CHARACTERIZATION OF FUNGAL LEAF SPOT DISEASE AND VARIETAL SUSCEPTIBILITY IN SWEET POTATO (*Ipomoea batatas* L.) GROWN IN PARTS OF WESTERN KENYA

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DECLARATION AND APPROVAL

Declaration

This thesis is my original work and has not been presented for a degree award in any other university.

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Approval

This thesis has been submitted for examination with our approval as the university supervisors.

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DEDICATION

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ABSTRACT

Increased human population has led to increased vulnerabilities to hunger and reduced sources of affordable food. Sweet potato (*Ipomoea batatas L.*) is a root vegetable with large, starchy, tuberous roots, consumed worldwide. It is a potential food security crop, with high nutritional and economic value. Production of sweet potatoes in Kenya is low due to disease constraints such as sweet potato leaf spot (SPLS). Fungal SPLS results in reduced tuber yield, since the pathogen attacks photosynthetic leaves, causing premature defoliation and senescence. Information on disease incidence, pathogen genetic variability and susceptibility of some locally cultivated varieties in western Kenya is not fully documented. A characterization and varietal susceptibility study of SPLS, presents an opportunity for increased production, improved food security and enhanced income. This study evaluated SPLS disease incidence, characterized the causative pathogens, assessed susceptibility of locally grown varieties to the infection and evaluated farmers' indigenous knowledge to sweet potato diseases. Infected leaf samples were obtained from farmers' fields in Kabondo-Kasipul and Rangwe Sub-Counties in Homa Bay County, and in Kajulu in Kisumu East Sub-County of Kisumu County where active cultivation of sweet potatoes is done. A multi-stage sampling design was used to identify exact sampling points at farmers' fields based on acreage of sweet potato grown. At each site, disease incidence and severity was evaluated in quadrats thrown on prescribed scales within the habitable space of diseased plants. Diseased leaf samples were collected and transferred to the Botany laboratory at Jaramogi Oginga Odinga University of Science and Technology, where pathogen isolation and characterization experiments were arranged in Completely Randomized Design with three replicates. Controlled pot-experiments were conducted in a green house to test the susceptibility of 1 farmer-held and 2 improved sweet potato varieties to one isolated SPLS pathogen (NF4 - Accession No. OK560339.1). The experiments were arranged in Randomized Complete Block Design with three replicates. A structured questionnaire was administered to 66 farmers to survey farmer's indigenous and perception of the disease. Data on disease incidence, severity and susceptibility were transformed into percentages and subjected to Analysis of Variance using General Linear Model and means separated using Least Significant Difference ($LSD_{0.05}$) on Scientific Analysis System (SAS) version 9.4. Morphological data was numerically transformed on MS Excel and hierarchical relationships determined using a dendogram. Genomic DNA was extracted from pure samples, and the Internal Transcribed Spacer (ITS) gene amplified by Polymerase Chain Reaction (PCR), followed by sequencing at Macrogen, UK. Molecular data were assembled and trimmed on CLC Main Workbench (CLC Bio, Version 6.8.3). Assembled sequences were transferred to MEGA Version 6 and aligned using CLUSTAL W. Data on farmer indigenous knowledge was analyzed using Chi-Square (χ^2) test on SPSS version 25. Results on disease incidence ranged from 11% to 30.38% at Kakelo and Kamollo villages respectively, while SPLS severity was significantly ($p<0.0001$) highest at Kokwanyo (28.37%) and lowest at Rapogi (15.27%). A total of 12 morphologically different fungal pathogens were isolated on Potato Dextrose Agar. Molecular identification of the fungi revealed *Fusarium chlamydosporum, Fusarium proliferatum* and *Fusarium verticillioides* as agents of SPLS infection. Variety 2 (Odinga) was more susceptible to the fungal pathogen NF4, with 33.18% leaf spots while variety 3 (Mugande) had the lowest susceptibility. Most farmers (90.91%) reported occurrence of sweet potato diseases on their farms, but more females were able to differentiate between the diseases. Integrated disease management approach should be adopted for improved sweet potato yields and the less susceptible Mugande variety be adopted by farmers. Farmer awareness programs on SPLS management is recommended. Policy makers should adopt the findings of this study for production of disease-free quality tubers for food, export and value addition to generate income.

