CHARACTERIZATION OF CANKER-CAUSING FUNGI ON *MELIA VOLKENSII* AND *AZADIRACHTA INDICA* TREES IN THE DRYLANDS OF KENYA.

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DECLARATION

Declaration by the Student

This thesis is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without the prior written permission of the author and/or University of Eldoret.

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I dedicate this thesis to my children.

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ABSTRACT

About 80% of Kenya's land area has been classified as Arid and Semi-Arid lands, and experiences high temperatures and low erratic rainfall throughout the year which leads to water scarcity, degraded and poor soils leading to resource conflicts. In the search for suitable tree species for agroforestry and landscape restoration in Kenya's ASALs two Meliaceae tree species, indigenous *Melia volkensii* (Geurke) and exotic *Azadirachta indica* (A. Juss.) were selected for health status research. Six (6) counties from Coastal and Eastern regions of Kenya were identified for the study. Systematic sampling was undertaken through a survey of the health status of the two tree species to identify fungi associated in the wild and on farms. In the laboratory, standard fungal isolation procedures were employed in culturing the emerging fungi on 2% Malt Extract agar (MEA) and incubated at 25^{o}C for 14 days. Emerging cultures were sub-cultured into pure cultures through single-spore isolation. Eighty-six (86) isolates were selected from the common fungal groups from the two species for Deoxyribonucleic acid extraction and amplification of the internal transcribed spacer (ITS) and translocation elongation factor (Tef) - 1-alpha gene regions Nine (9) species isolated from the Botryosphaeriaceae, were selected for pathogenicity tests under glass house conditions with average temperature of 28^{o} C. Statistical analyses used were analysis of variance and phylogenetic analyses. Symptoms found in the field associated with *Melia volkensii* were dieback and canker with resin flow while symptoms on *Azadirachta indica* were dieback with dry cankers. The combined percentages of canker and dieback were 32%, cankers only 13%, shoot die-back 10%, while 42% of the trees sampled were categorized as healthy and less than 2.5 % were dead or dying. The disease severity was higher in *Melia volkensii* >45 % than in *Azadirachta indica* >40 -<65 %. In total 484 pieces of diseased Melia were cultured using standard laboratory fungal isolation procedures out of which 1452 isolates were realized. Likewise, 694 isolates were made for *A. indica*. Morphological identification of the fungi isolated revealed 8 fungal groups from *M. volkensii* and 6 groups from *A. indica*. Molecular identification grouped the majority of isolates to the family Botryosphaeriaceae (51%). Three (3) of the Botryosphaeriaceae species belonged to the genus Lasiodiplodia namely *L. pseudotheobromae, L. theobromae* and *L. parva*. Phylogenetic analysis showed that most of the fungal groups identified occurred on both tree species and grouped into same clades. Pathogenicity tests showed that the *L. pseudotheobromae* species was most virulent to both *M. volkensii* and *A. indica* while *L. theobromae* was least virulent to both tree species. Wilting and necrosis was recorded within 7 days of inoculation, but wound healing occurred on both species after 12 weeks. *A. indica* had an average lesion of 8cm compared to *M. volkensii* with an average lesion of 14cm. The study gives insight into the two Meliaceae species and fungal attacks and could be suitable for dryland agroforestry. Management of the disease and plants in agroforestry in the drylands will further enhance the resilience of dryland agroforestry systems in Kenya.

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LIST OF ABBREVIATIONS

AEZ	Agro-ecological zones
ASALs	Arid and Semi-Arid Lands
CABI	Centre for Agriculture and Bioscience International
CTAB	CetylTrimethyl Ammonium Bromide
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
FAO	Food and Agriculture Organization
GFRA	Global Forest Resources Assessment
ITS	Internal Transcribed Spacer
JICA	Japan International Cooperation Agency
KEFRI	Kenya Forestry Research Institute
KFS	Kenya Forest Service
m.a.s.l.	Metres above sea level
MEGA	Molecular Evolutionary Genetics Analysis Software
PCR	Polymerase Chain Reaction
rDNA	Ribosomal Deoxyribonucleic Acid
RNA	Ribonucleic Acid
SDS	Sodium Dodecyl Sulfate
TNAU	Tamil Nadu Agricultural University, India
UNCCD	United Nations Convention on Combating Desertification
UNEP	United Nations Environmental Programme

OPERATIONAL DEFINITION OF TERMS

Canker: A sunken necrotic lesion of main root, stem or branching arising from disintegration of tissues outside the xylem cylinder concentric zonation may indicate successive host responses to advancing infection**.**

Climate Change: Changes in climate due to global warming which is a state of increase in atmospheric temperatures caused by the trapping of sun's radiation in the lower levels of the earth's surface. It is projected that the changes in climate may lead to prolonged droughts, intense storms, more severe pest attacks, wildfires, floods in other areas.

Dieback: Necrosis of a shoot beginning at the apex and spreading towards the older tissue, stem death may occur.

Disease Incidence: Percentage of diseased plants in a sample or population

Disease Resistance the prevention of substantial damage to a plant after infection by a pathogen. It can be due to preformed structures in the plant's cells or from infection-induced responses.

Disease Severity: the percentage of relevant host plant tissue that is covered by a symptom or lesion or damaged by the disease.

Disease Tolerance: host dependent mechanisms used to increase host plant health while neutralising or decreasing ability of a pathogen to cause disease or damage**.**

Host Range: the number of plants fungi is pathogenic to. Used in describing how many plant families or genera of fungi can infect and cause damage to including those it is endophytic on only causing infection at the onset of stress.

Pathogenicity Test: The inoculation of a fungi onto healthy seedlings or stems to confirm a pathogen and fulfil Koch's postulates as described by Agrios (2005).

Symptom: A visible or otherwise detectable abnormality arising from a disease or a disorder.

Virulence: The severity or harmfulness of a disease. The extent of damage it causes on its host.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Drylands cover over forty percent 40% of the earth's land mass (Feng & Fu, 2013; Schimel, 2010). They are described as areas where moisture loss through transpiration and evapotranspiration is consistently higher than the average rainfall (Zhang *et al*., 2024). Drylands are in four categories that include arid, semi-arid and dry sub-humid and on the extremes hyper-arid lands based on aridity index (Zomer, Xu & Trabucco, 2022). Aridity index is an indicator that defines the climatic dryness of a location and is calculated as the ratio of annual precipitation (P) to potential evapotranspiration (PET) in a given area (Hanan *et al*., 2021).

For drylands the ratio of P to PET is ≤0.65 meaning that the rate of moisture loss is higher than the precipitation received in drylands (UNCCD, 2017). The water deficit in drylands is caused by a moisture loss that is higher than the precipitation received and prolonged dry periods. This in turn reduces the number of days available for viable crop growth without additional measures such as irrigation (Piao *et al*., 2024). Over 37% of the world's population depends on drylands for livelihood and sustenance (Zhang *et al*., 2024). In Africa, drylands make up about forty-five percent 45% of the land mass and the proportion is increasing with changes in weather patterns (Kolding *et al*., 2016). Drylands are characterized by low average rainfall between 200 to 800 mm annually with the pattern being variable and erratic (Yao *et al*., 2020). Mean temperatures are high levels of evapotranspiration are elevated leading to degradation of soils, water scarcity and high salinity of soils (FAO, 2015; Wale & Dejenie, 2013).

In Kenya, eighty percent 80% of the land area is classified as arid and semi–arid and is home to thirty-five 35 % of the population (Birch, 2018). Life in the arid and semi-arid lands (ASALs) isn't easy. Rainfall is unpredictable and usually low. The soil doesn't help either—erosion, compaction, and salinity all make it tough to farm. Water is hard to find and even harder to get when you need it. All this puts a real strain on agriculture (Global Mechanism of the UNCCD, Conservation International, 2019). Because of these challenges, poverty and food insecurity are common in the ASALs. People often end up fighting over whatever little resources are left. Growing crops or planting trees is a serious struggle here (Plaza *et al*, 2018).

Most people in these areas rely on nomadic pastoralism, subsistence agro-pastoral farming, or ranching to get by (Coleman *et al*, 2021). Small-scale farmers especially have it rough, dealing with unpredictable weather and constant water shortages (FAO, 2015). Almost everything they grow depends on rainfall, and that's never reliable (Plaza *et al*, 2018). To cope, farmers usually plant drought-resistant varieties that grow quickly (Piao *et al*, 2024). But irrigated farming isn't common. High setup costs and salty soils—so salty they need regular treatment—make irrigation a tough sell (Mitchell-Mccallister *et al*, 2020; Karrou & Oweis, 2008). The big problems in these drylands? Water scarcity, long dry spells, frequent crop failures, and ecosystems that just keep getting more degraded (Zomer *et al*., 2022).

A mix of factors—climate swings, unsustainable land use like overgrazing and overstocking—keep pushing the ASALs closer to desertification (Mortimore, 2009). When too many animals graze and trample through these drylands, the soil loses its organic matter. That kills fertility and leaves the earth wide open to wind and water erosion (Yan

et al, 2024). Most land degradation actually comes from the punch: climate patterns and the way people treat the land. Soils lose their nutrients, can't hold water as well, and shed their top layer. They just stop bouncing back from damage (Christian *et al*, 2021). The result? Land becomes less productive, crop yields drop, livestock die off during long dry spells, and dust storms get worse, hitting both people and the environment hard (Wale & Denejie, 2013; Mortimore, 2009; FAO, 2004).

Planting trees for dryland agroforestry can really help. Trees offer all sorts of products, plus cultural and environmental benefits (Telwala, 2023; Mortimore, 2009). They feed livestock when grass disappears, provide food and fuel for families, and supply materials for building and small businesses. On top of that, trees cool the land with shade and actually fix nitrogen, cycle nutrients, and help soil hold onto its organic matter (Meena *et al*, 2022).

Humans are aware that trees can restore landscapes by storing carbon, reducing emissions and climate change adaptation (Ali *et al*, 2024). There are several projects planting high value tree species that are adapted to the drylands aiming at landscape restoration. These trees will also provide income to the households alongside food and energy.(Jhariya *et al*., 2021). Integration of trees and shrubs into the land use in drylands has potential to provide diverse products and services on the same plot of land (Atmadja *et al*., 2019). The agroforestry strategy is integral to Kenya National Climate Adaptation Plan (GoK, 2023).

In the humid highlands, *Grevillea robusta, Moringa oleifera, Leucaena leucocephala*, different Eucalyptus species, and *Calliandra calothyrsus* are commonly planted. In the drylands, species like *Melia volkensii, Sesbania sesban*, and *Azadirachta indica* have been introduced (Evans, 1990). Back in the 1980s and through the late '1990s, the Rural

Afforestation Extension Program really pushed for more agroforestry. The project promoted increased planting of trees on farms focusing on fast growing species for intermediate income. However, the effects of pests and diseases on the new land use were unknown at the time (Schroth *et al*., 2000).

Leucaena leucocephala, for example grows fast and produces fodder, and fuel wood while improving the fertility of the soil. But there is a psyllid known as *Heteropsylla cubana*, that attacked and finished *L. leucocephala* (Alene *et al*., 2012). The sap sucking psyllid attacks the tree's living part and young shoots. Their feeding produces large amounts of honeydew and forms sooty molds. From the attack leaves start falling and tree growth stops. The psyllid can also attack other tree species like *Albizia, Mimosa* and *Piptadenia* (CABI, 2014). Other tree species that were introduced for dryland agroforestry include, *Senna siamea*, *Carissa edulis*, *Strychnos spinosa* (Jama & Zeila, 2005).

Droughts, poor soils, and relentless pests—especially termites—have made it tough for agroforestry to take root in drylands (Evans, 1990). On top of that, tree diseases keep popping up more often in arid and semi-arid areas, making adoption even harder (Graziosi *et al*., 2020). Since the late '80s, pests and diseases have become a real headache for agroforestry systems (Schroth *et al*., 2000). The severity of diseases and pests in these systems is not thoroughly examined in many studies. Because agroforestry mixes a variety of species, people often assume that the trees protect one another in some way, preventing diseases and pests from entering easily (Pumarino *et al*.,2015). Monocultures, however, are a different matter. Attacks just keep coming, and pathogens spread quickly (Liu *et al.*, 2020). According to Kaur *et al.* (2024), this is particularly true for exotic species that are planted outside of their natural habitat because they simply do not adapt well and are more

susceptible to disease. The issue worsens in arid regions. Out there, disease outbreaks are more frequent and severe (Njuguna *et al*., 2011). The drylands' attempts at reforestation and regreening have been hampered by this.

Recent studies have shown that indigenous tree species that include *Melia volkensii, Adansonia digitata, Sclerocarya birrea, Vangueria rotundata, Berchemia discolor* and *Terminalia brownii* are well suited to the drylands and showed low incidences of disease attacks in the drylands (Njuguna *et al.,* 2011; Cherotich *et al.*, 2020, Karani *et al.* 2022; Okeyo *et al.*, 2024). The health status of the Meliaceae family was overlooked in previous research, despite the fact that these trees appear to have great potential for dryland agroforestry. Therefore, the purpose of this study was to investigate the health of two Meliaceae species: *Azadirachta indica* A. Juss, which has established itself as a naturalized species, and *Melia volkensii* Guerke, which is native. Both are excellent options for agroforestry and planting in Kenya's semi-arid and arid regions (ASALs). Among the native trees of Kenya, *M volkensii* is unique. In the arid regions of the nation, people have singled it out for promotion.

Earlier studies skipped over the health status of the Meliaceae family, even though these trees actually look promising for dryland agroforestry. So, this study set out to dig into the health of two Meliaceae species—*Melia volkensii* Guerke, which is native, and *Azadirachta indica* A. Juss, which has settled in as a naturalized species. Both are strong candidates for planting and agroforestry in Kenya’s arid and semi-arid lands (ASALs).

M. volkensii stands out among Kenya’s indigenous trees. People have singled it out for promotion in the country’s dry areas. It’s drought resistant, handles poor soils without a fuss, and you’ll find it growing all over tropical Africa, especially in Kenya’s drylands. It’s

handy, too—farmers use it for timber, firewood, and even as forage for bees. The tree grows fast and you can harvest decent timber in just about ten years. *A. indica* offers something different. People value it for herbal medicines, essential oils, and plant extracts that keep insects away.

Researchers checked both wild and farm-grown trees for signs of insect pests and diseases like canker, dieback, fruit rot, and different leaf problems. They'll use what they found to guide better ways to care for these trees and manage pests, especially for the two species growing in Kenya's drylands. The idea is to promote these two as better options than *Grevillea robusta* and Eucalyptus clones, which often struggle with pests and diseases.

For this study, we checked trees both in the wild and on farms, looking for insect pests and disease symptoms—things like canker, dieback, fruit rot, and leaf diseases. The results will help shape better silvicultural practices and pest management for these two species in Kenya's drylands. If all goes well, these trees could become solid alternatives to Grevillea robusta and Eucalyptus clones, which keep running into problems with pests and diseases.

1.2 Statement of the Problem

Arid and semi-arid lands cover 80% of Kenya's land, these are vast areas with low vegetation cover that offer great potential for afforestation and regreening in the drylands. Efforts to promote agroforestry in the drylands have been hampered by frequent drought, poor soil fertility and incidences of pests and diseases. Some of the common problems that have been identified include, termite damage, cankers, wilt, branch, stem and shoot dieback, root rots, leaf blights, leaf spots, powdery mildews, and death of seedlings and trees among others. Management of these challenges has also been difficult due to inadequate knowledge on the biology and type of pests and pathogens involved.

Incidences of canker and termite attacks have been reported on both *M. volkensii and A. indica* Voi, Makueni and Kitui (Njuguna *et al*., 2005). With continued promotion of *M. volkensii* and *A. indica* growing in the ASALs of Kenya, it is necessary to determine potential biotic stressors of the two tree species. Due to the fact that there is little knowledge on the diseases that affect *Melia volkensii* and *Azadirachta indica* in Kenya, the objective of this study is to investigate the incidence, severity and characterization of canker and dieback fungi associated with *Melia volkensii* and *Azadirachta indica* in selected arid and semi-arid lands of Kenya in order to support sustainable management of *M. volkensii* and *A. indica* for reforestation efforts in the ASALs of Kenya.

1.3 Justification of the study

Plant pests and diseases lead to over 30% yield losses annually amounting to about 220 billion dollars. The cost of disease management includes chemical and physical control methods. Understanding the mechanisms by which pathogens infect their hosts is crucial to developing effective integrated pest management strategies for the identified causal agent. Identifying pathogens protects native species from exotic pests and diseases. This study was designed to identify diseases and pests of the target species for proactive management. The study would also be used to develop integrated pest management protocols for the species in the drylands of Kenya. The research is crucial due to the existing challenges already faced in the drylands, as elucidated in the introduction. Understanding the potential biotic stressors of the target species will also support domestication efforts of the species to support efforts towards dryland and farm forestry.

1.4 Objectives of the Study

1.4.1 Major Objective

The major objective of this study was to assess the incidence, severity and characterization of canker and dieback fungi associated with Meliaceae trees in six (6) selected Arid and Semi-Arid Lands (ASALs) counties in Eastern and Coastal areas of Kenya.

1.4.2 Specific Objectives

i. To evaluate incidence and severity of canker and dieback disease affecting *Melia volkensii* and *Azadirachta indica* in six ASAL counties of Kenya.

ii. To isolate, identify and characterize canker and dieback fungal species associated with *Melia volkensii* and *Azadirachta indica* in the selected counties of Kenya.

iii. To carry out pathogenicity tests of selected fungal species associated with *Melia volkensii* and *Azadirachta indica* in ASALs of Kenya using Koch's Postulates.

1.4.3 Hypotheses

i. There is no significant difference in canker and dieback disease incidence and severity between different selected sites.

ii. There is no significant difference between fungi isolated from *Melia volkensii* and fungi isolated from *Azadirachta indica.*

iii. There is no significant difference in susceptibility to fungi between *Melia volkensii* and *Azadirachta indica*.

CHAPTER TWO

LITERATURE REVIEW

2.1 Drylands in Kenya

Drylands cover over forty percent (40%) of the earth's land mass (Mortimore, 2009; UNEP, 1992). Drylands are described as areas where moisture loss through transpiration and evapotranspiration is higher than the average rainfall (World Atlas of Desertification, UNEP 1992). Dryland categories include arid, semi-arid and dry sub-humid and on the extremes hyper-arid lands based on aridity index (FAO, 2015). Aridity index is defined as the ratio of annual precipitation (P) to potential evapotranspiration (PET) in a given area (FAO, 2004). For drylands the ration of P to PET is ≤0.65 meaning that rate of moisture loss is higher than the precipitation received in drylands (UNEP, 1992). The water deficit in the drylands caused by higher moisture loss than precipitation causes prolonged drought periods. This in turn reduces the number of days for viable crop growth without irrigation. Over 37% of the world's population depends on drylands for livelihood and sustenance (Thomas *et al*., 2014).

Dryland forests can be harnessed for a variety of ecosystem services for example, soil erosion control, shade, windbreaks, soil fertility improvement, water conservation and rehabilitation of water sources (FAO, 2004). In addition, these forests can provide products for income generation including timber, charcoal, medicines, fruits, gums and resins, wood for carving and timber for construction and furniture all which lead to supplemental income and better livelihoods for communities in the arid and semi-arid lands (Blackie *et al*., 2014). In Africa, drylands make up forty-five percent 45.01 % of the land mass (FAO, 2002). Further, Kenya's land area is classified as eighty percent 80 % arid and semi–arid and is

home to thirty-five 35 % of the population (Adoyo *et al*., 2024). Therefore, dryland forestry has a great potential to restore degraded landscapes and mitigate against negative climate change impacts (FAO, 2015). However, drylands are faced with challenges of water scarcity, erratic rainfall and degraded landscapes due to overgrazing, eroded and saline soils with low fertility (FAO, 2004). Tree species suitable for establishment in the ASALs can be used as fodder, a source of food and can improve the soil structure and fertility (Atmadja *et al*., 2019).

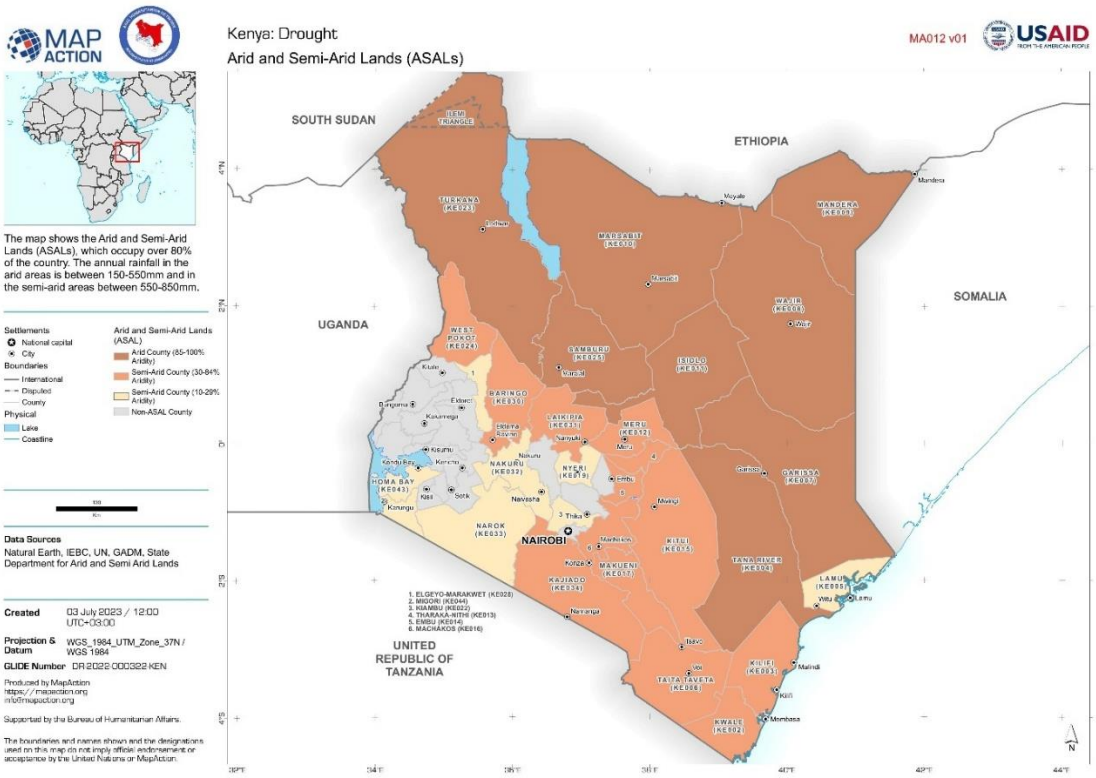

Figure 1: Map of Kenya showing Arid and Semi-arid Counties

(Source: ASAL Humanitarian Work, 2023)

2.1.1 Challenges faced in the Arid and Semi-arid lands of Kenya

Typically, drylands receive only 200 to 800 mm of precipitation annually (UNEP, 1992). The rainfall received is in small amounts and far between seasons being unreliable for food production. The hot temperatures quickly absorb moisture from the soil. The soils become salty and too dry and are often carried off by wind and water (Wale & Dejenie, 2013). These factors make tree planting and food production difficult in the arid and semi-arid lands (UNDP, 2013a). Food insecurity and poverty increase naturally when there is a shortage of water and the land continues to deteriorate (Creswell & Martin, 1998). People fight over the few resources that remain.

