264.

Kotzé, J.M. 2000. Black spot. *In*: Timmer, L.W, Garnsey, S.M and Graham, J.H, (eds) Compendium of citrus disease, 2nd edn. The American Phytopathological Society, St. Paul, MN, USA 23-25.

Kuldau, G and C. Bacon. 2008. Clavicipitaceous endophytes: their ability to enhance resistance of grasses to multiple stresses. *Biological Control* 46: 57-71.

Kumar, S and N. Kaushik. 2012. Metabolites of endophytic fungi as novel source of biofungicide: *Photochemistry Reviews* 11: 507-522.

Kumar, S and N. Kaushik. 2012. Metabolites of endophytic fungi as novel source of biofungicide: a review. *Phytochemical Reviews* 11: 507-522.

Kumar, S.S and N. Nagarajan. 2012. "Screening of preliminary phytochemical constituents and antimicrobial activity of *Adiantum capillus veneris*," *Journal of Research in Antimicrobial* 1(1): 56-61.

Kusari, S., Hertweck, C and M. Spiteller. 2012. Chemical ecology of endophytic fungi: origins of secondary metabolites. *Chemistry and Biology* 19: 792-798.

Kusari, S., Hertweck, C and M. Spiteller. 2012. Chemical ecology of endophytic fungi: origins of secondary metabolites. *Chemistry and Biology* 19: 792-798.

Lee, K.Y., Youn, K.H and H.J Kang. 1997. Cucumber scab caused by *Cladosporium cucumerinum* in Korea. *Korean Journal of Plant Pathology* 13: 288-294.

Lemmens, R.H.M.J. 2005. *Mimusops zeyheri* Sond. *In*: D. Louppe (ed). Prota 7: Timbers/Bios d'oeuvre. [CD-Rom]. Wageningen, Netherlands, Prota 89-94.

Lewis, G.C. 2004. Effects of biotic and abiotic stress on the growth of three genotypes of Lolium perenne with and without infection by the fungal endophyte *Neotyphodium lolii*. *Annals of Applied Biology* 144: 53-63.

Little, K.M and R.G. Payn. 2016. Screening of fungicides for the management of wattle rust (*Uromycladium acaciae*) in *Acacia mearnsii* plantations, South Africa. *Southern Forests* 78: 151-158.

Liu, K., Ding, X and W. Chen. 2009. “Isolation and characterization of endophytic taxol-producing fungi from *Taxus chinensis*.” *Journal of Industrial Microbiology and Biotechnology* 36(9) 1171-1177.

Loehrer, M., Botterweck, J., Jahnke, J., Mahlmann, D.M, Gaetgens,J., Oldiges, M., Horbach, R., Deising, H and U. Schaffrath. 2014. *In vivo* assessment by Mach Zehnder double-beam interferometry of the invasive force exerted by the Asian soybean rust fungus (*Phakopsora pachyrhizi*). *New Phytologist* 203: 620- 631.

Logan, J.A., Regniere, J and J.A. Powell. 2003. Assessing the impact of global warming on forest pest dynamics. *Frontier Ecology and the Environment* 1: 130-137

Lokesh, S., Raghavendra, V.B., Nagesh, K.S and M. Govindappa. 2017. First Report of Leaf Blight of Bakul (*Mimusops Elengi* Linn) Caused by *Pestalotiopsis Clavispora*(G.F.Atk.) Steyaert in India. *Journal of Plant Physiolology and Pathology* 5:1.

Lu, H., Xou, W.X., Meng, J.C., Hu, J and R.X. Tan. 2000. New bioactive metabolites produced by *Colletotrichum* sp., an endophytic fungus in *Artemisia annua*. *Plant Science* 151: 67-73.

Lugtenberg, B. J., Caradus, J. R and L.J. Johnson. 2016. Fungal endophytes for sustainable crop production. *FEMS Microbiology Ecology* 92: 1–17.

Macfadyen, S., McDonald, G and M. P. Hill. 2018. From species distributions to climate change adaptation: knowledge gaps in managing invertebrate pests in broad-acre grain crops. *Agriculture, Ecosystem and Environment* 253: 208-219.

Maher, E.A., Bate, N.J., Ni, W., Elkind, Y., Dixon, R.A and C.J. Lamb. 1994. Increased disease susceptibility of transgenic tobacco plants with suppressed levels of preformed phenylpropanoid products. Proceedings of the National Academy of Sciences. U.S.A 91: 7802-7806.