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ABBREVIATIONS AND ACRONYMS

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of Variance
BLAST	Basic Local Alignment tool
CIP	Center of International Potato
CRD	Completely Randomized Design
DNA	Deoxyribonucleic Acid
EDTA	Ethylene diamine tetra acetic acid
ISSR	Inter Simple Sequence Repeat
ITS	Internal Transcribed Spacer
KALRO	Kenya Agricultural and Livestock Research Organization
MEGA	Molecular Evolutionary Genetics analysis
NACOSTI	National Commission for Science, Technology and Innovation
NCBI	National Center for Biotechnology Information
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
RCBD	Randomized Complete Block Design
RAPD	Random Amplification of Polymorphic Deoxyribonucleic acid
RFLP	Restriction fragment length polymorphism
SAS	Scientific Analysis System
SPLS	Sweet Potato Leaf Spot
SPSS	Statistical Package for Social Sciences
UV	Ultra Violet

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Sweet potato (*Ipomoea batatas L.*) is a vegetable crop with starchy, large, tuberous roots that is consumed worldwide (Epeju and Rukundo, 2018). It is the sixth most important food crop after rice, wheat, potatoes, maize and cassava, while in developing countries it is the fifth most important food crop (International Potato Centre, 2022). Sweet potato is a potential food security crop with significant nutritional value and global production (Glenna *et al.,* 2017; Epeju and Rukundo, 2018). The crop is cultivable in marginal lands, diverse soils and environmental conditions, and is rich in dietary energy, proteins, vitamins (A and C) and amino acids such as methionine and cysteine (Chandrasekara and Kumar, 2016). China is the world's largest producer and consumer of sweet potatoes producing over 517,900,000 tons in 2019 (Shahbandeh, 2021). Sweet potato production is underexploited in Africa where it is regarded as a poor man's crop, grown mainly under marginal conditions (Abong' *et al.*, 2016). In Eastern Africa, Ethiopia with 1.76 million metric tons and Uganda with 1.75 million metric tons are the top producers, while Kenya produces about 0.8 million metric tons (Shahbandeh, 2021).

Sweet potato is majorly cultivated by small scale farmers in western Kenya counties of Busia, Kakamega, Siaya, Kisumu, Homa Bay, Nyamira, Kisii and Migori (Makini *et al.,* 2018). Kenya has the potential to produce up to 38 tons/ha with average yield ranges of 20-50 tons/ha (Farmlink Kenya, 2017). However, production in Kenya is below 10 tons/ha due to several constraints, including pest and diseases (Makini *et al.*, 2018; Infonet-Biovision, 2020), which contribute up to 75 percent of sweet potato yield losses (Musembi

et al., 2015). The sole effect of infectious diseases contributes to about 11.9% yield loss (Echodu *et al.,* 2019). Low production is exacerbated by lack of access to sufficient planting material at the beginning of each planting season (Njeru *et al*., 2004; Gichuki and Hijmans, 2005). Lack of access to disease-free planting materials of adapted cultivars in Homa bay and Kisumu counties is a challenge that needs to be addressed, in order to achieve wider adoption of improved sweet potato cultivars (Mudege *et al*., 2020; Low *et al*., 2020).