The main constraint in drylands? Water shortages, long dry spells, crops failing over and over, and ecosystems wearing down. Things only get worse when you combine climate fluctuations with bad land management practices like overgrazing and cramming too many animals onto too little land. Overgrazing destroys fertility, removes organic matter from the soil, and exposes the ground to wind and water erosion. Desertification eventually encroaches, and the cycle simply continues (Mortimore, 2009; FAO, 2004). The ASALs are getting closer to desertification as a result of all these factors, the erratic climate, and human land use practices like overgrazing and overcrowding animals on arid areas (Mortimore, 2009; FAO, 2004).

When animals eat the vegetation in the drylands, the soil loses its organic matter. This changes the soil fertility and leaves the soil open to both wind and water erosion (FAO,2004). Most of the land degradation here comes from how climate and people's choices wear down the soil. The soil starts to lose nutrients, can't hold water as well, and loses its organic matter and top layer. It gets weaker, less able to bounce back from stress or recover when things go wrong (FAO, 2004). All this makes the land less productive. Communities and the environment are particularly hard hit in the drylands, where crop yields decline, livestock deaths increase during extended dry spells, and dust storms worsen (Wale & Denejie, 2013; Mortimore, 2009; FAO, 2004). The majority of the people living in the ASALs are pastoralists who move around in search of pasture and water for their animals. Food insecurity results from their food types being negatively impacted by short cultivation periods as well as pest and disease attacks. Fruits and leaves from trees have long been used as a food source to prevent malnutrition during times of famine. To

encourage regreening and reafforestation, some farmers have participated in a variety of initiatives.

The communities in the ASALs are primarily pastoralists who migrate from one place to another looking for pasture and water for the livestock. Their types of food are severely affected by short cultivation periods and the effects of pest and disease attack hence leading to food insecurity. The trees leaves and fruits have always managed as source food to avoid malnutrition during the starvation periods. Some farmers have been involved in various projects to engage them in reafforestation and regreening. In Niger, farmers have successfully reclaimed over 5 million hectares of land by 2006 through natural regeneration. This effort not only boosted crop yields but also provided essential fodder for livestock (Pye-Smith, 2013). Agroforestry, which integrates trees and shrubs into existing land use, offers a promising way to improve livelihoods, increase incomes, and promote sustainable development by restoring degraded lands (Wale & Denejie, 2013).

2.1.2 Afforestation in the Arid and Semi-Arid Lands of Kenya

Twenty-eight (28) of Kenya’s 47 counties are classified as arid and semi-arid lands (ASALs) (Fig. 1). The actual socioeconomic and ecological status of ASALs is generally poor due to the degradation of soil and vegetation which are directly related to concentration of human and animal population (Mortimore, 2009).

Areas with lower elevations and adequate moisture are the primary targets of reforestation efforts in Kenya's arid and semi-arid lands (ASALs), which are frequently regarded as agriculturally low-potential regions (Fuchs *et al.*, 2019). These drylands can provide for the basic needs of the community when trees and forests are carefully incorporated (Castro Rodriguez *et al.*, 2016). With 80% of Kenya's land area covered by ASALs, the country

has a great chance to meet its 10% tree cover target and support international efforts to reduce emissions (GoK, 2023).

It is feasible to create woodlots and plantations of drought-resistant trees that can significantly contribute to climate change mitigation by choosing the appropriate tree species and implementing appropriate management techniques (FAO, 2015). In order to restore degraded areas in ASALs, local communities have partnered with NGOs, government agencies, and wood-based companies to encourage the planting of drought-tolerant tree species, such as Melia volkensii, Azadirachta indica, and Eucalyptus hybrids (Komaza, 2017).

In this endeavor, the Kenya Forestry Research Institute (KEFRI) has played a crucial role by creating superior tree seeds and seedlings for farmers. In order to assist farmers in effectively managing the woodlots they create, they also offer continuous technical assistance (KEFRI, 2017).

2.1.3 Achievements in Dryland Forestry Research

The promotion of tree growing is one of the ways of enhancing sustainable ecosystem services in Kenyan ASALs. Research efforts at KEFRI have concentrated on the propagation and genetic improvement of selected drought-tolerant tree species, particularly *Melia volkensii* and *Acacia tortilis* (JICA, 2013). Genetic improvement of the two tree species has mainly been aimed at increasing timber yield and widening the species propagation zones to cover more of the ASALs in Kenya (Mulatya, 2005b; Juma *et al*., 2005). Little research has been done on pest and disease resistance because both *Melia volkensii* a native species and *Azadirachta indica* a naturalized species are considered to

be naturally resistant to attack by termites and fungi (Perez-Flores, Eigenbrode & Hilje-Quiroz, 2012).

KEFRI, through its partnership with Japan International Cooperation Agency (JICA) and in its effort to promote the use of drought tolerant tree species as an adaptation strategy to climate change has established two clonal seed orchards following numerous generations of improved *Melia volkensii*, a fast-growing hardwood (Kariuki *et al.*, 2021). Further, KEFRI has published guidelines on how to grow improved *M. volkensii* stands (Muok, 2010). A seed processing and distribution unit has been established in Kitui to provide quality tree seed for commercial planting of *Melia volkensii* in Kenya (KEFRI, 2017; Kamondo *et al*., 2016). These efforts are aimed at promoting afforestation using *Melia volkensii* and *Acacia tortilis* for sustainable development and climate change mitigation in arid and semi-arid lands ASALs. This study is follow-up research to help in the management of *Melia volkensii* on farm.

2.2 Agroforestry as a land use system in the ASALs

Agroforestry refers to a term used to define a land use system where trees or shrubs are grown in the same land unit as agricultural crops and /or animals. More and more farmers are adopting agroforestry in the ASALs of Kenya. This practice protects the environment by providing shade and restores the soil while producing valuable products (Atmadja *et al*., 2019; Clough *et al*., 2011). Agroforestry can improve the lives of those living in the drylands and restore the ecosystem sustainably (FAO, 2015; Sharma *et al.*, 2016).

Trees in agroforestry are used as woodlots, fodder lots, alley cropping, shade trees, windbreaks, and improved fallows (Tengnas, 1994; Rocheleau *et al*., 1988). In agroforestry the elements determine its classification i.e. silvipastoral systems have livestock and trees,

agrisilvopastoral systems incorporate crops, trees, and livestock, and agrisilviculture uses crops and trees (Luedeling *et al*., 2014; Nair, 2011). Increased need for food and land for the growing population requires sustainable use of land (McMullin *et al.*, 2019).

2.2.1 Benefits of agroforestry as a land use system in the drylands

Agroforestry systems when properly designed and well managed provide a number of benefits. Trees planted on farm or rangeands with the aim to protect the environment and increase productivity (Dinesh *et al*., 2017; Huxley, 1983). Tree roots avail nutrients in the soil that enable pasture and plant growth (Dawud *et al*., 2016). Trees can also absorb heavy metals from soils reducing the salt levels (Castro-Rodriguez *et al*., 2016; Pajevic *et al*., 2016).

Trees can also offer shade in croplands and for livestock when weather is dry and hot (Atmadja *et al*., 2019; Tengnas, 1994; Rochleau *et al*., 1988). When pasture is limited during droughts, trees in rangelands serve as an essential source of fodder (Assouma *et al*., 2017). By binding soil particles and providing protection from wind and water erosion, they also aid in the control of soil erosion (Gregg, 2008). In addition to these advantages, trees help mitigate global warming by absorbing and storing carbon in the atmosphere (FAO, 2015; van Kooten *et al*., 2002). They supply vital resources like lumber and fuelwood for domestic use as well as revenue production.

According to Taha et al. (2012), certain trees and shrubs that grow in arid and semi-arid lands (ASALs) even have therapeutic qualities and essential oils that are used in the cosmetic and pharmaceutical industries. For instance, acacias yield gums and resins that are useful as stabilizers in medications and confections (Mulugeta, 2004). Thanks to its wide-ranging benefits, agroforestry has emerged as a climate-resilient land use system,

particularly suited for drylands that are more vulnerable to the impacts of climate change compared to higher-potential areas in Kenya (Wondie & Mekura, 2018).

Agroforestry when well-planned and managed is a most promising land use for profitability in ASALs which in most cases are not viable for forestry (Atmadja *et al*., 2019). Integrating trees into ASALs can help rehabilitate seasonal water sources and recharge groundwater during rainy periods (FAO, 2015). Introducing agro-silvicultural systems with nitrogen-fixing tree species can restore soil fertility, boost food production, and diversify diets (Koutika & Richardson, 2019; Thomas *et al*., 2014; Luedeling *et al*., 2012).

As Kenya strives for low-carbon, climate-resilient development, dryland forestry stands out as a promising solution with significant benefits for the environment, the economy, and ASAL communities (GoK, 2023). The success of tree planting for improving livelihoods depends on selecting tree species that hold value for local communities (Tengnas, 1994).

Agroforestry systems, though complex, often protect against economic losses by ensuring that if one component is affected by pests or diseases, the others remain unaffected (Njuguna, 2011). This makes agroforestry a sustainable approach to integrated pest and disease management, helping farmers safeguard the health of unaffected components (Jamnadass *et al*., 2013; Schroth, 2000). Due to their perceived tolerance to diseases the focus of this study was to assess the health status of *M. volkensii* and *A. indica* which are emerging popular agroforestry species in Kenya.

2.2.2. Challenges of growing trees in Arid and Semi-arid Lands

ASALs due to their low precipitation, strong winds and high temperatures experience great soil erosion leading to poor soils (Amtadja *et al.,* 2019). The ASALs also have been used mostly for pasture and have been over grazed over a number of decades leading to

degradation of the landscapes and the vegetation cover (FAO, 2015). The prolonged droughts and overexploitation lead to loss of biodiversity and increased desertification (Boitt & Odima, 2017). Weak land management policies that lead to conflict over the scarce water and land in Kenya worsen the challenges faced in the ASALs (FAO, 2015)**.**

Due to the low precipitation, poor soils and variable moisture content in the arid and semi-arid lands (ASALs), most tree species are not able to survive (Maluki *et al*., 2016). The high salt content, little rainfall and pest problems make tree planting difficult in ASALs (Amtadja *et al*., 2019; Boitt & Odima, 2017). Drought-tolerant species that can withstand poor soils are a prerequisite for successful implementation of large-scale tree planting in drylands (Cherotich *et al*., 2020). This adaptation also includes protection from regional pests and pathogens (Kaur *et al*.,2022).

Native species have been found to be well adapted to challenges in ASALs (Wondie & Mekuria, 2018). In Kenya, Meliaceae and Fabaceae species are suited to drylands (Koutika & Richardson, 2019). Agroforestry, through dryland and farm forestry initiatives, can address challenges faced in the ASALS and become an important contributor to the livelihoods of rural communities in the ASALs (Karp *et al.* 2013). The government of Kenya, through the Vision 2030 under Environment, water and sanitation, plans to introduce commercial tree species in ASALs in order to control desertification and improve livelihoods (GoK, 2008).

2.3 The family Meliaceae & Its Use in Agroforestry Systems of Kenya

Meliaceae is a family of leguminous trees that consists of trees and shrubs (Chen,1997). In both hemispheres, the Meliaceae family comprises about 50 genera and 650 species that are found in tropical, subtropical, and occasionally warm temperate regions (Hardin et al,

2000; Mabberley, 2010). The distinctive chemical characteristics of this family, including limonoids, azadirachtin, alkaloids, and phenols, are well-known and help explain the trees' innate resistance to insect damage (Perez-Flores *et al*., 2012). In Kenya's Eastern and Coastal provinces, where termite and insect attacks frequently cause other exotic tree species to fail, some species of this family are frequently planted on farms because of their resistance to insects.

The two most prominent Meliaceae species in Kenya are *Azadirachta indica*, a naturalized exotic species that originated in Burma, Asia, and *Melia volkensii*, an indigenous tree (Maundu & Tengnas, 2005). These species were picked because of their important contributions to insect control, medicine, and timber production. In Eastern and Coastal Kenya, both trees are extensively grown on farms and in the wild. Research by Njuguna *et al*. (2011) revealed that these species are relatively tolerant to canker and dieback diseases, which have posed challenges to the cultivation of Grevillea robusta and other exotic trees in Kenya’s drylands.

The Meliaceae family is a good choice for agroforestry in drylands because of its ability to adapt to hot, dry conditions as a result of climate change. For many years, Fusarium species were the main cause of seedling deaths reported in eastern Kenyan farmers' nurseries (Kamondo *et al*., 2016). However, in both natural and planted populations, mature *Melia volkensii* trees were found to have no notable pest or disease problems (Infonet Biovision, 2017a). However, the seedlings are susceptible to powdery mildew, which can result in yellowing, growth retardation, and, in extreme situations, death. Furthermore, if not adequately pre-treated prior to sowing, *M. volkensii* seeds are susceptible to rotting (Ulian *et al*., 2019).

2.3.1 *Melia volkensii* (Guerke) in Kenya

(a) Brief Botanical Description of Melia volkensii

In Kenya, *Melia volkensii* (Guerke), commonly referred to as Mukau, is native. It has branches that hang low making a rounded or spreading crown and is deciduous (Vandenabeele, 2015). Fully grown trees can reach a diameter of 25 com and height of up to 20 metres. The bark is gray in colour it is smooth in young age but furrows as the tree ages. Melia leaves are bipinnate with three to seven leaflets per pinna. The tree has both male and male flowers with dense clusters of white flowers. Petals are tetra- to pentamerous, white, free and may curl backwards. Fruits are drupe-like and oval up to 4 cm long, colour changes from green to yellow as they mature and are conspicuous on the bare tree (Orwa *et al*., 2009).

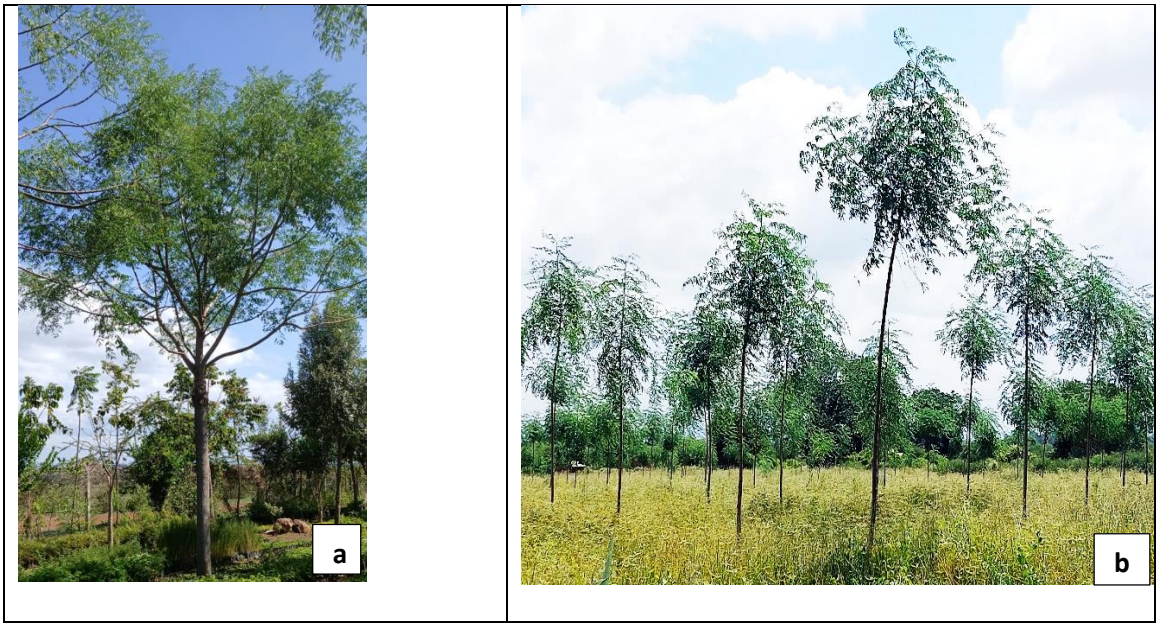

Plate 1: Showing *Melia volkensii* tree and plantation.

Source: Angela Muthama KEFRI 2023

(b) Ecology and Propagation of Melia volkensii in Kenya

Melia volkensii is a valuable tree species that is endemic to semi-arid areas of eastern Africa mostly found in the Eastern, North Eastern and Coastal regions of Kenya (Maundu & Tengnas, 2005). It is usually found in dry bushland or woodland and drier wooded grasslands, and in the coastal hinterlands, but not very near the coast. The species is frequently distributed within the altitude range of 400-1650 m.a.s.l. which lies in agroecological zone (AEZ) V-VI (Maundu & Tengnas, 2005; Jaetzold and Schmidt, 1982). *Melia volkensii* has been domesticated in agroforestry systems in Eastern Kenya (Kamondo *et al*., 2016). *Melia volkensii* is valued in Eastern Kenya for its hard wood which is termite-resistant (Muthike & Githiomi, 2020).

M. volkensii has seed dormancy that limits its propagation by seed (Kimondo & Kiamba, 2005). A number of methods have been tested to break seed dormancy such as acid, hot water, mist chamber use, mechanical crushing of the nut or seed nipping (Milimo, 1989). When the seed is stored for long or handled poorly the use of the seeds reduces by almost 60% (Olung'ati *et al*., 2023). Propagation by tissue culture has been used by KEFRI in collaboration with JICA and a protocol has been published on the same (Dushimimana *et al*, 2022; KEFRI, 2017; JICA, 2013)

(c) Reported Diseases of M. volkensii in Kenya

In the nursery *Melia volkensii* has been reported to be affected by a number of fungi *Alternaria spp., Colletotrichum spp., Fusarium spp., Phomopsis spp.* and *Botrytis spp.* (Njuguna *et al*., 2005). Mulanda et al. 2013 reported mold fungi *Aspergillus flavus* and *Rhizopus stolonifer* as causes of seed rots (Mulanda *et al*., 2013). The seeds are prone to seed borne pathogens and can be prevented using chemicals when sowing before the

dormancy is broken (Olung'ati *et al.*, 2023; Ulian *et al*., 2019). Use of correct protocols when planting seedlings and application of fungicides and control the diseases above (Muok, 2010).

In 2005, Njuguna et al., recorded a stem canker disease incidence in Kibwezi and the cankers yielded a Botryosphaeria species. This study will look into the fungi associated with trees on *M. volkensii* in the wild and on farm. The symptoms, incidence and proper identification will be recorded.

2.3.2 *Azadirachta indica* (A. Juss.) in Kenya

(a) Brief Botanical Description of Azadirachta indica

Azadirachta indica belongs to the family Meliaceae and is a fast-growing; small to medium sized tree and reaches to about 20 meters with a dense round crown usually evergreen except in very dry areas where its leaves may fall off as a water conservation measure (Maundu & Tengnas, 2005). Its bark is pale grey-brown and grooved. Its leaves are alternate, crowded at the end of branches, simply pinnate to 20-40 cm long, estipulate glossy green with two pairs of glands at the base, petiole 2-7cm long, margin serrate, apex acuminate, base unequal. Flower inflorescence is up to 30 cm long, bracts minute and caducous, flowers bisexual or male on same tree, actinomorphic, pentamerous, small fragrant, cream white spreading and ciliolate inside. Its fruits are oval yellow berries when ripe, 2 cm long thin skinned with oily pulp around 1-2 seeds (CABI, 2014; Orwa *et al.*, 2009; Maundu and Tegnas, 2005). *Azadirachta indica* is valued in coastal areas for its medicinal value in treatment of ailments and used in cosmetics and beauty industries. It is also planted as a wind break and shade tree.

(b) Ecology and Propagation of Azadirachta indica in Kenya

Azadirachta indica is an exotic tree species from North East India, Burma but has been naturalized in the world tropics where it has been widely planted. The species has been extensively planted in Tropical Africa especially in the arid and semi-arid regions (CABI, 2014). It is very drought resistant and performs well on poor soils but not water logging. In Kenya it is mostly found at the coast, eastern lowlands at altitudes of 0-1500 meters above sea level (Maundu and Tegnas, 2005; Jaetzold and Schmidt, 1982). Propagation of *A. indica* is usually by direct sowing of fresh seeds which exhibit good germination with no pretreatment required (Maundu and Tegnas, 2005). Drying kills the seeds and a fungicide must be used if the seeds have to be stored, to avoid seed rots (Baumer, 1983).