Makonda, A., Lungu, S., Maghembe, J.A and P.L .Mafongoya. 2003. Fruit and seed germination characteristics of Strychnos cocculoide an indigenous fruit tree from natural population in Zambia. *Agroforestry Systems* 58(1): 25-31.

Mashela, P.W and N. Mollel. 2001. Farmer – identified and selected indigenous fruit tree with suitable attributes for smallholder farming systems in South Africa's Northern Province. *South Africa Journal of Agricultural Extension* 30: 1-12.

Mashela, P.W., Pofu, K.M and B. Nzanza. 2013. Response of Mmupudu (*Mimusops zeyheri*) indigenous fruit trees to three soil types. *African Journal of Agricultural Research* 8: 1066-1069.

Mashela, P.W., Tseke, P.E., Pofu, K.M. and T.P. Mafeo. 2013. Potential commercialisation of *Mimusops zeyheri*: An indigenous tree selected for research and development through indigenous knowledge systems. African Crop Science Conference Proceedings, Entebbe, Uganda 11:667-669.

McGaw, L.J., Steenkamp, V and J.N. Eloff. 2007. In vitro antihelminthic, antibacterial and cytotoxic effects of extracts from plants used in South African ethnoveterinary medicine. *The Veterinary Journal* 173: 366-372.

McLeod, A., Labuschagne, N and J.M. Kotzé. 1995. Evaluation of *Trichoderma* for biological avocado root rot in bark medium artificially infested with *Phytophthora cinnamomi*. South African Avocado Growers' Association Yearbook 18: 32-37.

Mir, R.A., Kaushik, S.P., Chowdery, R.A, and Anuradha, M. 2015. Elicitation of Forskolin in Cultures of *Rhizactonia bataticola* – A Phytochemical Synthesizing Endophytic Fungi. *International Journal of Pharmacy and Pharmaceutical Sciences* 7: 10.

Mokhoelele, F., Dube, Z.P and P.W Mashela. 2018. Nemafric-BL phytonematicide with Dicerocaryum senocioides as a wettener in the management of Mealybugs on *Mimusops Zeyheri*. African Combined Conference. South Africa, Cape Town.

Morsy K.M., Abdel-Monaim, M. F. and Mazen, M. M. 2011. Use of abiotic and biotic inducers for controlling fungal diseases and improving growth of alfalfa. *World Journal of Agricultural Sciences* 7(5): 566-576.

Moses, T., Papadopoulou, K. K., and A. Osbourn. 2014. Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives. *Critical Reviews in Biochemistry and Molecular Biology* 49: 439-462.

Mousa, W. K and M.N. Raizada. 2013. The diversity of anti-microbial secondary metabolites produced by fungal endophytes: an interdisciplinary perspective. *Frontier in Microbiology* 4:65.

Nair, R and S.V Chanda. 2007. Antibacterial activities of some medicinal plants of the Western Region of India. *Turkish Journal of Biology* 31: 231-236.

Neudeck, L., Avelino, L., Bareetseng, P., Ngwenya, B.N., Teketay, D and M.R Motsholapheko. 2012. The contribution of edible wild plants to food security, dietary diversity and income of households in Shorobe Village, northern Botswana. *Ethnobotanical Research and Applications* 10: 449-462.

Neuwinger, H.D. 2000. African traditional medicine: A dictionary of plant use and applications. Medpharm Scientific Publishers, Stuttgart.

Newcombe, G. 2004. Forest Pathology Rust Diseases. *In*: Burley, J., Evans, J. and Youngquist, J., (eds). Encyclopedia of Forest Sciences. Elsevier Science, Asterdam.

Ntombela, S. and M. Moobi. 2013. South African Fruit Trade Flow. Pretoria, South Africa. National Agricultural Marketing Council (NAMC).

Ockels, F.S., Eyles, A., McPherson, B.A., Wood, D.L and P. Bonello. 2007. Phenolic chemistry of coast live oak response to Phytophthora ramorum infection. *Journal of Chemical Ecology* 33: 1721-1732.

Oelke L.M and J.A Kolmer. 2005: Genetics of leaf rust resistance in spring wheat cultivars Alsen and Norm. *Phytopathology* 95: 773-778.

Oksanen, T., P. Brita and Tomi, T. (eds). 2003. Forest in Poverty Reduction Strategies: Capturing the potential. European Forest Institute Proceeding No 47. European Forest Institute, Joensuu, Finland.