Sweet potato diseases include charcoal rot caused by *Macrophomina phaseoli,* java black rot caused by *Diplodia tubericola,* sclerotial blight and circular spot caused by *Sclerotium rolfsii* Sacc., soil rot caused by *Streptomyces ipomoea*, and leaf diseases caused by *Phyllosticta batatas, Coleosporium ipomoeae, Alternaria* sp. and *Fusarium* sp. (Martin, 2002). Studies relating to sweet potato diseases in East Africa have been skewed towards viral infections (Wokorach *et al.,* 2019; Tugume *et al*., 2016; Buko *et al.,* 2020; Onditi *et al*., 2020) and *Alternaria* blight (Anginyah, 2001; Alajo, 2009). Little scientific attention has been given to assess the occurrence of SPLS in parts of Western Kenya.

Sweet potato leaf spot has been reported in China and Nigeria, where fungal species *Neopestalotiopsis ellipsospora* and *Stemphylium solani* were identified as causative agents (Maharachchikumbura *et al.*, 2016; Chai *et al*., 2014; Ilondu, 2013). Symptoms of SPLS include small, irregular or circular brown lesions on the margin or center of lower leaves and sometimes upper leaves (Chai *et al*., 2014). The spots enlarge gradually and join together to form grey, brown necrotic lesions with yellowish halos. Diseased leaves eventually become senesced and drop off (Maharachchikumbura *et al.*, 2016). Few reports of the infection are available in Africa, except in west African countries such as Nigeria

(Ilondu, 2013; Ekhuemelo and Mariagoretti, 2020). There is scanty information on the occurrence of SPLS in western Kenya, and so far, little work has been done to isolate and characterize the causative pathogen.

Plant pathogen characterization involves laboratory isolation and evaluation using morphological or molecular markers (Adhikari *et al*., 2020). The sole use of morphological traits is considered inefficient due to possible divergent morphologies within different species and convergent morphologies among closely related species (Taylor *et al*, 2000). Confirmatory molecular markers are therefore useful in pathogen species delimitation, to alleviate variations due to morphology. This study utilized internal-transcribed spacer (ITS) gene region of the DNA for analysis of SPLS pathogens. The ITS gene is a spacer DNA situated between the small-subunit ribosomal RNA (rRNA) and large-subunit rRNA genes in the chromosome (Schoch *et al*., 2012). The ITS region is composed of two highly variable spacers, ITS1 and ITS2, and the intercalary 5.8S gene (Herrera *et al*., 2009). The region has primer sites shared by all fungi, has an appropriate length for efficient amplification and sequencing, and possesses a high interspecific variation (Lindahl *et al*., 2013). Thus, its amplified fragments contain enough variation that is informative at the phylogenetic level (Gazis *et al*., 2011). Indeed, the ITS region was designated as the formal barcode for fungal species (Schoch *et al*., 2012), and was adopted for molecular characterization of fungal pathogens isolated from sweet potato leaves cultivated in western Kenya.

Molecular characterization of sweet potato fungal pathogens has been carried out in different studies globally. For instance, *Fusarium oxysporum* and *Fusarium solani* were characterized in Uruguay and United States respectively (Scruggs and Quesada, 2016;

Scattolini *et al*., 2020). In China, *Stemphylium solani* was implicated as the causative agent for SPLS (Chai *et al*., 2014), while in Nigeria, *Macrophomina phaseolina, Fusarium verticillioides and Alternaria tamarii* were associated with SPLS infection (Ekhuemelo *et al*., 2020). Earlier studies in Nigeria revealed the involvement of *Cochlobolus lunatus*, *Fusarium solani* and *Fusarium lateritium* in SPLS infection (Ilondu, 2013). In Uganda, based on morphological markers, *Alternaria alternata* was isolated from leaf spot infected sweet potato plants (Osiru *et al.*, 2007). Clearly, SPLS is caused by a wide range of fungal pathogens. Yet, little work has been done in Kenya to identify the pathogens which cause SPLS in the varieties grown in parts of western Kenya.