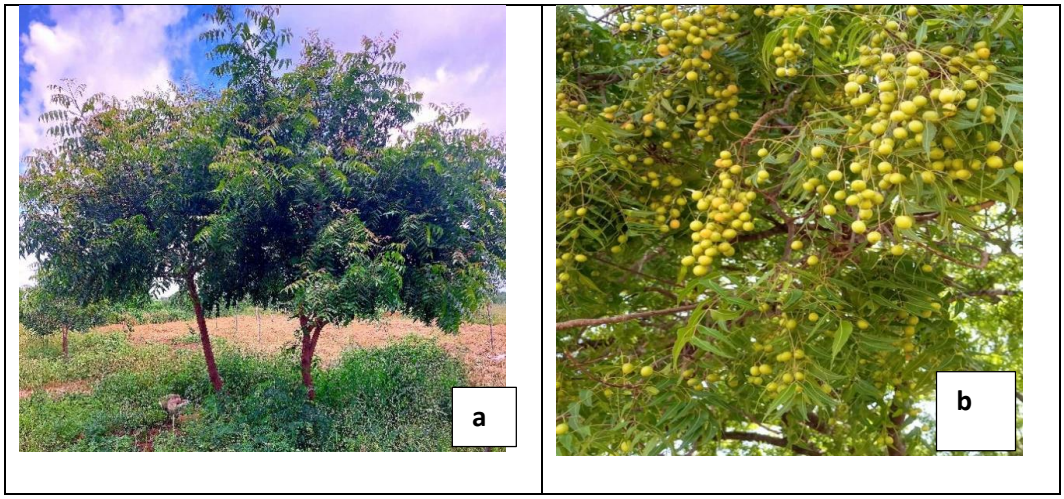

Plate 2*: Azadirachta indica (*Neem*) (a)* on farms and (b) Fruiting Neem at the Coast. Source: Angela Muthama, KEFRI 2023.

(c) Reported Diseases of Azadirachta indica in Kenya

Several fungi have been found to cause diseases on neem seedlings and plantations in Asia and West Africa. These are: *Alternaria sp., Fusarium sp., Colletotrichum sp., Cercospora sp., Oidium sp., Ganoderma sp.* and *Corticium sp.* (TNAU, 2016). These fungi cause

damping off in the nursery and leaf blight conditions leading to losses in the nursery and when seedlings are planted in the field. Bacterial wilt caused by *Xanthomonas azadirachtii* and *Pseudomonas viticola* has also been recorded in India (Bhanumathi and Rai, 2007). In another study, seedlings of *A. indica* were found to be mildly susceptible to *Botryosphaeria* species under hot conditions (Njuguna, 2011) in glasshouse experiments. There is little information on the diseases of mature neem trees in Kenya.

In India, *A. indica* seedlings have been reported to be affected by *Phomopsis azadirachtae* (Shirahati *et al*., 2019). The seedlings of the tree are also susceptible to powdery mildew fungi *Oidium azadirachtae*. Bacterial wilt caused by *Pseudomonas viticola* (Diatloff *et al.*, 1993) and *Xanthomonas azadirachtii* also cause leaf spot disease on the seedlings (TNAU, 2016). *A. indica* is also susceptible to *Ganoderma lucidum* which causes root rots (Adotey *et al*., 2024). All these were reported to cause mild infections of no economic significance (CABI, 2014). This is probably due to the perception that mature leaves and branches of *A. indica* produce compounds that inhibit fungal growth (Rodrigues *et al*., 2019).

Botryosphaeriaceae fungi on *Melia volkensii* were recorded in a seed orchard at KEFRI, Kenya in 2004 (Njuguna *et al*., 2005). This family of fungi has a wide host range and geographical distribution range globally and is mainly classified as a latent pathogen or endophyte known to cause infection only at the onset of physiological stress to its host (Osorio *et al.*, 2017; Phillips *et al.*, 2013; Liu *et al*., 2012).

2.4 Fungi associated with the Meliaceae

From literature about seventeen (17) fungal species belonging to 12 families have been reported to be associated with the Meliaceae. These include *Alternaria sp., Fusarium sp., Botryosphaeria sp., Phomopsis sp*., and *Corticium sp*. that occur on both *M. volkensii* and

Azadirachta indica (TNAU, 2016). *Cercospora sp*., *Botrytis sp*., *Oidium sp*., *Aspergillus sp*., *Colletotrichum sp.* and *Rhizopus stolonifer* have been recorded on *M. volkensii* (Mulanda *et al*., 2013; Njuguna *et al*., 2005).

Out of these, five (5) families are confirmed as fungi of economic importance in forestry. The families Nectriaceae, Botryosphaeriaceae, Diaporthaceae, Ganodermataceae, and Mycosphaerellaceae are of economic importance. Botryosphaeriaceae affect many crops and tree species globally (Cherotich *et al*., 2020; Slippers *et al*., 2017). For this reason, the family has been discussed in detail below.

Nectriaceae are also key in forestry and has been reported on agroforestry tree species in Kenya. Fungi is this family produce symptoms on leaves, branches, and roots of seedlings, and trees l(Njuguna *et al.,* 2011). The fungi are either yellow, orange red to purple or brown in nutrient media and have uniloculate perithecia (Zeng & Zhuang, 2022). Species of Nectriaceae have been found to be pathogens, endophytes while others are saprophytic on soils and plant debris (Gordon *et al*., 2015).

Diaporthaceae fungi have been known to be pathogens and saprobes of woody plants that cause cankers, leaf spots, fruit rots, among other symptoms (Karani *et al*., 2022). Some species are also isolated from unsterilized seeds of plants (Gilbert, Diaz & Bregoff, 2023). Diaporthaceae are also known as pathogens that cause pre- and post-germination losses of seedlings and seedling deaths at the nursery stage of horticultural crops (Chaisiri *et al*., 2022).

There are 120 known genera of fungal species that are detrimental to a broad range of plants in the Mycosphaerellaceae family, which is a member of the order Capnodiales (Videira *et al.,* 2017). This group of fungi is important to agriculture, horticulture, and

forestry. For example, in several regions of Europe, the production of olive oil has been adversely affected by Cercospora leaf spot (Lombardo *et al*., 2024). In a similar vein, *Pinus radiata* cultivation was stopped in East Africa due to Dothistroma red band needle disease (Barnes *et al*., 2016). Soft rot in woody plants is a common occurrence in the Ganodermataceae family, which includes sporophytes in the order Polyporales (Adotey *et al*., 2024). Rot fungi kills trees globally by attacking tthe heartwood and compromising the wood structure. The fruiting bodies left on wood debris prevents establishment of planted trees (Jazuli *et al.*, 2022). Some Ganoderma species have been cultivated for their medicinal value and as a dietary supplement (Lawal *et al.*, 2019).

The Botryosphaeriaceae family are important pathogens that affect woody plants globally (Marsberg *et al*., 2017; Slippers & Wingfield, 2007). Their impact is heightened in hot climates and it is imperative that their potential impact be assessed in dryland agroforestry (Pour *et al*., 2020).

2.4.1 Botryosphaeriaceae Fungi

The family Botryosphaeriaceae Theiss & Syd., Theiss. & Syd. [as 'Botryosphaeriacae'], belongs to the Kingdom Fungi, Division Ascomycota, Class: Pezizomycotina, Phylum: Dothideomycetes and the order Botryosphaeriales (Murill, 1918). It was first described in 1894. It is a family of fungi with a cosmopolitan distribution across the globe except in the Polar Regions known to cause severe die-back and canker leading to mortality of many woody plants (Zlatkovic *et al*., 2016; Phillips *et al.*, 2013; Slippers *et al*., 2013; Slippers *et al*., 2007).

The Botryosphaeriaceae has twenty-three (24) identifiable genera and two hundred (200) species known from culture isolations (Burgess *et al.*, 2019). The major genera are:

Lasiodiplodia, Dothiorella, Macrophoma, Sphaeropsis, Fusicoccum, Diplodia and *Botryosphaeria* (Phillips *et al*., 2013; Slippers &Wingfield, 2007). The group has been the subject of many studies due to their complex nature in the biology and taxonomy of the family. Constant renaming of some of the genera and species under this family has led to confusion in its naming, but that has since been cleared using DNA based identification (Phillips *et al*., 2013; Liu *et al*., 2012; Slippers & Wingfield, 2007). Majority of genera of Botryosphaeriaceae are described as endophytes that only become pathogenic at the onset of stress on the host (Slippers & Wingfield, 2007). The genus Botryosphaeria was proposed to be changed to a new name due to the presence of only one species namely *Botryosphaeria obtusa* (Slippers *et al.*, 2007). Seventeen (17) lineages were identified based on multi-locus phylogeny by Phillips *et al*., (2013) even though Liu *et al*. 2012, added Auerswaldia as a new genus in the family Botryosphaeriaceae which was disregarded by Phillips *et al*., (2013) due to inadequate data to support its differentiation from Lasiodiplodia and Dothiorella for the described species of Auerswaldia; *A. lignicola* and *A. dothiorella*.

Species from this family have been isolated from native, exotic and naturalized ornamental tree species in most tropical countries from both plantation and agro-forestry systems (Zlatkovic *et al*., 2016; Chen *et al*., 2014; Slippers *et al*., 2009; Sanchez *et al*., 2003). The *Botryosphaeriaceae* are known to attack many genera of plants including angiosperms and conifers (Sinclair &Lyon, 2005). The fungi attack all plant parts causing different symptoms with varying incidences. The fungi have been identified as seed-borne in some woody species include *Prunus africana* and *Podocarpus falcatus* in Ethiopia (Gure *et al.*, 2005).

The plurivorous nature of the Botryosphaeriaceae species is a threat to agroforestry systems since the disease can spread to agricultural crops causing symptoms a variety of symptoms that include necrosis, die-back and death of crops and fruit trees (Cherotich *et al.,* 2020; Machado *et al*., 2014; Urbez-Torres *et al*., 2014; Latinovic *et al*., 2013; Ismail *et al*., 2012; Pitt *et al.,* 2010). The Botryosphaeriaceae fungi are known to have significant economic impacts on agroforestry and agriculture by causing crop losses(Phillips *et al*., 2013; Pitt *et al.,* 2010; Slippers *et al*., 2007). These fungi can lead to the wilting of twigs and branches, the formation of longitudinal cankers on stems and twigs, girdling of stems, and the appearance of sunken holes on plant bark. Under favorable conditions, they may produce small fruiting bodies called pycnidia on the surface of stems, which allow them to survive through unfavorable seasons (Njuguna *et al*., 2011; Slippers *et al*., 2009). They are spread by wind and rain, especially during warm and humid weather (Sinclair & Lyon, 2005).

Trees are primarily infected through wounds caused by pruning, mechanical damage, or natural openings like stomata and lenticels (Njuguna *et al*., 2011). Once inside, the fungi germinate and damage the surrounding wood, causing stem rot and lesions. Botryosphaeriaceae fungi are versatile, acting as endophytes, latent pathogens, or saprobes (Mehl *et al.,* 2017; Slippers & Wingfield, 2007).

Endophytes live harmlessly within healthy plants but can become harmful when trees are stressed by factors like drought, insect damage, fire, frost, or waterlogging (Phillips *et al*., 2013; Slippers *et al*., 2007). These fungi have been identified as endophytic in species like *Melia azaderach* and various eucalypts, highlighting their adaptability and potential to cause damage under the right conditions (Xiao, 2014)

There are four major classes of fungal endophytes and they are grouped into two categories based on differences in evolutionary relatedness, plant hosts and ecological functions that is clavicipitaceous and non-clavicipitaceous (Table 1).

The Botryosphaeriaceae fungi have been described as class 3 endophytes which are defined as a non-clavicipitaceous endophyte; with broad host range, occupy shoots of plants and have limited *planta* colonization but are high in *planta* biodiversity (Rodriguez *et al.*, 2009). It has been found that the fungi germinate epiphytically, penetrates the plant and grows intercellularly and is accelerated by stress which weakens cuticle penetrability and endophyte persistence in plant tissue.

Table 1 Criteria used to characterize fungal endophytic classes

	Clavicipitaceos	Non- Clavicipitaceous		
Criteria	Class 1	Class 2	Class 3	Class 4
Host range	Narrow	Broad	Broad	Broad
Tissues colonized	Shoot and rhizome	Shoot, root and rhizome	Shoot	Root
In planta colonization	Extensive	Extensive	Limited	Extensive
In planta biodiversity	Low	Low	High	Unknown
Transmission	Vertical and horizontal	Vertical and horizontal	Horizontal	Horizontal
Fitness benefits	NHA	NHA and HA	NHA	NHA

> Non-habitat adapted (NHA) benefits such as drought tolerance and growth enhancements are common among endophytes regardless of the habitat of origin. (**Source:** Rodriguez *et al*., 2009)

In forestry and agricultural species of Botryosphaeriaceae are latent pathogens. In particular, *Botryosphaeria dothidea* has a prolonged latent infection with infection only becoming manifested at the occurrence of stress to the host (Masberg *et al*., 2017; Njuguna, 2011; Slippers *et al*., 2005). As a pathogen of great importance in woody plants, in its latent phases several species have been isolated from hosts showing a systematic infection though prolonged (Mehl *et al.*, 2017). However, some authors argue that the family cannot be strictly classified as latent pathogens due to the pathogenic nature of some of the species on some plants (Liu *et al*., 2012).

A number of species under the family Botryosphaeriaceae have been classified as saprobes on trees. The isolates collected from dead branches, leaves and stems were grouped into 5 genera *Diplodia, Neofusicoccum*, *Dothiorella*, *Botryosphaeria*, and *Eutiarosporella.* The activity of these fungi on dead tissue is saprobic (Dissanayake *et al.*, 2017). Dothiorella has previously been identified as a saprobe (Dissanayake *et al*., 2016; Phillips *et al*., 2013; Liu *et al*., 2012). The saprobes have been collected from dead tissue of Cupressaceae, Meliaceae and Rosaceae plant families.

In the context of a warming climate the Botryosphaeriaceae can be serious pathogens but because species of the Meliaceae are believed have coevolved with the natural environment of the drylands we expect the two species under study to tolerate the infection.

In Kenya, species of the Botryosphaeriaceae fungi have been found in various exotic plantation tree species across the country. One of the main reasons for this disease outbreak is poor site selection, where trees are planted outside their ideal ecological zones, leading

to high mortality rates. Affected species include *Eucalyptus camaldulensis*, Eucalyptus clones, *Grevillea robusta, Azadirachta indica*, and *Senna siamea* (Njuguna, 2011, Njuguna *et al*, 2011).

In earlier studies, an outbreak of *Diplodia pinea* (now known as *Sphaeropsis sapinea*) in pine plantations led to a ban on planting *Pinus radiata* in Eastern Africa (Barnes *et al*, 2016). Additionally, related fungi from the Teratosphaeriaceae family have caused significant issues like cankers, dieback, and foliar diseases in various Eucalyptus species (Machua *et al*, 2016). For instance, *Teratosphaeria zuluensis* creates circular, dark brown lesions with dark pycnidia, resulting in stunted growth and wood rot, which ultimately reduces timber yields (Jimu *et al*, 2016).

In Kenya, *Teratosphaeria gauchensis* has been identified in Eucalyptus trees in the central region. This species was first reported in South Africa in the late 1990s (Jimu *et al*, 2014). When fungal species spread to new regions, they can hybridize, making identification and management more challenging (Machua *et al*, 2016). The Botryosphaeriaceae fungi, in particular, are highly aggressive due to the close genetic relationships within the family, which likely explains their rapid and widespread distribution globally (Slippers *et al*, 2007). The interrelatedness also means that control mechanisms developed for the fungi will be widely effective against several species (Dissanayake *et al*., 2016; Raja *et al*., 2016; Liu *et al.*, 2012; Slippers *et al*., 2007).

2.4.2 Nectriaceae fungi

Nectriaceae Tul. &C. Tul. (1844) are a fungal family that comprises of uniloculate perithecia producing fungi that are either yellow orange red to purple or brown in nutrient media or lactic acid (Zeng & Zhuang, 2022). Established and circumscribed by C. and L.

R. Tulasne in 1865, Nectriaceae are in the order Hypocreales and are ascomycetes distributed in the tropics and sub-tropics (Wijayawardene *et al*., 2020). Some species of Nectriaceae are known plant pathogens but some genera have been found to be endophytes and saprobes found in soils and dead plant material (Lombard *et al*., 2015). Genera in this fungal family include *Fusarium, Nectria* and *Giberella* as examples. *Fusarium* has been widely studied due to its occurrence in both agricultural and forestry plants (Gordon *et al*., 2015).

The fungal family is associated with symptoms of leaf spots and blights, cankers, root and stem rot in seedlings (Lazarotto *et al*., 2014). The symptoms are significant in economic impact on some tree species for example pitch canker of pines caused by *Fusarium circinatum* causes tree mortality in Europe (Lazreg and Belabid, 2013).

2.5 Morphological and Molecular Characterization of Fungi

Morphological characterization is the primary basis of fungi identification. It is based on the phenotypic characters of the reproductive structures of fungi and is used to separate fungal groups up to the genus level (Agrios, 2005). However, this method is limited, requiring a more robust method of characterization to allow fungal identification up to the species level.

Molecular characterization was introduced in the early 1990s by White *et al*. (1990) to complement morphological identification of fungi for accurate separation of species (Raja *et al*., 2017). The use of specific gene regions to amplify DNA sequences from fungi with highly targeted primers has made molecular characterization a widely preferred technique among mycologists, including those in forest mycology (Slippers *et al*., 2007). This method focuses on ribosomal ribonucleic acid (rRNA) genes and relies on detecting conserved

sequences in ribosomal deoxynucleic acid (rDNA) genes (Ferrer *et al.,* 2001; Williams *et al.,* 1995). Molecular techniques also play a crucial role in population biology, offering insights into the ecology, phylogenetics, genomics, and transcriptomics of fungi. These findings help explain how fungi spread and are introduced to new sites or hosts by comparing species collected from different locations (Raja *et al*., 2017; Machua *et al*., 2016; Phillips *et al*., 2013; Liu *et al*., 2012). Accurate fungal identification is essential for selecting resistant host varieties based on pathogenicity studies, which are critical for future planting efforts (Machado *et al*., 2014; Latinovic *et al*., 2013).

2.5.1 Primary Identification Using Morphology

Spore structure, texture are examined using a microscope. In the petri dish the colony colour and appearance are used to identify fungi (Humber, 2001). Through the conidial morphology seen using a microscope fungal species can be identified up to genus level (Wang *et al.,* 2016; Agrios, 2005). The fruiting bodies of a fungi can also be observed together with the symptoms they cause.

2.5.2 Identification of Fungi Using Molecular Techniques

White *et al*., 1990 developed a methodology for identification of fungi using their genes. . The internal transcribed spacer (ITS) gene region has been widely used separate and identify species of fungi. Fungi possess genetic material in the nucleus of their cells (genome) (Stajich, 2017). This is known as deoxyribonucleic acid (DNA) and carries genetic information of the fungi including inheritance patterns of the fungi. DNA is extracted using chemicals and specialized equipment in the laboratory in repeated cycles. Once extracted, the ribosomal DNA is quantified using a high-definition spectrometer to determine the quality of the extracted DNA. The DNA is then multiplied using polymerase

chain reactions (PCR), a process known as DNA amplification. Amplification uses heat regimes and enzymes to multiply genes in a DNA sample. The amplified sample is then purified using columns of chemicals such as Sephadex 50© before sequencing and phylogenetic analyses of the sequenced DNA.

The basic gene regions, ITS and Translation Elongation Factor (Tef1α) 1 alpha are commonly used to solve problems in fungal taxonomy (Raja *et al*., 2017; Brown *et al*., 2014; Liu *et al*., 2012; Slippers *et al*., 2007). This involves sequence comparison of ribosomal Deoxyribonucleic acid (rDNA) found in the nucleus of cells of living organisms. Nucleotide similarity is studied with reference sequences of previously identified organisms. Phylogenetic analyses are carried out at various levels to show relationships between the different organisms under study (Guo *et al*., 2000).

There are three gene regions of nuclear ribosomal ribonucleic acid (rRNA) commonly used to differentiate fungal species. These are: Internal Transcribed Spacer (ITS) large subunit (LSU) and small subunit (SSU) first described by White *et al*., (1990). Later many authors have used this method including Raja *et al.*, 2017; Brown *et al*., 2014; and Phillips *et al*., 2013) to separate teleomorph and anamorphic stages of fungal reproduction and correctly identify fungal species.

Several software that include Molecular Evolutionary Genetics Analysis (MEGA) are used to analyse DNA sequences to show fungal interrelatedness (MEGA11: Molecular Evolutionary Genetics Analysis version 11 (Tamura, Stecher, and Kumar 2021). This is done by first aligning the sequences and later phylogenetic analyses are performed (Tamura, Stecher, and Kumar 2021). The neighbour joining method (Saitou & Nei, 1987) is extensively used to construct phylogenetic trees to analyze the relatedness of sequences

to each other. In this study, representative cultures of the most common species identified morphologically were further used for molecular identification of the fungi. This technique gives a more accurate identification of the fungi isolated up to the species level and also helps to show the "closeness" of the species to each other. To confirm identity, several databases are used to compare sequences that include Genbank (National Center for Biotechnology Information, Bethesda, ed MD, USA), and EMBL (European Molecular Biology Laboratory, Heidelberg, Germany).

2.6 Pathogenicity tests and Koch's Postulates

The virulence of a pathogen is assessed using pathogenicity tests to confirm that the isolated causal agent will induce the recorded symptoms on its hosts (Agrios, 2005). Koch's postulates require that when a fungus is isolated it should produce the same symptoms on a healthy host of similar and related species (Al-Jaradi *et al*., 2018). Pathogenicity tests involve the wounding of healthy seedlings with a cork borer and inoculation with the mycelium from the isolated fungi and then sealed with Parafilm (Agrios, 2005). Controls are also wounded but they are inoculated with sterile media plugs and then sealed with Parafilm. Disease symptoms developed are then recorded and the number of days taken to develop them are recorded (Njuguna *et al*., 2011). Common symptoms recorded in these tests include; chlorosis, necrosis, gummosis, wilting and mortality. The lesions formed are assessed and analyzed.

Virulence of a fungi is determined by the lesion lengths and the time taken to produce symptoms on a host (Cherotich *et al*., 2020). In some cases, the host plant forms tissues around the wound and this results in wound healing. Such occurrences allude to a plant's defences and susceptibility to the fungi used in the testing (Begoude *et al.,* 2010). At the

closure of the pathogenicity tests lesion measurements are taken and the symptomatic tissue is used for re-isolations from which the test fungi should be reisolated to fulfil Koch's Postulates (Agrios, 2005).

The steps in the Koch's Postulates are: Postulate 1: The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms. Postulate 2:The microorganism must be isolated from a diseased organism and grown in pure culture. Postulate 3: The cultured microorganism should cause disease when introduced into a healthy organism. Postulate 4: The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent (Koch,1890).