Ono, Y. 1984. A monograph of Maravalia. *Mycologia* 76: 892-911.

Osbourn, A. 1996. Saponins and plant defence—a soap story. Trends Plant Science 1: 4-9.

Osorio, J.A., Crous, C.J and Z.W. De Beer. 2017. Endophytic Botryosphaeriaceae, including five new species, associated with mangrove trees in South Africa. *Fungal Biolology* 121: 361-393.

Pal, K.K and B.M. Gardener. 2006. Biological control of plant pathogens. The plant health Instructor. DOI: 10.1094/PHI-A-2006–1117-02.

Panstruga, R. 2003. Establishing compatibility between plants and obligate biotrophic pathogens. *Current Opinion in Plant Biology* 6: 320-326.

Pateraki, I., Andersen-Ranberg, J., Jensen, N.B., Wubshet, S.G., Heskes, A.M., Forman, V., Hallstrom, B, Hamberger, B, Motawia, M.S and P. Olsen. 2017. Total biosynthesis of the cyclic AMP booster forskolin from *Coleus forskohlii. Elife* 6: e23001.

Petrini, O. 1991. Fungal endophytes of tree leaves. In: Andrews JH, Hirano SS (eds) *Microbial ecology of leaves*. Springer, New York.

Petrini, O. 1991. Fungal Endophytes of Tree Leaves. In: Microbial Ecology of Leaves, Andrews, J.H. and S.S. Hirano (Eds.). Springer Verlag, New York, USA 179-197.

Pichersky, E., and R.A. Raguso. 2018. Why do plants produce so many terpenoid compounds? New Phytologist 220: 692-702.

Ploetz, R.C. 2013. Disease of mango. In: Ploetz R.C (ed) Disease of tropical fruit crops. CABI Publishing, Wallingfords, 327-363.

Pofu, K.M. 2012. Potential use of indigenous Cucumis africanus and Cucumis myriocarpus as root-knot nematode-resistant rootstocks in watermelon (*Citrulus lanutus*) husbandry. PhD. Thesis Submitted to the University of Limpopo, Sovenga, South Africa.

Prabhat A., Navneet, D and C. Avnish. 2010. C. Evaluation of antimicrobial activity of six medicinal plants against dental pathogens. *Report Opinion* 2(6): 37-42.

Prats, E., Bazzalo, M. E., León, A and J.V. Jorrín. 2006. Fungitoxic effect of scopolin and related coumarins on *Sclerotinia sclerotium*. A way to overcome sunflower head rot. *Euphytica* 147: 451-460.

Probhat, A., Nneet and C. Avnish. 2010. Evaluation of antimicrobial activity of six medicinal plants against dental pathogens. *Report Opinion* 2: 37-42.

Radzuma, M.G. 2017. Using plant growth regulators and Vesicular Arbuscular Mycorrhiza to improve growth of the slow growing indigenous *Mimusops zeyheri* seedlings and accumulation of essential nutrients elements. MSc. Thesis Submitted to the University of Limpopo, Sovenga, South Africa.

Raghunath, R., Radhakrishna, A., Angayarkanni, J and M. Palaniswamy. 2012. Production and cytotoxicity studies of lovastatin from *Aspergillus niger* PN2 an endophytic fungi isolated from *Taxus baccata*. *International Journal of Applied Biology and Pharmaceutical Technology* 3 (3): 342-352.

Richardson, D.M and M. Rejmánek. 2011. Eucalypts encyclopedia of biological invasions (ed. By D. Simberloff and M. Rejmánek), University of California, Press, Berkeley 203-209.

Rodriguez, R.J., White Jr, J.F., Arnold, A.E and R.S. Redman. 2009. “Fungal endophytes: diversity and functional roles. *New Phytologist* 182(2): 314-330.

Roux, J., Granados, G.M., Shuey, L., Barnes, I., Wingfield, M.J and A.R. McTaggart. 2016. A unique genotype of the rust pathogen, *Puccinia psidii,* on Myrtaceae in South Africa. Australasian. *Plant Pathology* 45: 645-652.

Ruma, K. Sunil, H and Prakash. 2013. Antioxidant, anti-inflammatory, antimicrobial and cytotoxic properties of fungal endophytes from Garcinia species *International Journal Pharmacy and Pharmaceutical Science* 5: 889-897.