Different varieties of sweet potatoes are grown in Homa Bay and Kisumu Counties in western Kenya. *Kalamb Nyerere, Nyathi Odiewo, Nyakeya, Amina, Nyatonge, Kuny Kibuonjo* and *Odinga* are some of the local farmer-held varieties (Kivuva *et al*., 2014). Kemb 23, Kemb 10, SPK013, SPK004 and Mugande, developed by Kenya Agricultural and Livestock Research Organization (KALRO) in collaboration with International Potato Centre (ICP), are improved varieties that produce outstanding yields above the local varieties (Ruto, 2017). Although these farmer-held and improved varieties are actively grown in Western Kenya, little work has been done to assess their vulnerability to SPLS infection. Previous studies conducted have been skewed towards foliar and tuber diseases caused by viruses (Maina 2014; Maina *et al.*, 2017; Wanjala *et al.*, 2020) and fungal diseases such as *Alternaria* leaf blight (Tabu, 2000). Yet, identifying varieties which are tolerant or resistant to SPLS is an important prerequisite for integrated disease management. In order to increase sweet potato yield in areas where diseases are common, a study on susceptibility of available cultivars to a particular infection is necessary

(Nwankwo, 2015). Currently, there is scanty information on susceptibility of various sweet potato varieties grown in parts of western Kenya to fungal leaf spot pathogens.

Sweet potato farmers in western Kenya counties of Kisumu and Homa Bay utilize farming technologies which may be regarded as traditional or informal (Ochieng *et al*., 2017). Understanding the local farmer's indigenous knowledge and perception of sweet potato diseases is critical, as a pathway for infusion of modern techniques of integrated disease control (Echodu *et al.,* 2019). Local farmers have long been accustomed to indigenous agricultural practices in their farms, including detection and management of pests and diseases (Anyan, 2018). Although modernization of agriculture may result in increased yields, it seldom incorporate farmers' indigenous knowledge. Yet, farmers' knowledge of factors such as effect of environmental factors in disease progression, yield damage, identity of the causative pathogen and practiced control methods, may offer long term disease management solutions (Schreinemachers *et al*., 2015). Thus, a comprehensive understanding of farmers' indigenous knowledge may aid in effective disease management. However, information on indigenous knowledge and perception of farmers in western Kenya region regarding SPLS is still scanty.

This study sought to establish the incidence and severity of sweet potato leaf spot disease in parts of western Kenya, isolated and characterized the causative fungal agents, determined susceptibility of sweet potato varieties grown in the area to fungal leaf spot disease and assessed farmers' knowledge and perception of SPLS disease.

1.2 Statement of the Problem

Sweet potato is a highly nutritious starchy root vegetable and a major source of industrial starch. Kenya has potential to produce 20-50 tons/ha, but the actual production is below 10 tons/ha (Farmlink_Kenya, 2017). The low production has been attributed to devastating pests and diseases with the latter accounting for 11.9% of annual loss (Ochieng *et al.,* 2017). Sweet potato leaf spot is one of the diseases that has been implicated in low sweet potato production in Kenya. Indeed, a preliminary survey by Anginya *et al*., (2001) revealed *Alternaria* sp. as the fungi implicated in SPLS disease in Kenya. However, few studies have been conducted to identify the causative fungi for SPLS infection in Homa Bay and Kisumu counties.

Identification of the pathogen associated with crop loss is a significant step towards development of proper and precise disease management strategies. So far, few studies in Kenya have sought to identify the SPLS causative pathogens using morphological and molecular traits, which may affect effective disease management strategies. Challenges associated with inaccurate pathogen identification may result in non-targeted disease management and increased disease proliferation. Little information on SPLS fungi and its effects on production have resulted in continued yield loss, since previous studies in Kenya were skewed towards viral diseases (Buko *et al.,* 2020; Onditi *et al*., 2020) and *Alternaria* blight (Alajo, 2009).