2.7 Control of fungal diseases on trees and plants

Control of fungal diseases is a combination of plant hygiene, microclimate variation and elimination of predisposing factors that encourage growth of fungi (Agrios, 2005). Seed-borne fungi are eliminated through pre-treatment of seeds using fungicides (Fraedrich, 2009). The fungicides active ingredients inhibit the growth of fungi allowing germination of the seed (Moumni *et al*., 2023). Soil-borne fungi are often killed by fumigation of the soil. Armillara fungi can be controlled by use of preventive measures such as fumigation, crop rotation and leaving soil fallow (Aci *et al.*, 2025). Some bacterial species have been found to suppress fungi growth and are being studied for application to forestry (Mohammed & Ravishankar, 2016).

Disease management in nurseries can either be physical or chemical (Kamondo *et al.*, 2018). In plantations pruning and thining operations should be carried out when the humidity is low to reduce spread of pathogens (Agrios, 2005; Sinclair & Lyon, 2005). The

tools used for pruning and thinning must be sterilised using 70% Ethanol so as to avoid infecting healthy trees (Njuguna *et al.*, 2011).

In the case of opportunistic pathogens the plants must be protected from stress to prevent them from becoming disease causing agents. Trees must always be planted in the right areas to avoid stress from weather conditions and changes in soils (Njuguna, 2011). It is also important to avoid fires by ensuring fire breaks and preventing smoking near trees to protect the trees (Almeida & Purcell, 2003).

Hygienic seed collection can prevent introduction of seed borne pathogens (Moumni *et al*., 2023). Before sowing, seeds can be surface sterilised using sodium hypochlorite to remove surface contaminants and storage fungi (Gilbert *et al*., 2023). Some acetone-based fungicides have been used in treating seed borne fungi(Fraedrich, 2009; *Gure et al.*, 2005). Proper spacing, removal of weeds and shading of plants can protect seedlings from diseases (Hirooka & Ishii, 2013).

Some frequently used fungicides include, 0.01% Bavistin whose active ingredient is Carbendazim and 0.05% Blitox -active ingredient Copper oxychloride that are applied to young seedlings following manufacturers instruction give adequate control for most diseases of neem seedlings at the nursery stage (Hirooka & Ishii, 2013; Agrios, 2005; Negi, 1997).

Other ways to control plant diseases include cultural practices like ensuring good air circulation in tree nurseries, proper watering, regular weeding, and managing pests (Agrios,2005). Physical methods involve pruning and removing diseased parts, excavating infected trees, burning the infected material, and using disease-resistant plant varieties. When planting, it’s important to match the species to the site to provide the best growing

conditions and minimize stress on the plants (USDA, 2017; Eskalen *et al*., 2015). . This helps reduce their vulnerability to fungal infections, such as those caused by Botryosphaeriaceae species. Treating pruning wounds with fungicides has also been effective in preventing new infections in plantations. If trees are already infected, removing and burning the stumps is recommended to eliminate the source of infection and prevent the disease from spreading further(Eskalen *et al*., 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the study area

This study was carried out in six (6) selected counties in the Arid and Semi-arid Lands covering agro-ecological zones (AEZ) IV and V (Jaetzold *et al*., 2012). An agro-ecological zone (AEZ) is a land unit that is described by its suitability, environmental and edaphic factors. Kenya has seven (7) agro-ecological zones as described by Jaetzold *et al*. (2012). The environmental conditions that characterize various agro-ecological zones in Kenya are as summarized in table 2.

Table 2 Description of Agro-ecological Zones in Kenya

AEZ Zone	**Mean temperatures**	**Mean annual rainfall**	**Soil fertility**	**Agricultural potential**
I. Afro-alpine	2-10° C	Mountainous	Moderate to high	High
II. High potential	10-15° C	>1000mm	Moderate to high	High- moderate
III. Medium potential	15-18° C	950-1500mm	Moderate	Moderate
IV Semi-arid	18-21° C	500-1000mm	Variably moderate to low	Low to marginal
V Arid	21-24° C	300-600mm	Low to very low	Marginal
VI Very arid	>24° C	200-400mm	Very Low	Marginal

The agro-ecological zones are basically described by average mean temperatures, mean annual rainfall, soil fertility and the resultant agricultural production potential.

3.1.1 Characteristics of the study counties

Eight (8) counties were systematically surveyed for occurrences of diseases on the two Meliaceae species during this study whose locations are shown in table 3 below.

Table 3 Selected Eight ASAL Counties of the study sites

S/No.	Site Name and County	Location		Reference
		Longitude	Latitude	
1.	Tiva, Kitui County	37º 50' and 39º 00' East	0º 10' and 3º 00' South	GoK, 2015a
2.	Kibwezi, Makueni	37º 10' and 38º 30' East	1º 35' and 3º 00' South	GoK, 2013a
3.	Kilifi	39º 05' and 40º 14' East	2º 20' and 4º 00' South	GoK, 2013b
4.	Kwale	39º 23' and 39º 30' East	3º 45' and 3º 55' South	GoK, 1974
5.	Voi (Taita Taveta)	37º 14' and 37º 36' East	2º 46' and 4º 10' South	GoK, 2014a
6.	Lamu	40º 15' and 40º 35' East	1º 40' and 2º 30' South	GoK, 2014b
7.	Mbeere (Embu)	37º 03' and 37º 09' East	0º 08' and 0º 50' South	GoK, 2016
8.	Tharaka Nithi	37º 19' and 37º 46' East	0º 07' and 0º 26' South	GoK, 2015b

GPS coordinates of the study sites in Eastern and Coastal regions of Kenya

3.1.2 Location of the Study Sites in the Eight Counties

The study involved an extensive survey of the two tree species in eight (8) sites in eight counties which are located in three major geographical areas of the country i.e., Coast,

Upper Eastern and Lower Eastern as shown in figure 2.(Figure 2). The study was diverse because the two species were found in isolated areas. The selected counties were: Embu (Mbeere area), Tharaka Nithi, Kitui, Makueni (Kibwezi area), Taita Taveta (Voi area) and Lamu all of them in agro-ecological zone IV while Kilifi, and Kwale counties are in agro-ecological zone V.

Kitui County is home to the KEFRI Tiva on-farm station and has a large number of private farms where there are many woodlots and natural populations of Melia trees. Makueni County has woodlots of *Melia volkensii* where several projects have promoted the species for adoption in agroforestry. With an established trial at the KEFRI station and the early adopter farmers. Kilifi county has several populations of *M. volkensii* planted as woodlots in schools and on farms. *A. indica* is also widely planted as an ornamental across the county. An *A. indica* plantation was purposely selected in Arabuko Sokoke forest for the survey as all other trees were on farm.

Kwale county has *Melia volkensii* trees in woodlots on few farms and as stand-alone trees in the wild. A private farm with a large plantation of *M. volkensii* was available in Witu, Lamu County and was surveyed for disease occurrence. Tharaka Nithi County has some of the largest trees in diameter and height of *Melia volkensii*. In Embu County, seedlings and small woodlots of *M. volkensii* were available on farm and in nurseries, which were assessed in this study. Five sample plots were close to KEFRI stations where species trials were available and around early adopter farmers with established woodlots. Embu, Tharaka Nithi and Taita taveta counties have good documented natural populations of *Melia volkensii* (Kamondo *et al.*, 2016).

The study areas are characterized by bi-modal mean annual rainfall that is below 500 mm occurring in two seasons of March-May and October-December; prolonged dry periods and high mean temperatures of 23-24 ^{o}C (Jaetzold & Schmidt, 1982; UNDP, 2013b; GoK, 2012). The soils are predominantly sandy with variations of sandy loam classified as lithosols, regosols and xerosols with low to very low fertility (Infonet Biovision, 2017b; Gachene & Kimaru, 2003).

3.2 Sampling techniques

This study employed cluster sampling due to the uneven spread of the species in the wild. Both *Melia volkensii* and *Azadirachta indica*, the target species, exist in pockets and are scattered geographically due to overexploitation in the wild**.** Some samples were also collected from farms for comparison of disease incidence and severity between the populations in the wild and those that were found on-farm.

3.2.1 Field Experimental design and sampling techniques

Experimental design for natural resources differs depending on the availability of the target species, scope of the study and objectives of the study (Seltman, 2018; Birhane *et al*.,2014). Cluster sampling was used in this study due to the isolated nature of the populations of *M. volkensii* and *A. indica* in the selected sites (Dudovskiy, 2016; Kowalski *et al*., 2015).

Cluster sampling method was considered to be cost efficient and suitable for large geographical areas. Sites were found within the county area but were at least 5 kilometres (km) away from one to the other. Clusters of each tree species (*M. volkensii* and *A. indica*) were randomly selected at each site. All trees within a cluster were assessed for disease incidence and severity.

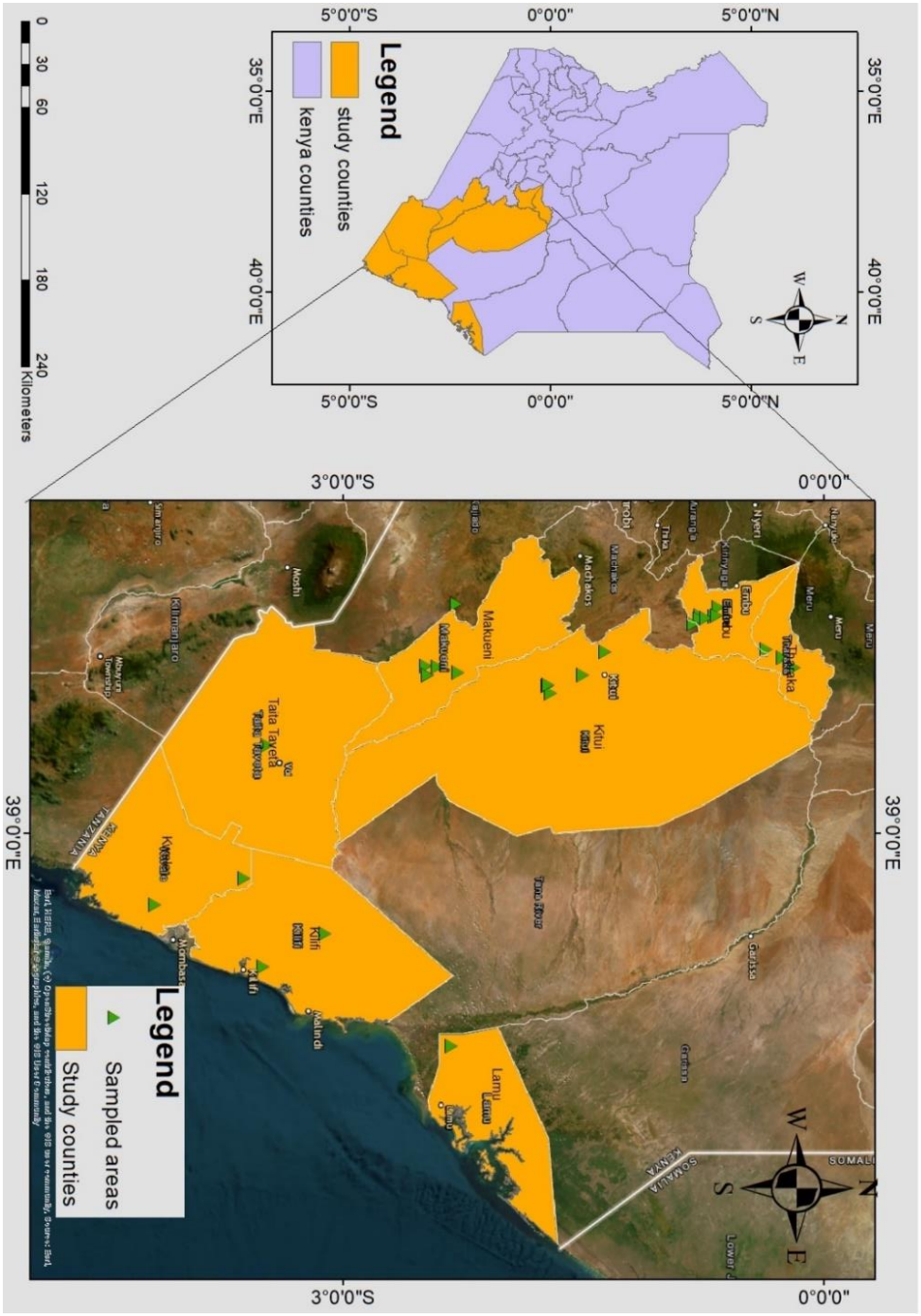

Figure 2: A Map of the study sites.

Source: Author, 2024

3.3. Assessment of Tree Disease Symptoms in the Field

Visual assessment was used for disease symptoms in the field. Samples of leaves, stem, branches and twigs of affected trees were collected for further analysis in the lab. The symptoms recorded in each sample area were recorded. Common symptoms of disease are twig dieback, stem cankers and root rots. The disease infection can lead to drying, discoloration and death of the tree (Agrios, 2005). Other symptoms may include presence of resin, rotting of fruits and leaf spots. We also sampled healthy trees to examine the presence of opportunistic pathogens.

(a) Field Procedures for disease incidence assessment

In each site a minimum of 8 trees and a maximum of 20 trees where available were sampled. The total number of trees samples were 146 for *Melia volkensii* and 62 for *Azadirachta indica*.

In each site disease incidence was assessed and samples of bark, twigs and leaves showing symptoms and signs of disease were collected and preserved in cooler boxes for further laboratory analyses. In this study at least ten trees were sampled per site or cluster.

Two main types of disease symptoms were used to assess the disease incidence: 1) leaf and shoot dieback and 2) twig, stem/trunk cankers. Disease incidence was determined as the total number of trees with disease symptoms that included die-back and canker symptoms expressed as a percentage of the total number of trees in each site sampled (Cherotich *et al*., 2020).

(b) Field Procedures for disease severity assessment

Disease severity of each tree sampled was determined by two people standing five (5) m away from the tree from different angles as described by Karani *et al*. (2022); as adopted

from Njuguna *et al*. (2011). Disease severity assessment was done by visually assigning and recording the proportion (percent) of the above ground tree biomass affected by canker and dieback diseases and assessing the mortality of the tree (Agrios, 2005).

Six (6) severity categories were used for scoring disease severity according to Njuguna *et al*. (2011) as follows

Table 4 Disease severity categories

Category	Disease severity	Description
1	0%:	Healthy no canker or dieback symptoms
2	1-5%	showing dieback of shoots
3	5-25%	of tree crown showing dieback
4	25-50%	showing cankers and dieback
5	50-65%	showing shoot dieback and severe dieback and severe resin flow
6	>65%	showing very severe shoot dieback and resin flow

3.4 Collection of Samples

Samples of twigs with leaves and bark were collected from the assessed trees within the clusters of 30 m from trees perceived as healthy and those exhibiting disease symptoms such as die-back which is the browning and dying of a tree from its leaves and shoots downwards (Sinclair & Lyon, 2005). Other symptoms observed on diseased trees were cankers, sunken holes on twigs or on the bark that were darker in colour than surrounding wood and could be accompanied by resin exudation or a gum like substance produced by infected cells (Agrios, 2005). Trees in some cases had fruit rots which in turn affect seed quality.

Plant parts collected included twigs and stem cuttings and fruits with symptoms. Tools used to collect samples were sterilized with 70% ethanol to avoid re-infection and infection of other trees. Trees whose trunks or stems were scraped were sprayed with a copper-based fungicide to prevent re-infection by other pathogens and spread to neighboring trees. Four widely spaced sites per county were sampled within the same county; that is five (5) sites in Kibwezi. A minimum of three (3) samples were collected from each tree both healthy and diseased to give 8 pieces for isolation on culture media and for moist chambering.

3.5 Isolation of Fungi

Three steps were taken in isolation of the fungi including isolation of pathogens, identification of the pathogens using conidial morphology, and identification of the pathogens by molecular characterization.

3.5.1 Isolation of Pathogens

Two methods were employed in isolation of pathogens including moist chamber and surface sterilization.

(a) Moist Chamber Isolation

The cut sections were placed in surface sterilized glass Petri dishes. The Petri dishes were then placed in sterile airtight plastic boxes containing wet sterile blotting papers and the containers covered to form a damp chamber to encourage microbe growth. The plastic boxes were left at a temperature of 21°C for at least 5 days until the fungal mycelia grew on the sampled material. Once fungal mycelium formed on the cut sections it was transferred to 2% Malt extract Agar amended with Streptomycin and then incubated in a

growth chamber at 23 ^{o}C. The emerging fungi were then transferred to fresh culture media to obtain pure cultures.

(b) Surface sterilization Isolation

This method was used to isolate fungi after surface sterilization by cutting small pieces of plant tissue approximately 1 cm long from the disease leading edge. The cut pieces were then surface sterilized by rinsing for one minute using 33% hydrogen peroxide and rinsed 3 times using sterile distilled water. The pieces were then blotted dry on sterile filter papers in the laminar flow before being aseptically placed in Petri dishes containing 2% Malt Extract Agar amended with Streptomycin. The emerging fungi were transferred to culture media to obtain pure cultures of the fungi according to Okeyo *et al*., (2024).

3.5.2 Identification of Isolated Fungi

Two methods were used to identify the fungi namely conidial morphology and molecular identification.

a) Primary identification by fungal conidial morphology

Once a pure culture was obtained, morphological characteristics were used to group the fungi into their genera according to colony growth characteristics. Colony characteristics including shape, form, size and pigmentation (colour) of the spores, colony elevation, opacity and morphology were studied under a compound microscope in order to identify the pathogen.

Colony and spore characteristics were also studied by staining mycelia using Anylene blue and observing the slide under a compound microscope. Sketches of the spores observed were made and used to identify the genus of the fungus by reference to (Barnette & Hunter,

1972). Conidial morphology aided in grouping the fungi according to genus together for easier selection of isolates for further identification using ribosomal Deoxyribonucleic acid (DNA) based methods.

b) Fungi Identification by DNA characterization

DNA characterization is important for a near accurate separation of species of fungi usually grouped together after morphological characterization (White *et al*., 1990). Ribosomal Ribonucleic acid (rRNA) sequences are used for microbial identification as it is the portion that is changed during mutation therefore, it can be used to distinguish species. Ribosomal RNA (rRNA) was extracted from a single spore culture of isolated fungi for use in characterization of pathogens to get accurate identification of the species. The process of DNA extraction, amplification, purification and sequencing was done as described by White *et al*., (1990).

The CetylTrimethyl Ammonium Bromide (CTAB) method as described by Phillips *et al*., (2013) was used in extracting rDNA material from fungal mycelia. These were Internal Transcriber Space (ITS) and Trans elongation Factor (Tef1-alpha). In sequencing the ITS region, ITS3 and ITS4 were used as forward and backward primers respectively. For Tef1 – alpha pairwise primers were used (EF 728 and Tef1). Polymerase chain reaction (PCR) products were purified using the Sephadex® 50.0 Purification Kit and gel electrophoresis done using Agarose gel for the PCR products. Purified products were then run-on ABI PRISM® 310 Sequencer machine using big Dye and the sequences downloaded and analyzed using capillary electrophoresis method. The downloaded sequences were subsequently compared with existing sequences from Genbank and other sequence collections to find the closest relative at 100% bootstrap values. Other fungi that had above

60% relation within the cladogram were also considered. The phylogenetic analysis was created using Molecular Evolutionary Genetics Analysis Software (MEGA) Tamura *et al*. 2021, version 11.0 Software to produce a cladogram to further analyse relationships between the isolates.

Representative cultures of the fungi used in this study were sequenced and the sequences submitted to Genbank. Molecular identification of the pathogens to the species level is a pre-requisite to guide prescription of specific management and control methods. It is for this reason that molecular characterization of canker and dieback disease forming fungi was carried out in this study.

3.6 Pathogenicity tests

Pathogenicity tests are used to assess the virulence (ability to cause disease) of a pathogen and to confirm that the isolated fungi are the ones causing the symptoms seen in the field (Agrios, 2005). The representatives from 3 of the most frequently occurring species of fungi isolated were tallied and further grouped into either pathogenic fungi or saprobes. From this characterization, the potentially pathogenic fungi with their associated symptoms were identified. Isolates from the most frequently isolated pathogen group were then cultured for use in glass house pathogenicity tests. From the isolations, the most frequently occurring fungal group of potential economic importance known to cause cankers on woody plants was the Botryosphaeriaceae with a total of 125 out of the 247 total isolates.

Pathogenicity tests were carried out in the glass house using one-year-old seedlings of *Melia volkensii* and *Azadirachta indica* to fulfill Koch's Postulates as described by Agrios, 2005. A wound was made mid-stem on the seedlings before inoculation with 1 mm mycelia plugs from a pure culture of the test fungal species using a 1mm cork borer. After wounding

and placing the mycelial plug the point of inoculation was sealed with sterile Parafilm® and placed on benches in a controlled glasshouse.

Completely randomized design was used where each isolate was inoculated into five seedlings of the two species. Each treatment was replicated 3 times. Controls were wounded but not inoculated and were sealed with sterile laboratory film Parafilm® to prevent reinfections. Symptoms developing on the inoculated seedlings were recorded for 12 weeks. Assessments were carried out every two days and the symptoms recorded in a data sheet for record and analysis. At the closure of the experiment at 12 weeks, lesion length and diameter were assessed on each seedling and recorded. Mortality and other observations made on the seedlings were recorded such as wound healing. Re-isolations were carried out from the tissues of the seedlings using the surface sterilization isolation method.

Table 5 Botryosphaeriaceae isolates used for the glass house pathogenicity tests

Fungal Species	**Code**	**Location**
Lasiodiplodia theobromae a	KzB1Mv1	Kibwezi
Lasiodiplodia crassispora	MB4Ti20	Mbeere
Lasiodiplodia pseudotheobromae a	CB1Mv20	Coast
Lasiodiplodia pseudotheobromae b	MB4Vp10	Mbeere
Lasiodiplodia sp. A	CB1At11	Coast
Lasiodiplodia sp. B	KZB1As14	Kibwezi
Lasiodiplodia sp. C	KZB1As10	Kibwezi
Neofusicoccum parvum	MB4As11	Mbeere
Lasiodiplodia parva	CB1Aq1	Coast

3.7 Data Analysis

All data analysis was done using R statistical package (R Core Team, 2023) and Microsoft Excel. Prior to ANOVA analysis the percentage data on disease incidence and disease severity was arcsine transformed to conform to the assumptions of ANOVA (Uneven data). One-way ANOVA was used to test the significance of the sites and tree species on the disease incidence and severity data. Correlations between disease incidence and severity on the two tree species across sites and zones were tested using Pearson's correlation coefficient and using least significant differences (LSDs) and the Tukeys Test.