Satish, S., Raghavendra, M.P., Mohana, D.C and K.A Raveesha. 2008. Antifungal activity of a known medicinal plant *Mimusops elengi* L. against grain moulds. *Journal of Agriculture, Science and Technology* 4(1): 151-165.

Schmidt, S., Lotter, M and W. McCleland. 2002. *Trees and Shrubs of Mpumalanga and the Kruger National Park.*

Schultz, T.P and DD. Nicholas. 2000. Naturally durable heartwood: evidence for a proposed dual defensive function of the extractives. *Phytochemistry* 54: 47-52.

Schulz, B., Haas, S., Junker, C., Andree, N and M. Schobert. 2015. Fungal endophytes are involved in multiple balanced antagonisms. *Current Science* 109: 39-45.

Scott, A., Chung, H.Y and F.G. Whitby. 2005. Structural and Mechanistics studies of VPS4 proteins. *European Molecular Biology Organisation press* 24(20): 3658-69.

Shalini, S and P. Sampathkumar. 2012. “Phytochemical screening and antimicrobial activity of plant extracts for disease management.” *International Journal of Current Science* 209-218.

Siddhuraju, P., Mohan, P.S and K. Becker. 2002. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): A preliminary assessment of crude extracts from stem bark, leaves, and flower and fruit pulp. *Food Chemistry* 79: 61-67.

Sieber, T.N. 2007. Endophytic fungi in forest trees. Are they mutualists*? Fungal Biology Reviews* 21: 75-89.

Silva, G. H., Teles, H. L., Zanardi, L. M., Young, M. C. M., Eberlin, M.N and R. Hadad. 2006. Cadinane sesquiterpenoids of *Phomopsis cassia*e, an endophytic fungus associated with *Cassia spectabilis* (Leguminosae). *Photochemistry* 67:1964-1969.

Simelane, M.B.C., Shonhai, A., Shode, F.O., Smith, P., Singh, M and A.R. Opoku. 2013 AntiPlasmodial activity of some Zulu medicinal plants and of some triterpenes isolated from them. *Molecules* 18: 12313-12323.

Stone, C.L., McMahon, M.B., Fortis, Nuñez, A., Smythers, G.W., Luster, D.G and R.D. Frederick. 2012. Gene expression and proteomic analysis of the formation of *Phakopsora pachyrhizi* appressoria. *BMC Genomics* 13: 269.

Stone, J.K., Bacon, C.W. and J.F. White. 2000. An overview of endophytic microbes endophytism. *In*: Bacon, C.W and J.F, White (eds). Microbial Endophytes, Marcel Dekker, New York, 3-29.

Sun, B., Richardo-Da-silvia,J.M, and I. Spranger. 1998. Critical factors of vanillin assay for catechins and proanthocyanidins. *Journal of Agricultural and Food Chemistry* 46: 4267-4274.

Sun, J.Q., Guo, L.D., Zang, W., Ping, W.X and D.F. Chi. 2008. Diversity and ecological distribution of endophytic fungi associated with medicinal plants. Science in China Series C. *Life Sciences* 51: 751-759.

Suryanarayanan, T.S. 2011. Diversity of fungal endophytes in tropical trees. In: Prittila, A.M., Carolin, F.A. (edn) endophytes of Tropical Trees, Forestry Sciences Series. *Springer* 67-80.

Suryanarayanan, T.S. 2013. Endophyte research: going beyond isolation and metabolite documentation. *Fungal Ecology* 6: 561-568.

Suwannarach, N., Sujarit, K., Kumla, J.,Bussaban, B and S. Lumyong. 2013. First report of leaf spot disease on oil palm caused by *Pestalotiopsis theae* in *Thailand. Journal of general plant pathology* 79: 277-279.

Talhinhas, P., Azinheira, H.G., Vieira, B., Loureiro, A., Tavares, S., Batista, D., Morin, E., Petitot, A.S., Paulo, O.S, Poulain, J., *et al*. 2014. Overview of the functional virulent genome of the coffee leaf rust pathogen *Hemileia vastatrix* with an emphasis on early stages of infection. *Frontiers in Plant Science* 5: 88.

Tarigan, M., Roux, J., Van Wyk, M., Tjohjono, B and M.J. Wingfield. 2011. A new wilt and dieback disease of Acacia mangium associated with Ceratocystis manginecans and Ceratocystis acaclivora sp. nov. in Indonesia. *South African Journal of Botany* 77: 292-304.