Several improved and farmer-held sweet potato varieties are grown in Homa Bay and Kisumu Counties. The susceptibility of these varieties to the fungi that cause SPLS disease is yet to be determined. According to media press reports (The Star Newspapers, 19th May 2020), sweet potato farmers in parts of western Kenya complained of a rapid decline in

farm outputs and household incomes due to lower yields of sweet potatoes. This is because SPLS infection targets the photosynthetic area which contributes to lower yields by reducing transport of nutrients and products to storage roots, leading to low quality tubers with reduced marketability and palatability (Adam *et al*., 2015).

1.3 Justification of the Study

Assessment of the incidence and severity of any plant disease is an important strategy to its epidemic analyses as a foundation to its management. Thus, a diagnostic study of SPLS in western Kenya, presents an opportunity for increased production, improved food security and enhanced income. The data obtained from this study provided reliable estimates of the extent of disease occurrence within the targeted counties, and thereby informed strategic interventions for its control and management.

Scientific information regarding the genetic nature of the pathogen(s) which cause SPLS through morphological and molecular markers is vital to disease diagnosis, complementing the singular use of visible plant symptoms. Proper diagnosis of the causative pathogen of SPLS is an important pre-requisite to better prevention and management strategies. The prevalence of SPLS disease is known to vary with season, environmental conditions, and the varieties grown.

Selection of leaf spot-tolerant sweet potato varieties is important in identifying breeding materials suitable for improved productivity. The tolerant varieties were recommended to farmers to enhance quality tuber production for consumption and income.

Further, understanding of local farmers' knowledge and perception about SPLS infection is critical, since the livelihood of these farmers depend on their ability to make accurate agronomic assessment.

1.4 Significance of the Study

The outcome of this study is of benefit to sweet potato farmers in parts of western Kenya through determination of the incidence and severity of SPLS and recommendation of efficient disease management measures. Information on identity of the causative fungi is a significant step towards development of proper and precise management strategies. Suitable methods for loss reduction were prescribed for economic empowerment of farmers. Sweet potato varieties that are tolerant to SPLS were determined and recommended to farmers in the region and policy makers for production of quality tubers that can be exported and used in value addition to generate more income. The study also added to the body of knowledge on sweet potato production, and serves as a source of reference to farmers and scholars.

1.5 Objectives

1.5.1 Broad Objective

To characterize sweet potato leaf spot fungi, assess occurrence and varietal susceptibility to the infection and evaluate farmer's knowledge and perception of the disease in parts of western Kenya.

1.5.2 Specific Objectives

1. To assess the incidence and severity of SPLS infection in parts of western Kenya.
2. To characterize the fungal pathogens which cause SPLS in parts of western Kenya using morphological and molecular markers.
3. To assess the susceptibility of selected farmer-held and improved sweet potato varieties to leaf spot disease in parts of western Kenya.
4. To evaluate farmers' perception and indigenous knowledge of SPLS in parts of western Kenya.

1.6 Hypotheses

1. There is no significant difference in the incidence and severity of sweet potato leaf spot infections in parts of western Kenya.
2. Fungal species which cause SPLS in parts of western Kenya are not genetically diverse.
3. There is no significant variation in the resistance profiles of selected farmer-held and improved sweet potato varieties to leaf spot disease in parts of western Kenya.
4. Sweet potato farmers in parts of western Kenya do not have varying perceptions and knowledge of SPLS infection.

1.7 Scope and Limitations of the Study

The study involved isolation and characterization of sweet potato leaf spot fungi obtained from diseased sweet potato leaves in farmers' fields. It also determined varietal susceptibility of the three preferred sweet potatoes to the fungal leaf spot pathogen in the greenhouse. The effects of soil pH, soil type, climate and other sweet potato varieties on disease incidence and severity in the field was not considered in this study. The culture media used for isolation was Potato Dextrose Agar, which may have not supported the growth of all culturable fungi. Only sweet potato leaves were used in isolation of the fungi and not other plant parts like petiole, stem and roots. Greenhouse experiment used to establish varietal susceptibility did not allow accurate determination of the effect of the fungi on root growth due to the confining nature of small pots. Given that the research was done in only two sub-Counties in Homa Bay County and one Sub County in Kisumu County, it only covered a small segment of the sweet potato industry across the country.