Isolations revealed that there were different fungal species occurring on the various diseased and healthy plant parts. The fungal occurrences from the different plant parts of the two tree species were expressed as a percentage of the total number of isolations. All analyses were done at a 95% confidence level with data being assumed to have a normal distribution.

Ribosomal RNA based phylogenetic analysis of selected isolates for identification was done using MEGA 7.0. The resultant sequences from the study were aligned using ClustalW method and a dendrogram was produced using the Maximum-Likelihood method (Yang, 1993) to analyze the evolutionary relationships of the isolates. This was used to help establish the diversity of the fungi isolated from samples in Kenya. The sequences from the representative isolates were subjected to nucleotide database NCBI to find closest matches. The resultant DNA sequences and corresponding names or identity were used in the dendrogram development.

The pathogenicity of potential pathogens was assessed based on symptoms recorded in glasshouse experiments. Lesion lengths and widths were recorded at the closure of the tests and used for analysis of virulence of different fungal species on 6 different tree species. A one-way analysis of variance (ANOVA) was done to test the significance of differences between the species as defined by the lesion lengths.

CHAPTER FOUR
RESULTS

Objective 1: To assess and determine incidence and severity of canker and dieback disease on *Meliaceae* trees in selected sites in ASALs of Kenya.

4.1 General Overview of the study sites

The survey revealed that populations of *Melia volkensii* and *Azadirachta indica* in the counties of Kitui, Embu, Tharaka Nithi and Kibwezi which fall in AEZ IV showed good growth both on-farms and in the wild. Good growth encompasses stem form that is straight without girdles, with height, diameter at breast height and a plant that is relatively free of diseases and pests. This was inferred from the presence of a large number of candidates plus trees of *Melia volkensii* in the four counties that had been selected for breeding studies by the Kenya Forestry Research Institute, KEFRI.

Further, higher disease incidences were recorded in Kwale, Kilifi and Taita Taveta counties which fall in AEZ V compared to the above counties in AEZ IV. This was evidenced by gum exudation, stem girdling, stunted growth and in extreme cases death of trees. In addition, fruits of *M. volkensii* had severe brown necrotic lesions and were sampled for further laboratory analysis. The three counties are lower in elevation and experience higher temperatures and relative humidity.

4.2 Field disease symptoms observed

Symptoms recorded in the field were observed in the farms visited but at varying intensity. Most trees surveyed were healthy making almost a half (42%) of the sample population. As seen in Plate 3a, dieback and cankers were among the disease symptoms that were noted. The fruits of Melia volkensii on infected trees showed brown spots, which were

necrotic lesions (see Plate 3b). Additionally, *Melia volkensii* showed resinous superficial cankers, as shown in Plate 3c, with gum exudation. Since there were few or no obvious symptoms on the stems and branches of the majority of the trees at the time of sampling, they were categorized as healthy. However, as shown in Plates 4a and 4b, cankers were observed on either the stems, the branches, or both. The size of these cankers ranged from tiny lesions to huge cracks in the bark that were leaking resin. In extreme situations, as shown in Plate 4c, the impacted trees were either already dead or dying.

Field data results show that there were higher incidences of a combination of both canker and dieback on trees assessed than the dieback or canker symptoms alone as shown in Figure 5. Dieback was recorded where there was partial or entire drying up of a twig or branch. In partial dieback some leaf blights and spots were observed as possible precursors for new infections.

Where a tree part was sampled, rotting wood was visible on the woody tissue of the sampled part as shown in Plate 3d. There were also a few cases of stem girdling, insect damage and in some cases brown discoloration and necrotic lesions were observed on infected fruit of *M. volkensii* as shown in Plate 3b. Cankers and dieback are the most common symptoms occurring on approximately 32% of the sampled trees and usually co-occurred together.

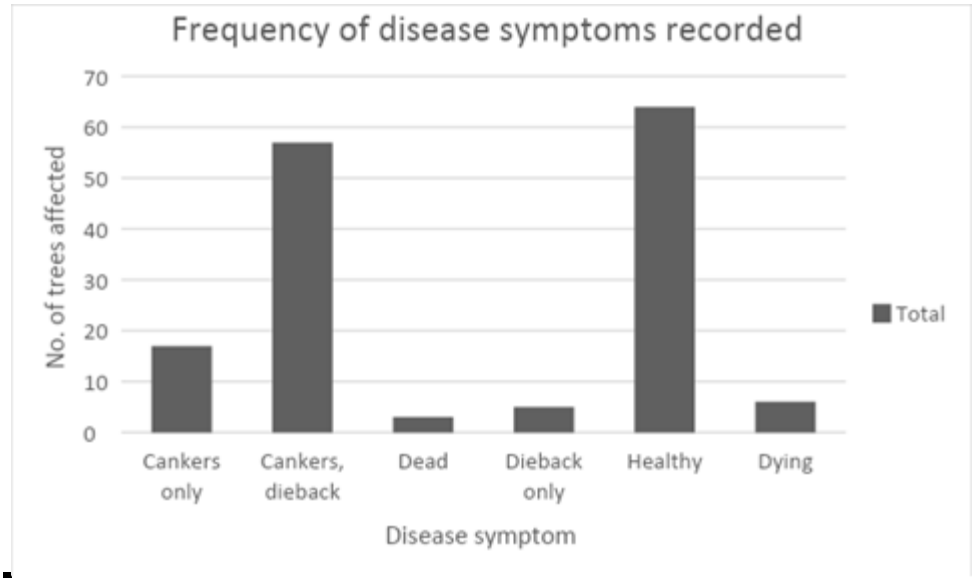

Figure 3: Frequency of the disease symptoms recorded in the field

4.2.1 Disease symptoms observed on *Melia volkensii*

In all the six sites sampled disease symptoms recorded on *M. volkensii* were shoot and twig dieback, cankers on either twig or stem characterised by brown necrotic areas on stems or branches, sometimes sunken areas or as cracks, resin /gum exudation from cankers (Plates 3a and 3c), and five (5). At the time of sampling, there were no dead *M. volkensii* trees recorded in all the study sites. On some infected trees *Melia volkensii* fruits were also infected showing necrosis and dark brown spots on their surface (Plate 3b).

Where infection broke through the bark there was resinosis /gummosis or heavy gum exudation and when the section was cut transversely revealed dark brown rotting xylem tissue inside the stem (Plate 3a). On *Melia volkensii* cankers appeared brownish red in colour and caused swelling (Plate 3c and 3d) and cracking of the bark in the affected area.

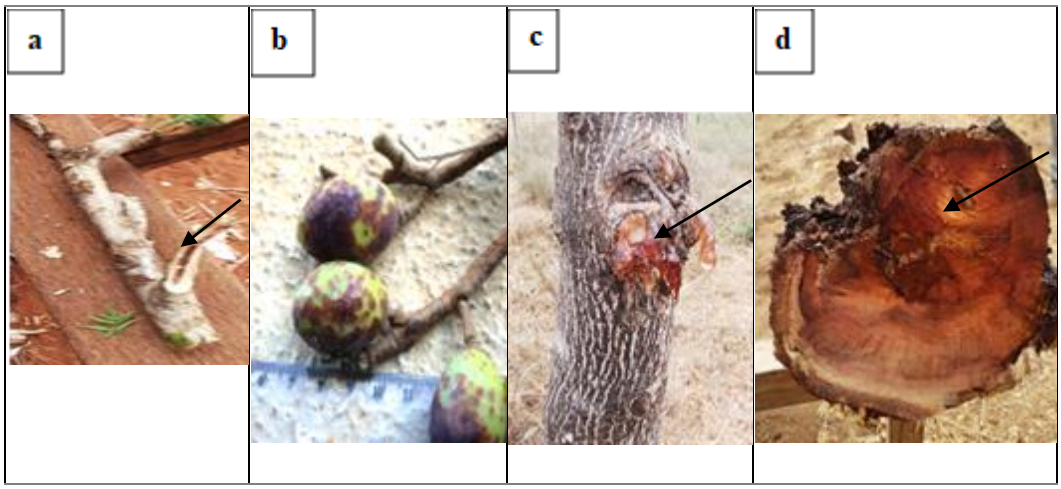

Plate 3: Showing different symptoms on *Melia volkensii,* a) brown cankers and internal lesion on a twig of *M. volkensii*, b) Infected fruits of Melia showing brown discoloration c) Resin /gum exudation from a canker on a *M. volkensii* stem, d) Dead wood from infection with fungi on a *M. volkensii* stem.

Source: Author, 2023

It was notable that one plantation of Melia in Lamu site recorded over 95% disease incidence with resinous cankers and observation showed that poor and unhygienic pruning methods had been used causing openings for disease infection and spread. For this reason, the site was treated as an outlier and removed from subsequent data analysis.

4.2.2. Disease symptoms observed on *Azadirachta indica*

In all the sites sampled *Azadirachta indica* showed low incidences of stem cankers and die-back of branches and twigs. On. *A. indica*, cankers were observed as sunken and reddish to dark brown in colour see Plate 4b. In some cases, there was presence of black pycnidia on the lesions surrounding the canker and there was little or no gum exudation associated with the tree species (Plate 4a). Canker infection caused surrounding tissue to rot and dry

up making it hard to cut through. In severe cases, fungal infection led to death of the tree as shown on plate 4c.

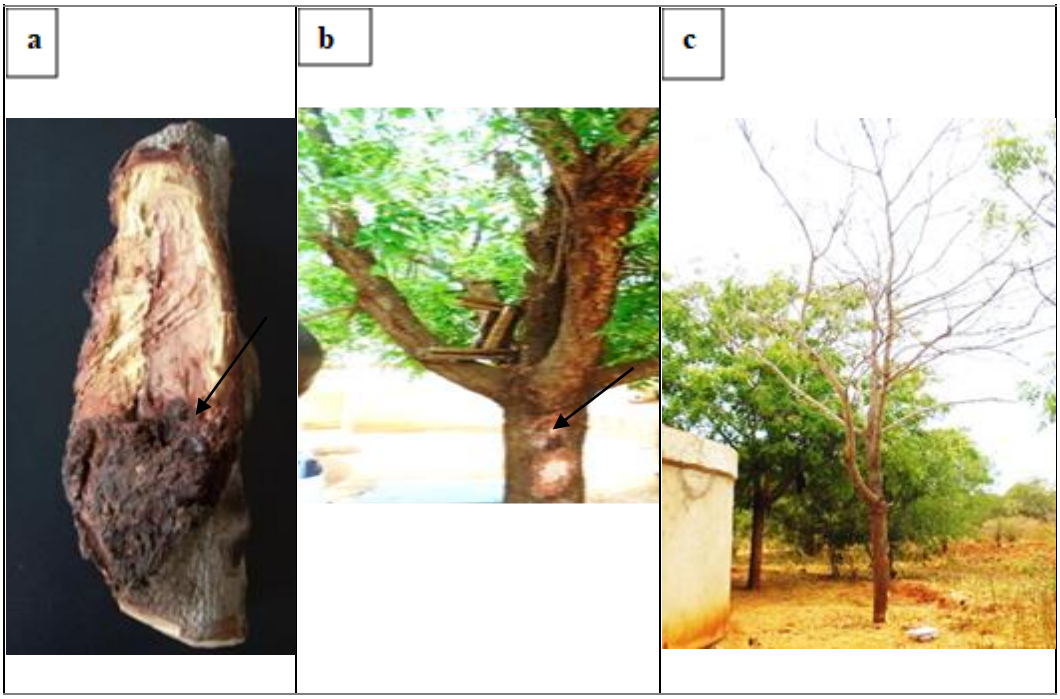

Plate 4: Showing different symptoms on *Azadirachta indica* a) Dark brown canker on *A. indica* stem with gum pockets appearing shiny and Stained wood b) *A. indica* (neem) tree showing cankers on stem and branches c) A dead neem tree caused by disease infection. Source: Author, 2022

Both *Melia volkensii* and *Azadirachta indica* showed similar disease symptoms across the sites and therefore the frequency of the symptoms was grouped into similar categories as shown in Figure 5 below.

4.3 Disease incidence and severity across sites

4.3.1. Disease incidence of *Melia volkensii* across sites

Disease incidence is the percentage of diseased plants in a sample or population. Disease incidence was determined for each of the clusters assessed by counting the number of symptomatic trees and dividing by the total population in the sample.

Disease incidence varied significantly ($p<0.001$) across the study sites and between the two species. The results also showed that there was a significant difference between the sites (alpha = 0.05).

From table 5, it is clear that the lowest disease incidence on *Melia volkensii* was recorded in Tharaka Nithi site while Kwale recorded the highest disease incidence on the species in both dry and wet seasons. In these two sites trees were on average over 20 years old and could explain the disease incidence variation. It is worth noting that these two sites are in different agro-ecological zones.

Table 6 Disease incidence and severity on 12 sites surveyed in the six counties in two seasons

County	Site	Tree species	Incidence %		Severity %		Remarks
			Wet	Dry	Wet	Dry	
Kibwezi	Kinyambu	*M. volkensii*	50	30	15	10	On farm
	Manyanga	*M. volkensii*	50	30	20	10	On farm
	Kibwezi	*M. volkensii*	40	20	20	10	KEFRI
Kitui	Ikanga	*M. volkensii*	30	20	5	0	On farm
	Kisasi	*M. volkensii*	40	20	25	10	Wild
	Mutomo	*A. indica*	40	20	55	30	On farm
Kilifi	Chakama	*M. volkensii*	50	20	55	20	Wild
	Sokoke	*A. indica*	40	20	30	10	On farm
Kwale	Silaloni	*M. volkensii*	60	30	40	20	KFS
	Mwatate	*M. volkensii*	35	20	40	20	On farm
Mbeere	Rwakinanga	*M. volkensii*	25	15	15	5	Wild
Tharaka Nithi	Mutugo	*M. volkensii*	40	20	20	10	Wild

The Tharaka site had impressively tall trees with less branching and is the site where most of the KEFRI *Melia volkensii* candidate plus trees used for breeding programs are located. Further multiple comparisons done using ANOVA confirmed that there were significant differences between sites using Lsd (Tukeys test), p-value=≤0.001 as shown in Table 6 below. There was no significant difference within counties.

Table 7 Multiple comparisons of diseases incidence on Melia volkensii between sites using Tukey's Test

Incidence per SITE	Mean	s.e.mean	Confidence interval
Mutugo	31.62 a	0.000	31.62±0
Mwatate	47.74 b	0.784	47.74±0.7838
Chakama, Sokoke	55.28 c	0.213	55.28±0.2126
Manyanga,Kinyambu. Kibwezi	56.12 cd	0.452	56.12±0.4524
Ikanga, Kisasi, Mutomo	58.17 e	0.640	58.17±0.6395
Rwakinanga	59.51 e	0.000	59.51±0
Silaloni	89.96 f	0.000	89.96±0
Test statistics: $f_{(7,266)}$=656.21, p-value=≤0.001, lsd=1.856			

4.3.2. Disease incidence between the *Melia volkensii* and *Azadirachta indica*

It is to be noted that *Azadirachta indica* was found in good numbers (>10) in three sites that are Kilifi, Kibwezi and Kitui and very negligible (<3) in the other sites. However, disease incidence was not significantly different between the two species in Kibwezi (Manyanga), Kilifi (Sokoke) and Kitui (Mutomo) sites.

4.3.3. Disease severity between the *Melia volkensii* and *Azadirachta indica*

Disease severity is the percentage of relevant host plant tissue that is covered by a symptom or lesion or damaged by the disease. An Analysis of Variance (ANOVA) was carried out to test the significance of the sites based on the severity of diseases. The results showed that there was a significant difference in disease severity between the sites ($p<0.001$).

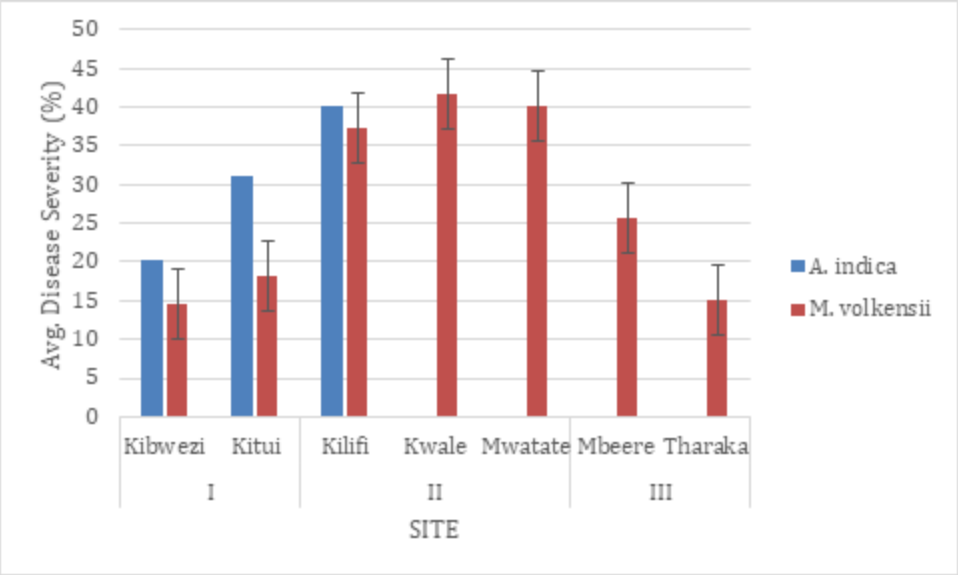

Figure 4: Bar chart showing differences in disease severity between sites

Disease severity varied within the different sites and between the two tree species see Figure 4. The highest disease severity recorded on *A. indica* was in Kitui, while the highest severity for *Melia volkensii* was recorded at the coastal regions (Table 7)**.** The lowest severity on *M. volkensii* was recorded in Mutugo in Tharaka Nithi County. Moderate severity levels were recorded in Ikanga and Mutomo sites in Kitui County as well as Manyanga and Kinyambu sites in Kibwezi.

Table 8 Comparison of Disease severity on *M. volkensii* and *A. indica* across sites

Severity per SITE	Mean	s.e.mean	Confidence interval
Mutugo	19.35 a	1.958	19.35±1.958
Ikanga,Kisasi, Mutomo	22.31 a	2.783	22.31±2.783
Rwakinanga	29.94 ab	2.519	29.94±2.519
Kinyambu,Manyanga,Kibwezi	35.08 bc	2.655	35.08±2.655
Chakama, Sokoke	45.55 d	3.033	45.55±3.033
Mwatate	48.39 d	3.387	48.39±3.387
Silaloni	49.71 cd	2.57	49.71±2.57
Test statistics: $f_{(7,266)}$=14.03, p-value=≤0.001, lsd=10.779			

4.4 Correlation Between Disease Incidence and Severity Per Site

Kendall's rank correlation tau was used to analyse the correlation between disease incidence and disease severity (Figure 5). A weak positive correlation was found between the variables with tau being 0.145. There was a significant difference (p=0.009) between the sites on the correlations.

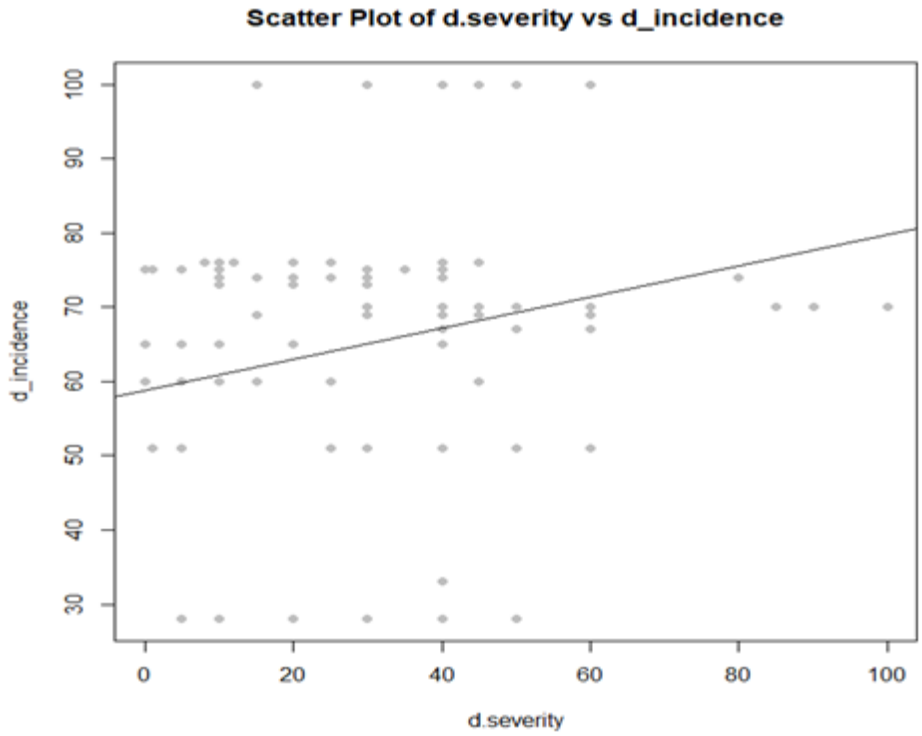

Figure 5: Regression line showing correlation between disease incidence and disease severity

From the normality plots, we conclude that both populations are not from normal distributions as points lie away from shaded areas (Figure 5). This was influenced by the cluster sampling method used where the sample numbers were uneven on the different sites depending on availability of the target species at each site.

Objective 2: To isolate, identify and characterise fungi associated with *Melia volkensii* and *Azadirachta indica* in selected sites in ASALs of Kenya.

4.5 Fungal identification using morphological characteristics.

From the 152 samples collected, a total of 3888 fungal isolations were realised. The isolations yielded 4120 fungal isolates that were grouped into 25 groups representing different morphotypes. Using colony morphology, the fungal cultures were further grouped into four basic groups: dark, whitish, whitish pink and whitish black coloured as shown on Table 8. The dark coloured group included cultures which were brownish, light to dark grey cultures with black pigment. The whitish coloured group included cultures which were creamy white, whitish pink and whitish with black pycnidia (Sinclair & Lyon, 2005). The whitish black group carried the light greyed as well as brownish cultures without black pigment.