Terhune, B.T., Allen, E.A., Hoch, H.C., Wergin, W.P and E.F. Erbe.1991. Stomatal ontogeny and morphology in phaseolus vulgaris in relation to infection structure initiation by *Uromyces appendiculatus*. *Canadian Journal of Botany* 69: 477-484.

Theerthagiri, A., Thiruvergadam, R., Gandhi, K., Velapan, P and S. Ramasamy. 2007. Chemically and biologically mediated systemic resistance in cucumber (*Cucumis sativus* L.) against *Pseudoperonospora cubensis* and *Erysiphe cichoracearum*. *Phytopathologia Mediterranea* 46: 259-271.

Thomson, L.J., Macfadyen, S and A.A. Hoffmann. 2010. Predicting the effects of climate change on natural enemies of agricultural pests. *Biological Control*, 52: 296-306.

Treutter, D. 2005. Significance of flavonoids in plant resistance and enhancement of their biosynthesis. *Plant Biology* 7: 581-591.

Venter, F and J. V. Venter. 1996. Making the most of indigenous trees. Briza Publication, Cape Town, South Africa 55: 303-304.

Voegele, R.T and K. Mendgen. 2003. Rust haustoria: nutrient uptake and beyond. *New Phytotologist* 159:93-100.

Voegele, R.T., Hahn., M and K. Mendgen. 2009. The Uredinales: cytology, biochemistry, and molecular biology. *In*: HB Deising, ed. The Mycota, 5 Plant relationships. Berlin, Germany: Springer, 69-98.

Von Maydell, H.J.1986.Trees and Shrubs of the Sahel: Their Characteristics and Uses. GTZ, Eschborn, Germany. *Open Journal of Forestry* 5(4): 1102-1107.

WHO. 1999. “Consultation meeting on traditional medicine and modern medicine: harmonizing the two approaches,” (WP)TM/ICP/TM/001/RB/98-RS/99/GE/32(CHN).).

Wingfield, B.D., Ericson, L., Szaro, T and J.J. Burdon. 2004. Phylogenetic patterns in the Uredinales. *Australasian Plant Pathology* 33: 327-335.

Wingfield, M.J., Crous, P.W and T.A. Coutinho. 1996. A serious canker disease of Eucalyptus in South Africa caused by a new species of Coniothyrium *Mycopathologia* 136: 139-145.

Wood, A.R and M.J Morris. 2007. Impact of the gall-forming rust fungus *Uromycladium tepperianum* on the invasive tree Acacia saligna in South Africa: 15 years of monitoring. *Biological Control* 41: 68-77.

Wu, L., Han, T., Li, W., Jia, M., Xue, L and K. Rahman. 2013. Geographic and tissue influences on endophytic fungal communities of *Taxus chinensis* var. mairei in China. *Current Microbiology* 66: 40-48.

Yin, C., Downey, S.I., Klages-Mundt, N.L., Ramachandran, S., Chen, X., Szabo, L.J., Kigawa, T., Kamoun, S and S.H. Hulbert. 2015. Identification of promising host-induced silencing targets among genes preferentially transcribed in haustoria of *Puccinia*. *BMC Genomics* 16: 579.

Zhishen, J., Mengcheng, T and W. Jianming.1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* 64: 555-559.

APPENDICES

Appendix 3.1 Analysis of variance for the level of tannins contained in the *Mimusops zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	3	4.538	1.51259	0.71	0.5545
Error	64	131.627	2.05667		
Total	67	136.164			

Appendix 3.2 Analysis of variance for the level of flavonoids contained in the *Mimusops zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	15	3.44283	0.22952	455.35	0.0000
Error	32	0.02658	0.00083		
Total	47	3.46941			

Appendix 3.3 Analysis of variance for the level of phenolics contained in the *Mimusops zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	15	4.82248	0.32150	5.32	0.0007
Error	47	3.26675	0.06951		
Total	62	8.08923			

Appendix 3.4 Analysis of variance for the level of proteins contained in the *Mimusops zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	16	15.8783	0.99239	4.75	0.0001
Error	34	7.1070	0.20903		
Total	50	22.9853			

Appendix 4.1. Phylogenetic tree based on ITSrDNA sequence variants of endophytic fungi isolated from *M. zeyheri* groves at University of Limpopo.