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy, History and Agronomy of Sweet Potatoes

Sweet potato (*Ipomoea batatas* L.) is the world's sixth most important food crop, after rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), potato (*Solanum tuberosum* L.), maize (*Zea mays* L.) and cassava (*Manihot esculenta* L.) (Stathers *et al*., 2018). It is an underground tuber rich in starch, fibers, minerals such as potassium and manganese, vitamins B5, B6, C, E and an antioxidant called *beta* carotene which is very effective at raising blood levels of vitamin A, particularly in children (Megan, 2017). According to the Centre for Agriculture and Bioscience International (CABI), sweet potatoes belong to the Kingdom *Plantae*, Phylum *Spermatophyta*, Class *Dicotyledonae*, Order *Solanales*, Family *Convolvulaceae*, Genus *Ipomea* and Species; *Ipomoea batatas* (https://www.cabi.org). The crop originated from South and Central America about 5000 years ago, and in 1492, Christopher Columbus took the crop to Europe (Tai *et al*., 2019). Sweet potato was introduced into China in the late 16th century and spread through Asia, Latin America and Africa, during the 17th and 18th centuries (http://www. history-origin-sweet-potato.html).

Sweet potatoes are short cycle crops that mature after 3 to 4 months (FAO, 2020). This cycle allows for flexible planting and harvesting times in high rainfall regions or in drier areas, thus well suited to the double cropping seasons particularly in rain-fed systems (Mukhopadhyay *et al*., 2011). The crop has a high demand in local and national markets since it is cheap and contributes to a more stable food system, and a predictable source of income (Degebasa, 2019). Sweet potatoes ensure good groundcover, thus prevent soil erosion and smoothers weeds, ensure availability of surplus biomass from vines for animal feed and the possibility of using planting material from own fields (Afzal *et al*., 2021).

Therefore, the crop enhances the resilience of smallholder farmers that is frequently affected by low yields or crop failure of other staple crops due to bad weather or diseases (Motsa *et al*., 2015). Sweet potato is not a regionally or internationally traded crop hence not affected by food price fluctuations like most of the main cereals. It therefore provides affordable food during times of high food prices (Van Jaarsveld *et al*, 2005).

2.2 Sweet Potato Production in Kenya

In Kenya, sweet potato production is practiced in the Western, Central and Coastal areas of the country (CIP, 2018). Out of this, over 80% is grown in the Lake Victoria basin with Kakamega, Bungoma, Busia, Homa Bay and Kisii Counties having high acreages of this crop (Gruneberg *et al.*, 2004). This is because sweet potatoes can adapt to a wide range of environmental conditions and can be grown on marginal areas with poor soils of limited fertility and inadequate moisture (Ochieng *et al,* 2017). The production of sweet potatoes in Homa Bay County is greatly enhanced by conducive conditions including sandy loam soil, and an abundant almost continuous rainfall pattern. The crop is used for human consumption, livestock feed, and for industrial processes to make alcohol, starch and other products such as desserts and flour (Lebot, 2010, Hazra, 2010; Low *et al.*, 1997).

In Kenya, sweet potatoes are often considered "subsistence", "food security" or "famine relief" crop (Günter *et al*., 2010), or as an alternative food crop among households whose main staple food is maize (Low *et al*., 1997). Its importance becomes evident when there is shortage in maize supply due to shortfall in production or immediately before harvest (Shiferaw *et al*., 2011). In such cases it forms the main diet in many rural households. On the other hand, demand for sweet potatoes among the urban population is growing rapidly due to changing consumption patterns and population growth (Kilui, 2016). Therefore, the

importance of sweet potato in Kenya cannot be overemphasized, due to the potential that it holds for both producers as an income generating enterprise and consumers as a source of nutritious staple food.