Table 9 Colour differences between key morphological groups of the fungi isolated.

Fungal Groups	**Colony Characteristics**	**Fungal Genera Isolated Under this Group**	
		Melia volkensii	*Azadirachta indica*
Group 1	Dark coloured mycelia	*Botryosphaeria spp.* *Alternaria spp.* *Phoma spp.*	*Botryosphaeria spp.* *Alternaria spp.*
Group 2	Whitish mycelia	*Fusarium spp.* *Nectria spp.* *Pestalotia spp.*	*Fusarium spp.* *Pestalotia spp.*
Group 3	Whitish Pink	*Nectria spp.* *Fusarium spp.*	*Nectria spp.* *Fusarium spp.*
Group 4	Whitish Black	*Botryosphaeria spp.* *Alternaria spp.*	*Botryosphaeria spp.* *Alternaria spp.*

The most frequently isolated fungal family using conidial morphology was the dark coloured group making up 39.7% of the total number of isolations see Table 9. The dark colored group represented the Botryosphaeriaceae, see Figure 6. The whitish-to-whitish pink whose spores resembled those of *Fusarium* and *Nectria* were the second most frequently isolated from leaf diseases and some dieback symptoms at 33.1% of the total isolations. The third group was *Pestalotia spp*. and made up 17.9%. Lastly, *Alternaria* and *Phomopsis* groups based on colony and spore characteristics were few and in total made up 5% of the total isolations. The genus *Lasiodiplodia* was the most abundant among the Botryosphaeriaceae comprising 87% of the total Botryosphaeriaceae isolates.

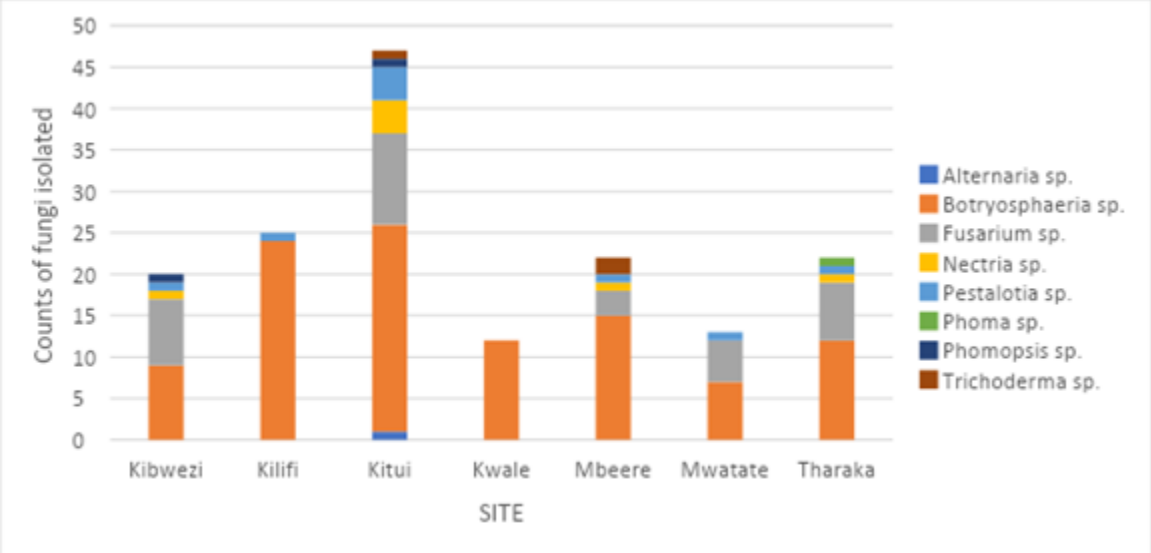

Figure 6: Frequency of isolations of different fungi according to morphotypes in the study

Table 10 Frequency of different fungal families isolated in the study and the associated species

Fungal Family	**Species Identified**	**Frequency of isolation**	**% of the total**
Botryosphaeriaceae	*Lasiodiplodia theobromae*	49	10.7
	Lasiodiplodia parva	20	4.4
	L. pseudotheobromae	27	5.9
	Lasiodiplodia crassispora	19	4.1
	Lasiodiplodia sp. KR1	44	9.6
	Neofusicoccum parvum	10	2.2
	Macrophoma theicola	9	1.9
	Dothiorella viticola	4	0.9
	Subtotal	182	39.7
Nectriaceae	*Fusarium spp.*	95	20.7
	Nectria spp.	60	13.1
	Subtotal	155	33.8
Amphisphaeriaceae	*Pestalotiopsis spp.*	82	17.9
	Subtotal	82	17.9
Pleosporaceae	*Alternaria spp.*	23	5.0
Diaporthaceae	*Phomopsis spp.*	16	3.5
Total		**458**	**100**

Some fungal species isolated from the two tree species looked different morphologically but turned out to be the same fungal species after molecular characterization, as shown in Plates 5 a and b below.

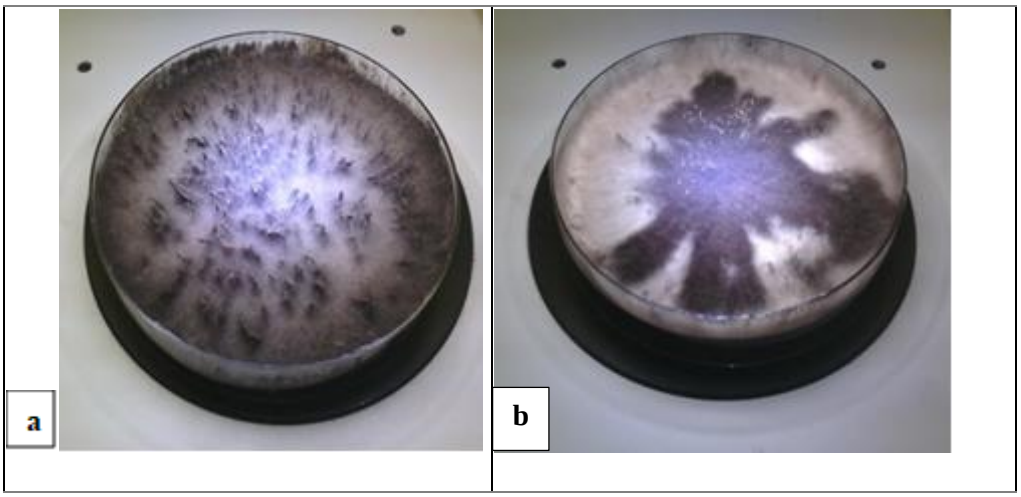

Plate 5: Showing colony morphology of two *Lasiodiplodia* sp. cultures from the two species sampled a) *Lasidiplodia* sp. from *Melia volkensii* and b) *Lasiodiplodia* sp. from *Azadirachta indica*. Source: Author, 2023

Morphological characteristics such as colour of mycelia and the colony as formed on nutrient media were used to group the cultures of fungi isolated from the target tree species. For morphological grouping spores were mounted on glass slides using lactic acid and observed under a microscope. The dark coloured group had the greatest variation of fungal species and the summary of results and frequencies of individual fungal species is shown in tables 8 and 9 above.

Botryosphaeriaceae species found in this study comprised 39.7 % of the total isolates made in the study making it the most frequently isolated species. Lasiodiplodia genus was most abundant in the isolations made (34.7%) of the total isolations. The species is associated with canker and dieback symptoms of a wide range of hosts globally.

In total, four hundred and fifty-eight (458) cultures were used for morphological groupings in this study. Thirty-eight (38) cultures of Botryosphaeriaceae and twelve 12 cultures

representing the Nectriaceae and Amphisphaeriaceae families were further used for molecular characterization.

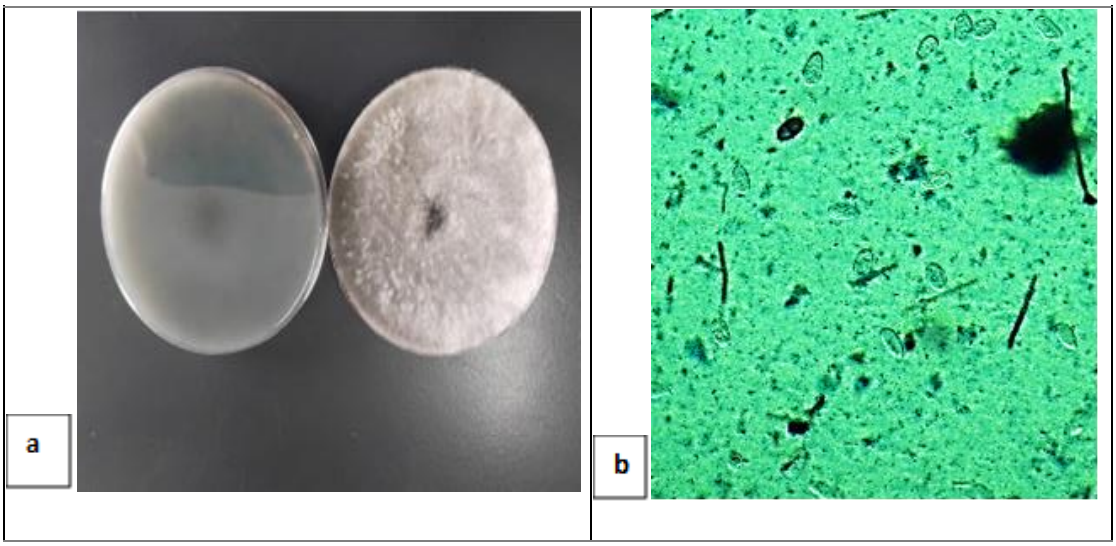

Plate 6 showing a) fungal colony and reverse side of *Lasiodiplodia theobromae* and b) showing mature and immature conidia of *L. theobromae*.

Source: Author, 2024

Lasiodiplodia theobromae was the most common species of Botryosphaeriaceae in this study making up 10.7 % of the total isolations. The species was majorly isolated from bark and stem samples and was associated with cankers and dieback symptoms. Conidia are ellipsoid with one septation and have striations. Young colonies were light grey turning to dark grey in colour with time with aerial mycelia and greyish black in petri dish reverse Plate 6 (a). Immature conidia are transparent and ellipsoid in shape with thick walls as shown in Plate 6b.

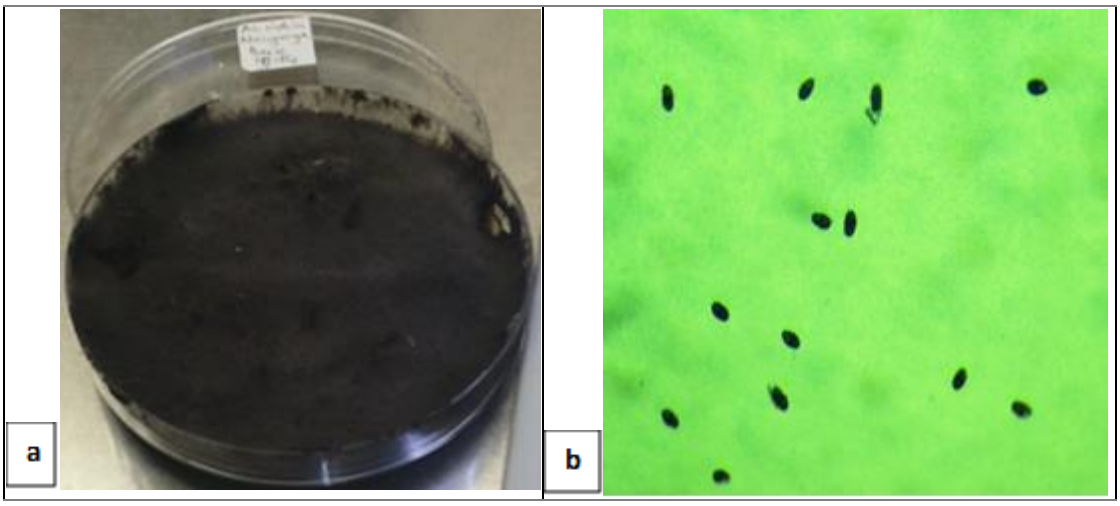

Plate 7: Showing a) a colony and b) spores of *Lasiodiplodia pseudotheobromae on* MEA. Source: Author, 2024

Lasiodiplodia pseudotheobromae made up 5.9% of the total isolates made from this study. It was isolated from symptomatic tissues of both tree species. Symptoms associated with this species included stem canker and dieback. Conidia are dark coloured without septation and oval in shape see Plate 7b. Fungal colony is dark black in colour with abundant aerial mycelia and dark black pigmentation in MEA see Plate 7a.

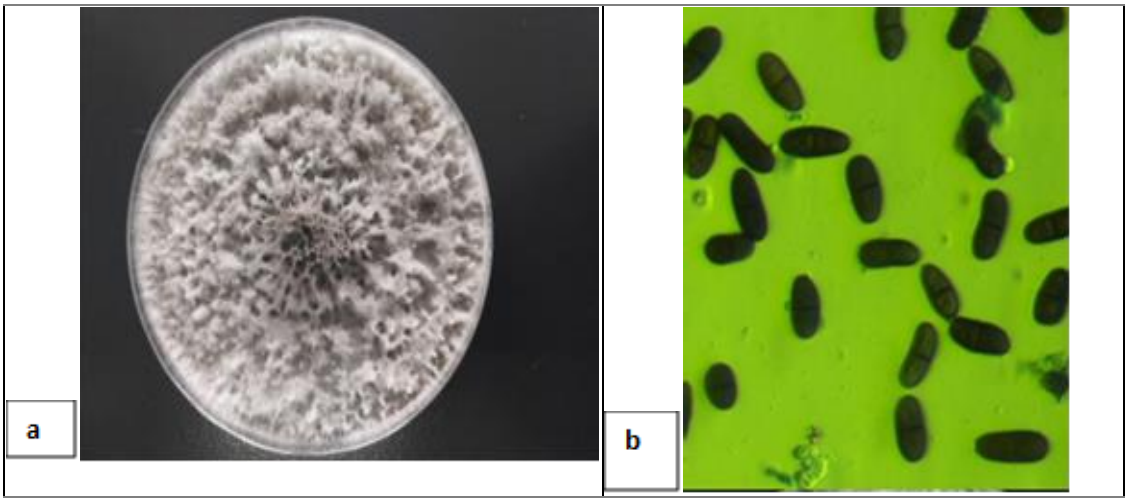

Plate 8: a) One-week old Fungal culture and b) spores of *Lasiodiplodia parva.* Source: Author, 2024

Lasiodiplodia parva was associated with high disease severity in the hotter sites within Coast and Kitui. The species was isolated from symptomatic tissues on *Azadirachta indica*. The colony was whitish when young appearing light grey after one week see plate 8a. The species grew fast covering a 90mm plate in 7 days. Spores were septate and dark brown in colour with an elongated oval appearance (Plate 8b).

Neofusicoccum parvum was isolated from *Melia volkensii* twigs with canker symptoms. Of the total number of isolates *N. parvum* made up 2.2%. This species was mainly isolated from twigs. A colony of *N. parvum* was light grey then darkens with age to dark grey with whitish cover mycelia being sparse and aerial (see plate 9a). Conidia were light brown in colour and double walled however they lacked septation (see Plate 9b).

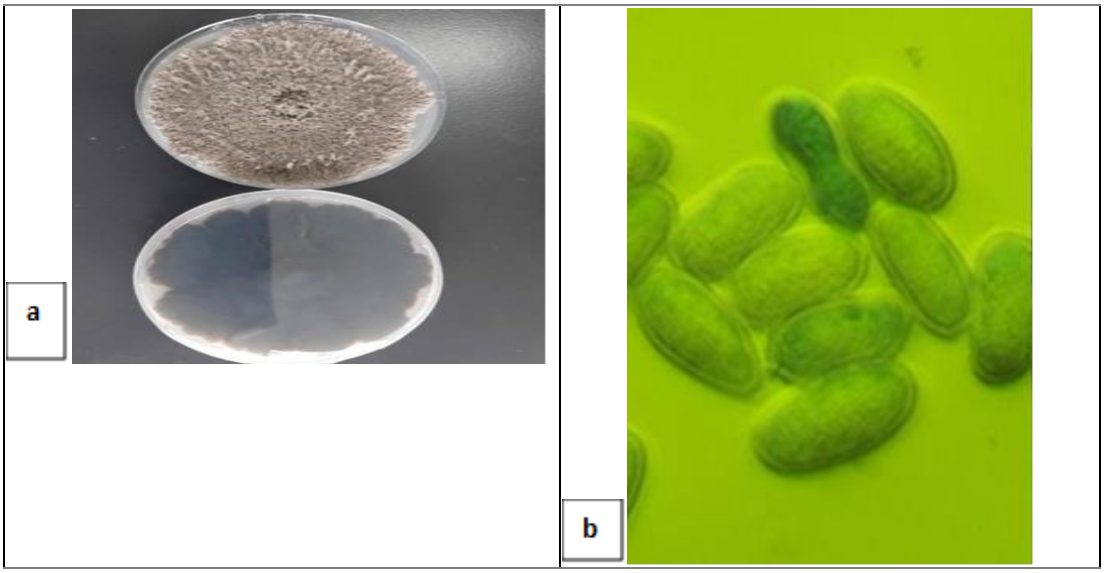

Plate 9: a) Colony and reverse appearance of *Neofusicoccum parvum* and b) *N. parvum* spores. Source: Author, 2024

Species from the family Nectriaceae were the second most abundant in the isolated fungi (33.1 %). Nectriaceae cause a range of symptoms including necrosis, blights, dieback and cankers on some species. The presence of Botryosphaeriaceae and Nectriaceae on the two

species alludes to a disease complex causing more severe symptoms and higher incidence levels. *Fusarium oxysporum* and *F. equisetti* were isolated in this study. *Nectria spp*. was also found on a number of samples. Conidia of *Fusarium sp.,* appear elongated and have multiple cells see Plate 10 (b&c). Colony colour in 2 % Malt Extract Agar varies from white, to brown to pink with abundant non-aerial mycelia. *Fusarium sp.* is found in soil and on plant tissue including fruits causing a number of symptoms such as root rots, fruit rots, cankers and blights. In this study *Fusarium sp.* was mostly isolated from symptomatic twigs and leaves.

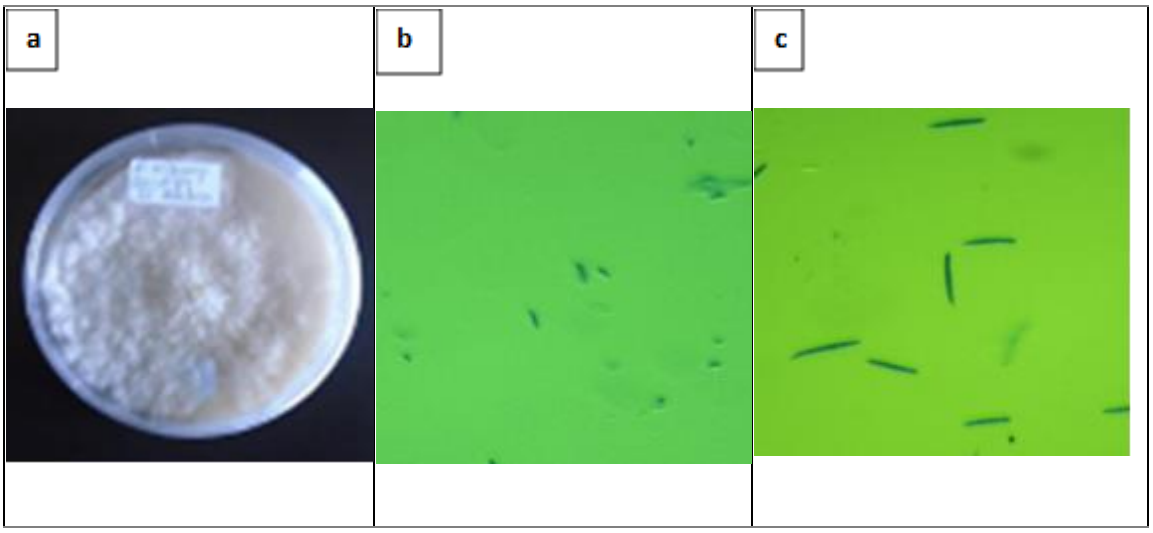

Plate 10: a) Fungal culture of *Fusarium sp.*, (b)micro conidia and c) macroconidia of *Fusarium sp.* Source: Author, 2023

Fungi from the family Amphisphaeriaceae comprising mainly of *Pestalotia sp.* were the third most abundant group making up 17.9% of the fungal isolates. The species is white in culture. It was isolated from healthy plants and diseased leaves with blight symptoms. Five-celled conidia with dark brown mid-part cells distinguish the species with ends being pointed see Plate 11b. It is also isolated from seeds of a number of tree species. Only five

(5) cultures of *Pestalotia sp.* were used for molecular characterization yielding *Pestalotia funerea* which has since been reclassified *Pestalotiopsis funerea* a proven pathogen.

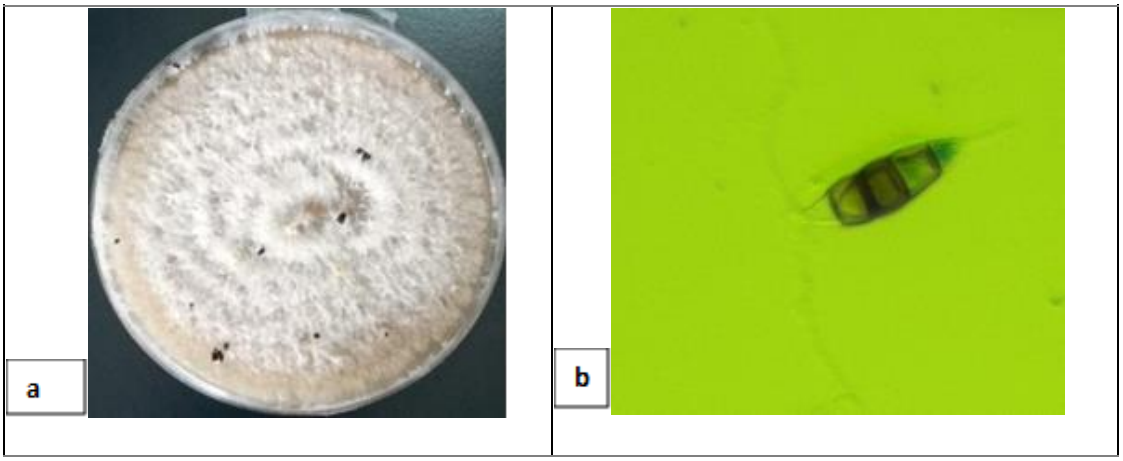

Plate 11: a) Fungal colony with black specs from aggregated conidia and b) fungal spore of *Pestalotia sp.* Source: Author, 2024

Alternaria alternata comprised 5% of the fungal isolates and was associated with fruit lesions and necrosis. Fungi culture is grey in colour and slow growing. The conidia are septate and dark brown in colour with many cells and are club-like as shown in Plate 12b. *A. alternata* was the only species isolated in this study and is known to cause leaf spots.

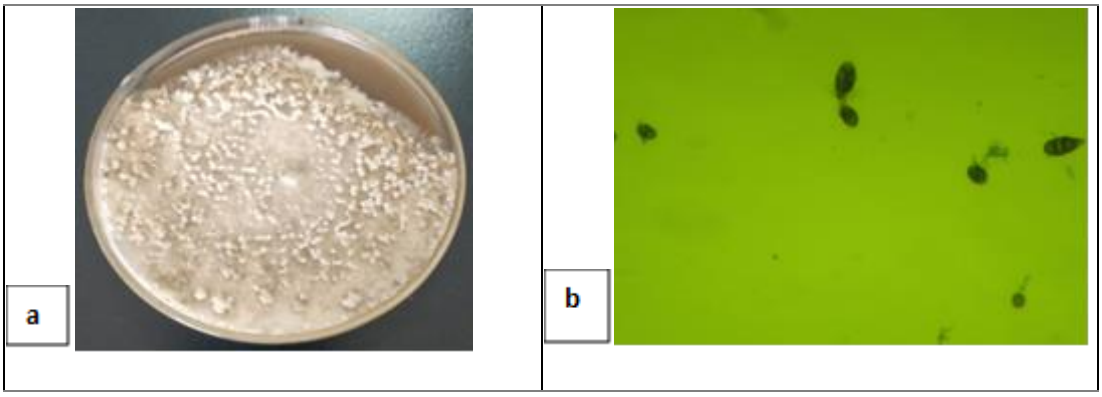

Plate 12: a) Fungal culture of *Alternaria sp.* in MEA, b) spores of *Alternaria sp.* Source: Author, 2024

Representative isolates from various groups within the three main morphological groups were characterised by their rRNA sequences. Two gene regions (ITS and Tef-1 alpha) were targeted for comparison of identities of the sequences. The downloaded sequences were between 533 and 1656 base pairs long.

All sequences from the study were submitted to Genbank and their unique accessions assigned as shown in table 10 below. Nine (9) species of Botryosphaeriaceae were obtained and submitted from the study. One species of Nectriaceae and 2 species of Trichocomaceae were also submitted. Some of the species identified using rRNA sequences had bootstrap levels below 60% and were eliminated from the phylogenetic analysis to get the final tree in Figure 7 below molecular level identification confirmed the most isolated group was Botryosphaeriaceae.

Additional sequences were downloaded from Genbank for comparison with the isolated fungal gene region sequences obtained. The comparison sequences used in the phylogenetic analysis were: *Lasiodiplodia theobromae* KC964548, FJ904841, KM406107, NG062745, FJ478103, FJ904844, *L. pseudotheobromae* JX914479, MW341580, KM006447, NG062746, KF766193, *Macrophoma theicola* KP179222, *Macrophoma sp.* KP004441, *L. parva* GQ469961, GQ469964, KF766192,NG067414, *Lasiodiplodia sp.* KR1 KC623566, *Allanphilipsia aloeigena* KF777137, NR137121, *Penicillium griseofulvum* EU497954, NR103692, *Fusarium solani,* JX914481, NR163531, FJ867936, *Neofusicoccum parvum* FJ904817 , KJ190278, *Spencermartinisa viticola (Dothiorella viticola)* JF271752, KF766313, *L. crassispora* GU799456, NG062741, EU918710, *Alternaria alternata* KP271958, *Talaromyces purpureogenus* KJ528885, NR121529, *Wrightoporia tropicalis* FJ904857, *Amycosphaerella africana* DQ267577, *Botryosphaeria*

sp. FJ904821, *Diplodia corticola* GU799458, *Diaporthe macintoshii* KJ197289, *Cytospora sp.* KF746102, *Pestalotiopsis sp.* KP120986, *Nigrospora sp.* JF819161,*Curvularia sp.* KF624777, *Penicillium chrysogenum* LN809047.

A cross reference of the identified samples from molecular analysis revealed that the dark coloured fungal species belonged to the Botryosphaeriaceae, the cream white to whitish pink belonged to the Nectriaceae while those from whitish mycelia with black pycnidia belonged to the Amphisphaeriaceae group. Other fungal groups identified included the Pleosporaceae and Diaporthaceae. The dendrogram below was developed after alignment using ClustalW method on MEGA 11.0 and using the neighbour joining method to quantify closeness of the fungal species to one another as shown in Figure 7.

Table 11: Details of cultures used for molecular characterization for this study

Culture No.	Accession no. (ITS)	Species	Host	Locality
AMM1A	MZ182299	*Lasiodiplodia theobromae*	*Melia volkensii*	Chakama
AMM1B	MZ182298	*L. pseudotheobromae*	*Melia volkensii*	Silaloni
AMM2	MZ182300	*L. pseudotheobromae*	*Melia volkensii*	Witu
AMM10	MZ208812	*L. theobromae*	*Melia volkensii*	Kibwezi
AMM63A	MZ182301	*Macrophoma theicola*	*Melia volkensii*	Silaloni
AMM69	MZ182303	*Lasiodiplodia parva*	*Melia volkensii*	Silaloni
AMM71	MZ182308	*L. crassispora*	*Melia volkensii*	Witu
AMM73	MZ182309	*Talaromyces purpureogenus*	*Melia volkensii*	Mwatate
AMM98B	MZ208811	*L. pseudotheobromae*	*Azadirachta indica*	Gede
AMM100	MZ182313	*Alternaria alternata*	*Melia volkensii*	Kibwezi
AMM102	MZ208814	*Dothiorella viticola*	A. *indica*	Manyanga
AMM126A	MZ208810	*Macrophoma theicola*	A. *indica*	Gede
AMM126B	MZ182353	*L. theobromae*	*A. indica*	Kibwezi
AMM160	MZ208813	*L. pseudotheobromae*	*A. indica*	Manyanga
AMM217	MZ182360	*Lasiodiplodia parva*	*A. indica*	Manyanga
AMM232A	MZ183449	*Alanphillipsia aloeigena*	*A. indica*	Manyanga
AMM234	MZ183972	*Penicillium griseofulvum*	*Melia volkensii*	Kibwezi
AMM257	MZ183974	*Fusarium solani*	*Melia volkensii*	Mutugo
AMM274	MZ183975	*Neofusicoccum parvum*	*Melia volkensii*	Kirangare

The rRNA sequences from this study were blasted in comparison to TYPE material sequences from existing databases including CBS-Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands CMW- FABI, University of Pretoria, South Africa and WAC-Department of Agriculture Western Australia Plant Pathogen Collection. All sequences were downloaded in FASTA format and then aligned online using Multiple Alignment using Fast Fourier Transform (MAFFT) before being used to develop a phylogenetic tree at 1000 bootstrap values using the Kimura 2 model with Invariant and Gamma separation methods (K2+G+I).

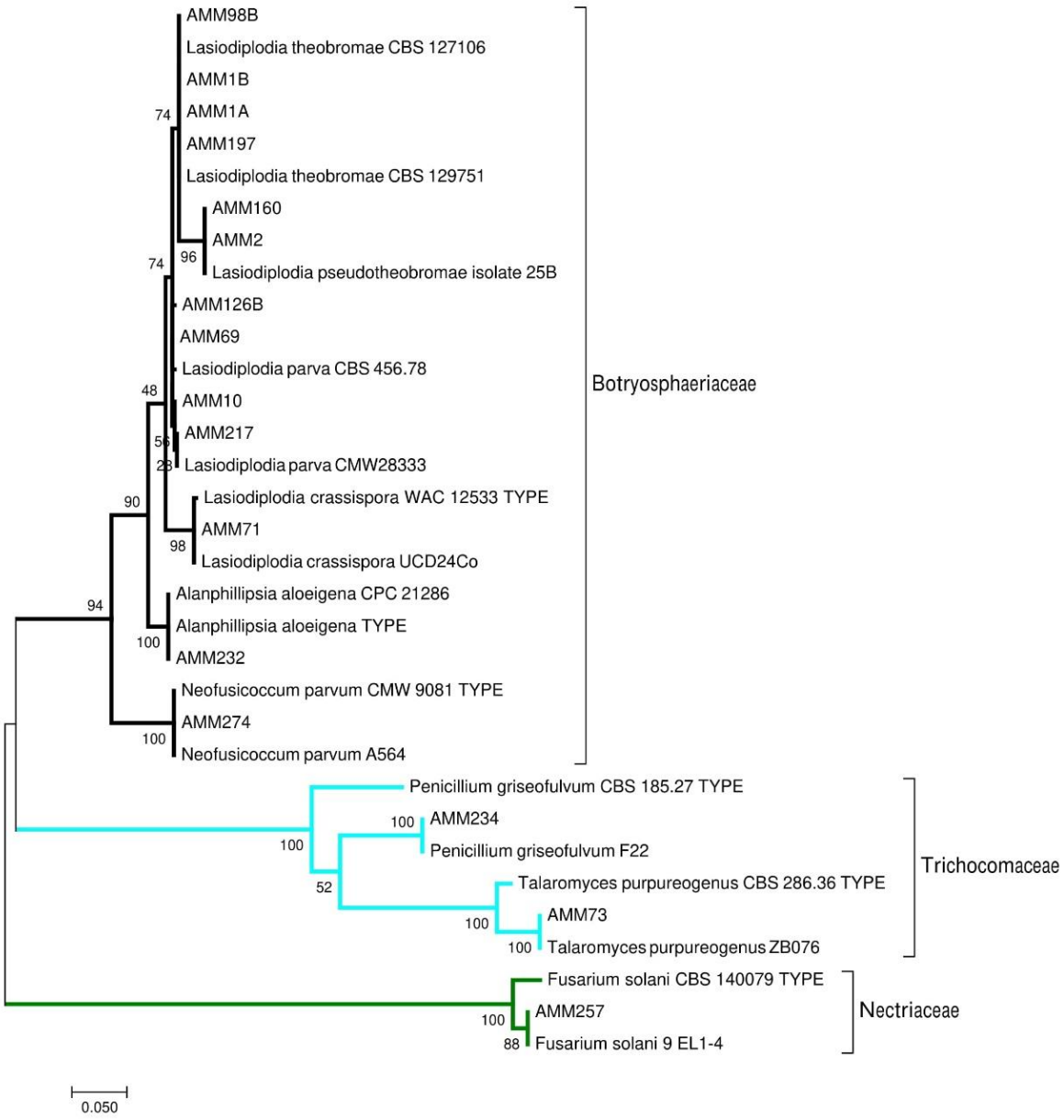

Figure 7: Simplified Dendrogram of ITS gene region fungal sequences isolated from *Melia volkensii* and *Azadirachta indica* from the study.

Objective 3: To carry out Pathogenicity tests of isolated fungi on seedlings of the same species in the glasshouses

Seedling mortality and lesion measurement were used to test pathogenicity of the selected isolates. Of the 130 *Melia volkensii* seedlings inoculated with the different fungal species 80 developed chlorosis and wilted and only 5 died. All the dead seedlings exhibited symptoms of necrosis and gummosis with black pycnidia growing around the point of inoculation. Fifty (50) seedlings were still healthy with slight wilting at the close of the experiment. Of the healthy-looking seedlings some had lesions of up to 5cm but were still growing giving an implication of tolerance to the fungi inoculated by the species.

Out of the 130 *A. indica* seedlings inoculated, 63 wilted and only 8 died by the close of the experiment. The other 59 seedlings were still growing with a range of 3.1-12cm mean internal lesion. The same fungi that were used to inoculate the seedlings were re-isolated in the lab from the symptomatic tissue at the close of the experiment.

4.6 Mean lesion development

In general, *Melia volkensii* had longer mean lesion measurements of 0.1–19.5 cm than *Azadirachta indica* at 0.1-14.8cm as shown in Fig. 8. Isolate 4 representing *Lasiodiplodia sp. (a). KR1* previously isolated from *Melia volkensii* in Voi caused the longest lesion on *M. volkensii* (19.5 cm) while isolate 5 previously isolated from *A. indica* in Kibwezi representing *Lasiodiplodia sp. (b). KR*1 caused the longest lesion on *A. indica* (14.8cm).

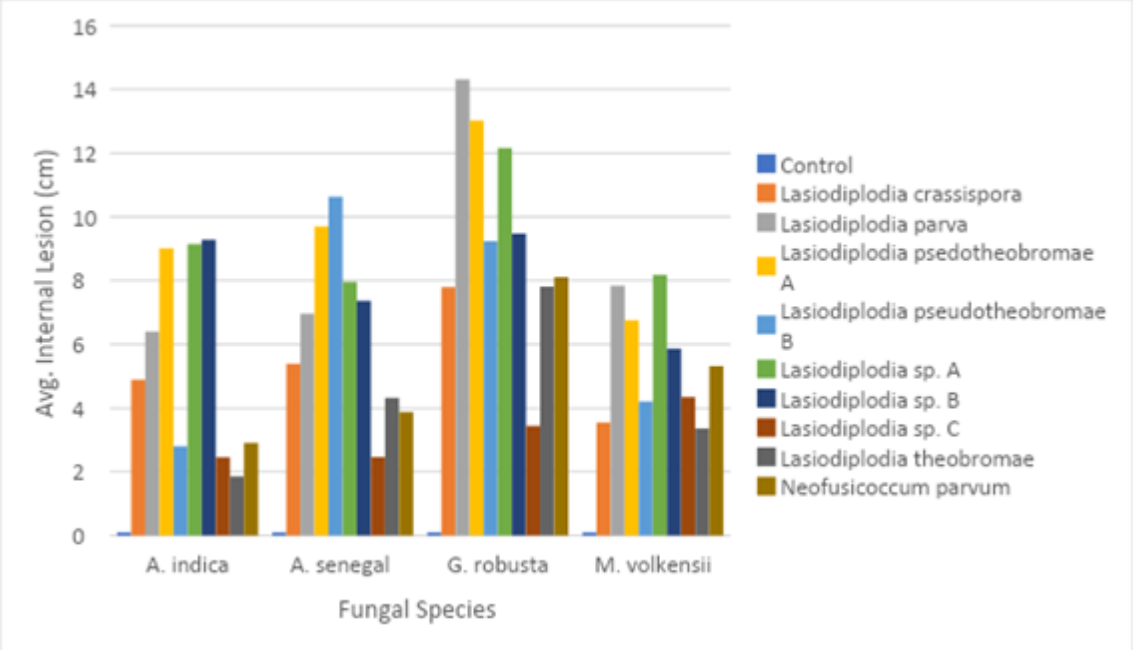

Figure 8: Graph showing mean internal lesion lengths of the nine (9) *Botryosphaeriaceae* species on four tree species in the glasshouse pathogenicity tests

Different fungal isolates caused different lesion measurements between the two tree species. An example of lesions is illustrated on Plate 14. *Lasiodiplodia parva* caused the longest mean internal lesion (20 cm) in *M. volkensii* and a total mortality of 22% of the seedlings inoculated. *Neofusicoccum parvum* was the second most pathogenic fungus with 17% mortality of the seedlings inoculated. The *Lasiodiplodia crassispora* isolate was the least pathogenic with no mortality and the least average lesion measurements of 0.5cm on *M. volkensii* and 0.1cm on *A. indica*.

4.7 Mortality of seedlings

Melia volkensii recorded higher mortality than *A. indica* under glasshouse conditions closely resembling our field observations, where higher disease severity and disease incidence were recorded on *M. volkensii* than in *A. indica* especially in the coastal sites.

There are significant differences between fungal isolates and their ability to cause mortality on the two tree species see Table 11. *Lasiodiplodia parva, Neofusicoccum parvum,* and *Lasiodiplodia sp. (a) KR1* respectively caused the highest mortality on both species at 20%, 13% and 8% for *L. parva, N. parvum,* and *Lasiodiplodia sp. (a) KR1* respectively on *M. volkensii.* In comparison, the same fungal species caused 12%, 8%, and 5% mortality on *A. indica* respectively.

Table 12 One-way Analysis of Variance for the Pathogenicity Tests

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Tree species	17	4947	291.03	5.66	0.000
Error	247	12704	51.43		
Total	264	17652			

From the pathogenicity tests conducted using the isolated fungal species both *Melia volkensii* and *Azadirachta indica* were found to be disease tolerant to allow infection rates below economic injury level see Plate 13.

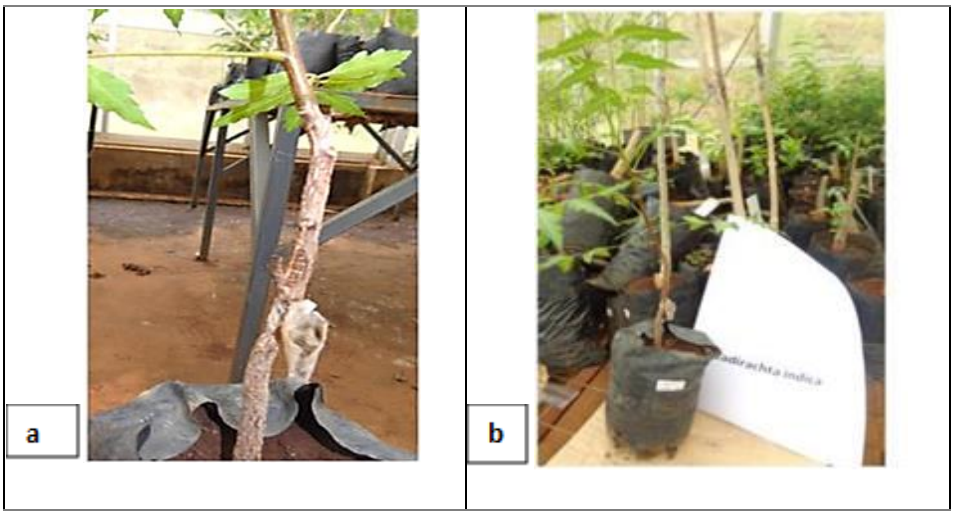

Plate 13: Showing callus tissue and wound healing on a) *M. volkensii* and b) *A. indica* after inoculation with *Lasiodiplodia crassispora* Source: Author ,2024

During the field surveys other tree species identified in the drylands included *Gmelina arborea*, *Tamarindus indica* and *Vitex payos* having low disease incidence and severity. *G. arborea* and *Vitex payos* are native; while *Tamarindus indica* is naturalised in Kenya. These species have multiple uses and should be considered for mixed planting in the drylands together with *M. volkensii* and *A. indica*.

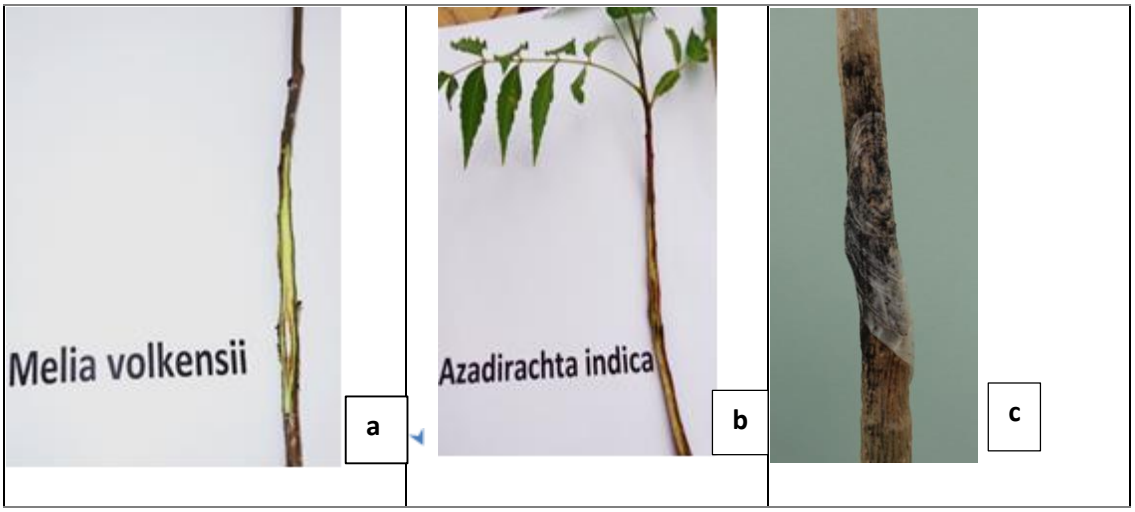

Plate 14: Showing internal lesions on a) *M. volkensii* and b) *A. indica* inoculated with *L. pseudotheobromae* and c) black *pycnidia* on *M. volkensii* Source: Author, 2024

CHAPTER FIVE
DISCUSSION

5.1 Disease severity, incidence and symptoms recorded in the field .

Plant diseases require a virulent pathogen, favourable environmental conditions and a susceptible host to develop (Agrios, 2005). The two tree species; *Melia volkensii* and *Azadirachta indica* in the present study were in their optimum growing conditions as they are drought tolerant and the study area is generally dry with sandy soils and annual rainfall below 500 mm with mean temperatures between 24 and 32 degrees Celsius. *M. volkensii* is indigenous and *A. indica* is naturalized but both tree species are in the same family Meliaceae and are suited to the same agro-ecological zones (Maundu & Tengnas, 2005).

On the two tree species both dieback and canker symptoms were recorded most frequently across the sites. This is consistent with studies showing the Botryosphaeriaceae fungal family causing stem cankers, branch and shoot die-back (Batista *et al.*, 2021; Dissanayake *et al*., 2016; Abdollazadeh *et al*., 2013; Phillips *et al.*, 2013; Liu *et al.*, 2012; Slippers *et al.*, 2009). The fungi also cause fruit rots and this symptom was recorded on *Melia volkensii* fruits. The dieback and canker symptoms coincided with the high presence of Botryosphaeriaceae and Nectriaceae fungi on the trees sampled. The higher the proportion of the host showing symptoms of the disease the higher the incidence as mature conidia are easily spread when there is optimum temperatures and relative humidity (Talley *et al*., 2002). Most trees sampled with both canker and dieback symptoms resulted in isolation of *Fusarium* spp. and species of Botryosphaeriaceae. The Nectriaceae and Botryosphaeriaceae fungi are known for their canker and dieback causing ability on a wide host range (Cherotich *et al*., 2020; Graziosi *et al*., 2020; Gezahgne *et al.*,2004).

There was a high occurrence of both cankers and dieback on the two species. Canker causing fungi mode of spread is through natural openings and wounds on the host (Sinclair & Lyon, 2005). Both Botryosphaeriaceae and Nectriaceae which were the frequently isolated fungi in this study cause both dieback and cankers on their hosts (Dissanayake *et al*., 2016; Liu *et al*., 2012; Luo & Zhuang, 2010; Slipper & Wingfield, 2007). Twigs and new shoots had higher disease severity due to their susceptibility to scorching by the hot sun compared to older parts on the same plant (Zhao *et al*., 2016). The actively dividing or growing cells may be killed by the high temperatures and due to their tenderness are easily penetrated by disease agents and exposed to physiological stress thus easily exhibiting symptoms of the disease (Cherotich *et al.,* 2020).

The presence of canker causing fungi in healthy plant tissue shows that the isolated fungi are also endophytic in the two tree species. Endophytic fungi have been found to become pathogenic at the onset of stress to the plant (Franic *et al*., 2024; Jami *et al*., 2014; Njuguna *et al*., 2011; Slippers &Wingfield, 2007; Slippers *et al*., 2005). The canker causing fungi that were abundantly isolated in this study are common and cosmopolitan with a global distribution including many economically important forest tree species and economically important crops (Batista *et al*., 2021; Slippers *et al*. 2017). *Lasiodiplodia theobromae* was isolated from both symptomatic and asymptomatic tissue in this study showing that it is endophytic. This is consistent with previous studies (Batista *et al*., 2021; Cherotich *et al.,* 2020; Xiao, 2014; Slippers & Wingfield, 2007) which also isolated *L. theobromae* from different tree species.

This study confirmed the ability of the canker and dieback causing fungi to be pathogenic to the two Meliaceae species; *Melia volkensii* which is indigenous and *Azadirachta indica*

a naturalized species in Kenya. However, most of the isolated fungi are not thought to be pathogens of economic importance because the two species exhibited good levels of potential disease tolerance to Botryosphaeriaceae through wound healing and low mortality to some of the fungal species.

Meliaceae as a family has been studied for its pest-repellent properties. Botryosphaeriaceae species are known to have a wide host range and are also widely distributed worldwide. The fact that they were found on healthy trees further demonstrated that they are latent pathogens of these two tree species and could develop into dangerous ones in the event of severe weather conditions, such as protracted droughts and high temperatures. This is in line with research on the pathogenicity of certain fungal species conducted under controlled circumstances (Zhou et al., 2022; Cherotich et al., 2020; Njuguna et al., 2011). For both tree species, disease incidence varied with altitude, with Tharaka Nithi having the lowest incidence and Kilifi having the highest. The incidence and severity of dieback and canker disease varied significantly among the various sites that were chosen.

The alternative hypothesis was accepted at p=0001, rejecting the null hypothesis that there was no significant variation in disease incidence and severity between sites. The incidence and severity of the disease varied significantly between the two species and between sites. There was no statistically significant difference in disease incidence between the Voi, Kilifi, Kwale, and Lamu sites (LSD=01225).

Disease incidence was not significantly different statistically for Voi, Kilifi, and Kwale sites. This is attributed to the fact that they are in the same agro-ecological zone with conditions characterized by high humidity and hot weather. Kitui, Kibwezi, and Mbeere sirtes were also not statistically different. The sites are in the same agroecological zone and

geographical area. However, Tharaka Nithi site had the lowest disease incidence, and is a potential site for *Melia volkensii* planting (Kariuki *et al*., 2021).

The low altitude, high humidity and temperatures could have led to the higher disease levels in the coastal area. Other studies have noted the impact of high heat areas on plant disease infections (Cherotich *et al*., 2020; Dissanayake *et al*., 2016; Njuguna *et al*., 2011; Abdollahzadeh *et al*., 2010). Plant types, their extractives and other characteristics determine the level and response to disease infection (Slippers & Wingfield, 2007; Slippers *et al*., 2005).

The weak positive correlation between disease incidence and disease severity in this study shows that the species could have a perceived tolerance to the fungal species or have co-evolved with the fungus thereby producing less severe disease symptoms. Disease incidence was moderate for both *M. volkensii* and *A. indica* appears to have co-evolved with fungi in its environment, allowing it to tolerate infections, as noted by Schafer (1971). This suggests that, in such cases, fungal infections may not lead to significant economic losses or severe damage to the trees (Li & Shao, 2024). From this study, Melia volkensii seems better suited to the Eastern lowlands compared to the Coastal sites. On the other hand, Azadirachta indica thrives in the coastal areas, where it showed lower disease incidence and severity than in the Eastern lowlands.

Interestingly, there was a weak positive correlation (0.309) between disease incidence and severity, suggesting that one factor might influence the other. For instance, high disease incidence could indicate a highly virulent pathogen or a susceptible host. A highly

pathogenic fungus may also produce large amounts of inoculum, infecting more plant parts and increasing disease severity (Sacristan & Garcia-Arenal, 2008).

Among the study sites, Tharaka stood out with significantly lower average disease incidence compared to the other five locations. Additionally, trees in Tharaka experienced less insect and parasite damage, grew taller, had larger girths, and displayed better stem form. These observations suggest that Tharaka is an ideal site for growing Melia volkensii, likely due to higher rainfall and fewer dry months, which reduce physiological stress on the plants. In this site *M. volkensii* should therefore be promoted for agroforestry since the species was also observed to grow well with crop mixtures including cereal and legume crops. In Kitui there was little termite damage as opposed to the heavy insect damage in Chakama evidenced by presence of galls on the stems and twigs of the trees. Insect damage may have been a predisposing factor that opened up the trees to opportunistic pathogen attack as has been seen in many species susceptible to *Botryosphaeria* attack (Li *et al*., 2018; Slippers *et al*., 2009).

5.2 Fungi isolated, morphological and molecular characterization

The isolated species of *Botryosphaeriaceae* have been found on other native *Fabaceae* and *Myrtaceae* in South Africa, Venezuela, China and India (Li *et al*., 2018; Chen *et al*., 2011; Slippers *et al*., 2009; Mohali *et al*., 2007). The presence of *Neofussicoccum parvum, Lasiodiplodia theobromae* and *L. pseudotheobromae* in this study as well as on *Grevillea robusta* in a previous study (Njuguna, 2011) presents a possibility of host jump from one species to the other (Slippers *et al*., 2017; Slippers & Wingfield, 2007). However, it was notable that the incidence levels on the two Meliaceae species were lower than those reported on *Grevillea robusta* by Njuguna *et al*. (2011 within the same regions. This could

be due to the fact that *Melia volkensii* and *Azadirachta indica* are drought-tolerant as compared to *Grevillea robusta*. The presence of dead *G. robusta* trees in dry and humid sites may have been a case of poor site species matching.

Lasiodiplodia parva has been increasingly isolated and found to be pathogenic to different species (Zhou *et al*., 2022; Cherotich *et al*., 2020). It has been found to cause lesions on inoculated plants resulting from dieback and canker symptoms. In this study it was found to produce the longest internal lesions on *M. volkensii* and *A. indica*. *L. parva* was previously categorized as *L. theobromae* but increasing phylogenetic studies have differentiated the species as distinct (Alves *et al.*, 2008).

Lasiodiplodia pseudotheobromae has been previously isolated from other native species within Africa *(*Cherotich *et al.*,2020*;* Adetunji & Oloke, 2013*).* Its host range is wide and covers both forest trees and fruit trees. In this study *L. pseudotheobromae* made up 5.9% of the total isolations realized. In the pathogenicity tests the species was intermediate causing wilting within 3 days of inoculation and killing seedlings of *G. robusta* within 10 weeks.

Lasiodiplodia theobromae is an important species with a wide host range and was the most isolated species of Botryosphaeriaceae in this study. This is consistent with literature on Botryosphaeriaceae (Zhou *et al*., 2022; Batista *et al*., 2021; Cherotich *et al*., 2020; Slippers *et al*., 2017). In the pathogenicity tests *L. theobromae* was second most virulent fungi causing symptoms within 3 days of inoculation and resulting in long internal lesions on inoculated seedlings. This was similar to two other studies on dryland tree species (Cherotich *et al*., 2020; Njuguna *et al*., 2011). *Neofusicoccum parvum* was only isolated

from *Melia volkensii* and caused short internal lesions on the four test species in the pathogenicity tests under glasshouse conditions. The species causes damage to exotic species in East Africa (Njuguna *et al*., 2011; Mohali *et al*., 2007) but has less severity on native species.

Fusarium sp. had been previously isolated from *Melia volkensii* in previous surveys of the tree species (Njuguna *et al*., 2004) and recorded on seedlings of Neem in India (Harrison *et al*., 2003). *Fusarium solani* and *F. oxysporum* have been known to cause root rots and wilts on woody plants (Chliyeh *et al*., 2017; Suga *et al*., 2000). Nectria species have also been recorded as causing canker and dieback symptoms on trees. Though rarely fatal, Nectria species cause damage to the affected parts and are listed on many extension materials as key disease to be managed on trees (Cherotich *et al*., 2020; Luo & Zhang, 2010). The presence of several fungal species in the sites studied is perceived as the emergence of a disease complex that could increase the disease severity due to compromised plant immunity and vigor in growth (Jami *et al*., 2014; Njuguna *et al*., 2011; Sinclair & Lyon, 2005).

The null hypothesis that states there is no significant difference between fungi isolated from *Melia volkensii* and those from *Azadirachta indica* was rejected at p=0.045 there was some difference between the fungi isolated from *Melia volkensii* and *Azadirachta indica.* The species shared several species of Lasiodiplodia namely*: Lasiodiplodia parva, L. theobromae, L. pseudotheobromae, L. crassispora, Lasiodiplodia sp. KR1.* However, *Neofusicoccum parvum* and *Spencermartinsia viticola* were only isolated from *Melia volkensii*. A different strain of *L. theobromae* species and *Macrophoma theicola* were only isolated from *Azadirachta indica.* This is consistent with literature that has found some

Botryosphaeriaceae species to be host specific (Slippers *et al*., 2017; Phillips *et al*., 2013; Liu *et al*., 2012; Slippers & Wingfield, 2007).

There is a relationship between fungi causing disease in *Melia volkensii* and *Azadirachta indica* in the selected ASALs of Kenya. The phylogram showed that the species were not closely related though some were similar. From the phylogenetic analyses, the species affecting *M. volkensii* formed one node branching away from those affecting *A. indica*. The species that were shared also grouped together in a node and were closely related. The null hypothesis was therefore rejected and the alternative hypothesis adopted with bootstrap values at 100%. *Botryosphaeria dothidea* as a species was not isolated from the two tree species studied despite being known for its wide host range across the globe (Slippers *et al*., 2017; Dissanayake *et al*., 2016; Phillips *et al*., 2013; Njuguna *et al*., 2011; Denman *et al*., 2000).

5.3 Pathogenicity tests under glasshouse conditions

The initial response recorded on both *Melia volkensii* and *Azadirachta indica* seedlings in the glasshouse experiments was chlorosis and wilting within three days of inoculation. By the closure of the experiment (46%) of the seedlings had healed at the point of injury. Wound healing caused by plant chemicals fighting the entry of a pathogen infers the ability of the host to overcome pathogen invasion (Pagan & Garcia-Arenal, 2018). Examination of the internal lesions showed that callus tissue had grown over the inoculated lesion after 5 weeks indicating some level of tolerance by the two tree species. These findings further confirmed that wound healing is common to these *M. volkensii* and *A. indica* as reported by (Njuguna *et al.,* 2011). However, since mortalities have been observed on some trees in

the field, this phenomenon is worth further investigation implying that some plant families and individuals may be more susceptible to the canker and dieback pathogens.

Melia volkensii was slightly more susceptible to infection than *Azadirachta indica* both in the field and under glasshouse conditions as indicated by the mean length and width of lesions formed and mortalities recorded in the pathogenicity tests and the disease incidence and severity recorded in the field. These results contradict pathogenicity tests done in glass house by Njuguna *et al.,* 2011 in which *Azadirachta indica* was more susceptible to fungi inoculated than *Melia volkensii*. The difference can be explained by the differences in the species and strains of the fungi used and their origin within the country (Mehl *et al*., 2017; Slippers *et al.,* 2009; Slippers and Wingfield, 2007). The phenomenon needs further investigation for clarification.

The ability of the two tree species to tolerate infection by the canker and dieback forming fungi presents hope for agroforestry in the semi-arid areas (Huxley, 1983). This aspect can also be used to enhance resilience of dryland agroforestry and also for climate change mitigation in the dry lands. For climate change mitigation the two species could be planted together with other native agroforestry species that are drought resistant hardy species such as *Tamarindus indica*, *Vitex payos* etc for their interaction to be studied and appropriate integrated pest management protocols to be developed (Buttoud, 2013).

5.4 Management of associated diseases of *M. volkensii* and *A. indica*

According to the National Climate Change Adaptation Plan 2023 (GoK, 2023), agroforestry and establishment of plantations in various ecosystems should be based on sound silvicultural and integrated pest management practices to enhance sustainability of

tree growing. The results of this study further support this strategy. Further studies should be conducted on the host-pest relationships to identify shifts that can be exploited for integrated pest management strategies (Slippers &Wingfield 2007; Toljander *et al*., 2007; Agrios 2005).

Native species have been promoted for rehabilitation and landscape restoration due to their adaptation to the environment (Kenichi *et al*., 2007). They have adapted water use efficiency and are perceived to coevolve with the environment and other biotic factors helping them grow vigorously while keeping levels of pathogens and pests low (Rey *et al*., 2013). Indigenous trees are a key component of resilient ecosystems as the trees promote biodiversity and create sustainable habitats and have been promoted in climate change and carbon sequestration programs for landscape restoration and conservation (Rey *et al*., 2013).

The presence of the fungi which are mostly classified as latent pathogens on the two tree species provides a challenge for integrated pest management in the phase of changing weather patterns (Dissanayake *et al*., 2016; Phillips *et al*., 2013; Liu *et al*., 2012; Slippers *et al*., 2009). The best way to manage and control outbreaks of endophytes is stress management for the trees which will in turn control the pathogen population to below pathogenic infection and symptom development. Increased vigour of the trees can be brought about by use of high-quality propagules, appropriate spacing and proper silvicultural management (Cherotich *et al*., 2020; Njuguna *et al*., 2011; Agrios, 2005).

It is important to study the invasion pathways of isolated species to determine their origin and spread in the country and the region for effective disease control mechanisms.

However, the latent infection mechanism of most of the endophytic Botryosphaeriaceae species presents a challenge in Phytosanitary control measures as their endophytic characteristic makes them seldom detected in asymptomatic plant parts (Cherotich *et al*., 2020; Slipper *et al*., 2017; Phillips *et al*., 2012; Slippers *et al*., 2009; Slipper & Wingfield, 2007).

Certified seed tested for seed borne pests and pathogens should be used for plantation establishment (Tuquerrez *et al*., 2023). The results of this study show that there is eminent risk in introduction of any pathogen on the trees right from the nursery to the field. All means should be used to avoid introduction of inoculum into planting material and subsequent treatments such as using sterile tools when pruning the trees and controlled pest damage as they form openings for disease infection (Agrios, 2005). Use of resistant/tolerant cultivars in tree breeding programmes will help reduce disease susceptibility and produce less infected seed for subsequent stands (Lamicchane, 2020). Further research on additional disease management strategies for tree species in the arid and semi-arid lands is ongoing.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The present study collected samples of plant tissue from *Melia volkensii* and *Azadirachta indica* from 6 counties in Eastern and Coastal Kenya. Both symptomatic and asymptomatic tissues were collected for the study. Different fungal families were identified from 247 isolates with Botryosphaeriaceae being the most frequently isolated family followed by *Nectriaceae* and *Amphisphaeriaceae.*

The most common symptoms recorded on the trees in the study sites were canker and dieback. There were also fruit rots noted on the fruits of *Melia volkensii* at the time of the study. *Botryosphaeriaceae* are known to cause canker and dieback symptoms on their hosts once pathogenic. The isolation of Botryosphaeriaceae from asymptomatic tissue is consistent with studies which have classified members of the fungal family as endophytic in nature only becoming pathogenic at the onset of physiological plant stress.

Disease severity and incidence differed between the two tree species and among the different sites. The trees showed different severity levels on different farms alluding to the design of tree planting or site characteristics. Plantation establishment will have to be designed based on optimum spacing and development of silvicultural regimes for the two tree species for optimum growth and vigor to control plant stress which could make the tree susceptible to endophytic pathogens.

The most commonly isolated genus was *Lasiodiplodia* followed by *Neofusicoccum* from the *Botryosphaeriaceae* family. The two genera have been isolated from other woody

species in the region and classified as both endophytes and latent pathogens and pathogenic.

From the phylogenetic tree the species differentiated into two distinct clades. The B*otryosphaeriaceae* differentiated into one clade and the other species comprising mostly *Nectriaceae* and *Trichocomaceae.* The isolated *Botryosphaeriaceae* species were different with bootstrap values of 80 to 100%.

Glass house tests done on seedlings in glass house conditions using the isolated fungi confirmed the isolated pathogens as the causative agents with dieback, gummosis and death being recorded as symptoms on the inoculated seedlings. The controls remained healthy and asymptomatic. *Botryosphaeriaceae* and *Nectriaceae* species were re-isolated from the inoculated seedlings fulfilling Koch's postulates.

The study provides insight into expected diseases of drought tolerant *Meliaceae* tree species *Melia volkensii* and *Azadirachta indica*.

6.2 RECOMMENDATIONS

Further studies need to be done on the health status of the trees sampled to confirm resistance and tolerance to the canker and dieback disease. Timely silvicultural operations are required in plantation of the species to reduce occurrence of disease on the trees. Use of clean and superior germplasm in plantation establishment will give the tree species an advantage over pests and diseases.

Since the species have gained popularity for plantation establishment periodic monitoring of established woodlots will be crucial to avert outbreaks over large areas in the event of a disease or pest attack. Optimum spacing in planting and prevention of fire outbreaks to minimize physical injury to the tree will help control attack by opportunistic pathogens. The occurrence of new species such as *Macrophoma theicola* and *Spencermartinsia viticola* requires further studies to identify other hosts of these species in the country.

Farmer outreach and training on the management of the indigenous *Melia volkensii* must be intensified on the use of pesticides after pruning and the rinsing of pruning knives and secateurs to prevent infection of the plantation.

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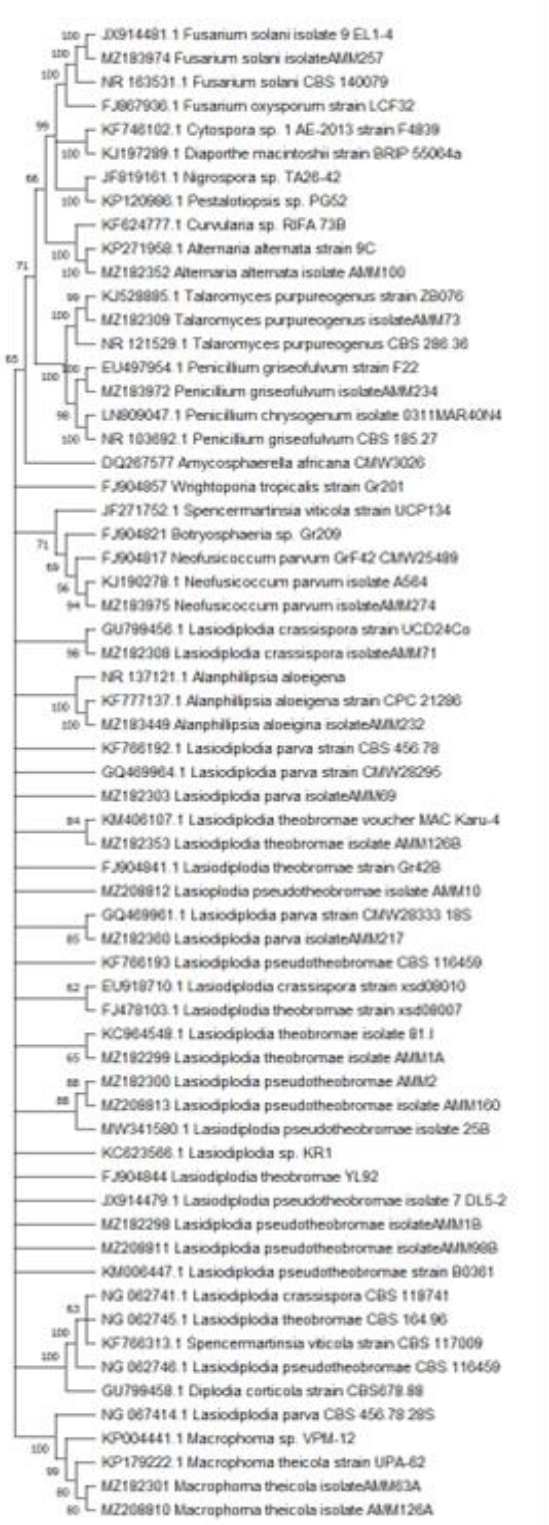
APPENDICES

APPENDIX I: COMPLETE DENDROGRAM OF DNA SEQUENCES FROM THE STUDY



APPENDIX II: SIMILARITY REPORT

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Department	Type here...
Acceptable Maximum Limit	Type here...
Submitted By	titustoo@uoeld.ac.ke
Paper Title	CHARACTERIZATION OF CANKER-CAUSING FUNGI ON Melia volkensii AND Azadirachta indica TREES IN THE DRYLANDS OF KENYA.
Similarity	4%
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Total Pages	136
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