2.3 Constraints to Sweet Potato Production

Sweet potato yields in western Kenya has been low due to several abiotic (drought, low rainfall and poor soils) and biotic (insects, pests and diseases) factors (Gibson *et al.*, 2002). The main production constraints include pests that accounts for 32.6%, drought (21.6%), diseases (11.9%) and lack of clean planting materials (6.8%) (Echodu *et al*., 2019). Poor access to quality vines of suitable varieties, drought/weather and poor agronomic practices affect yield, food availability and income for households (Low *et al*., 1997). There is also limited land area for cultivation as the human population increases, and this leads to proliferation of pests and diseases (Kansiime *et al*., 2015). Despite these challenges, there is a need to increase productivity and tackle these constraints, by ensuring access to good quality and disease free vines accompanied by improvements in plant nutrition and post-harvest management.

Most notably, sweet potato diseases have a large impact on its productivity, food security and income generation (Kivuva *et al*., 2014). For instance, viral diseases can lead to reduction of sweet potato yield by up to 98% (Mukasa *et al*., 2003). Fungal diseases are major biotic constraints to crop production (Clark *et al*., 2009). Pathogenic fungi cause plant diseases such as anthracnose, leaf spot, rust, wilt, blight, coils, scab, gall, canker, damping-off, root rot, mildew, and dieback (Farr and Rossman, 2013; Iqbal *et al*., 2018). Most pathogenic fungi remain dormant on living and dead plant tissues for a long time, until conditions are conducive for their proliferation (Jain *et al*, 2019). Fungal spores are

readily dispersed by wind, water, soil, insects, and other invertebrates hence they can infest an entire crop (Lazarovits *et al*., 2014). Therefore, fungal infections of crop plants require greater scientific attention, to determine the incidence and severity and identify the causative pathogen, as a prerequisite for integrated disease management.

2.4 Occurrence of Leaf Spot Disease of Sweet Potatoes

The SPLS has been reported in major sweet potato growing areas in China, with disease incidence of up to 70% (Chai *et al*., 2014). Several fungi have been reported to cause SPLS in parts of Nigeria and elsewhere in the world (Ilondu, 2013). Anginya (2001), reported *Alternaria* leaf spot in sweet potato grown in Kenya. However, there is limited information on the occurrence of SPLS as well as characterization of the associated fungi in parts of western Kenya. There is the need to identify these fungi and determine their pathogenicity on sweet potatoes, as well as determine susceptibility of sweet potato varieties grown in the area to the infection.

The SPLS disease manifestation is characterized by large, irregular, brown, necrotic lesions on the margin or in the centre of leaves (Chai *et al*., 2014). As the spots grow, some join together to cause a large scar, which reduces the photosynthetic area and consequently reduces yield (Ilondu, 2013). The lesions are common on mature leaves and may be surrounded by a yellow ‘halo’. Diseased leaves eventually become senesced and drop off (Chai *et al*., 2014). The infection is devastating to sweet potatoes, since it targets and destroys photosynthetic leaves, which reduces the amount of assimilates which enter the plant sinks (Ilondu, 2013). This results in low quality tubers with reduced marketability and palatability.

Several fungal species have been implicated in the cause of SPLS. In China, *Stemphylium solani* and *Neopestalotiopsis ellipsospora* were identified (Chai *et al*., 2014; Maharachchikumbura *et al.*, 2016); in America and Nigeria, *Cercospora bataticola* was isolated (Clark *et al*., 2015; Ilondu *et al*., 2010), while in Nigeria *Aspergillus flavus*, *Aspergillus tamarii*, *Macrophomina phaseolina* and *Fusarium verticillioides* were the main causes (Ekhuemelo and Nsobundu, 2020). In Kenya, *Alternaria bataticola* and *Alternaria alternata* have been implicated in the cause of *Alternaria* leaf spot, petiole and stem blight of sweet potatoes (Anginya *et al*., 2001). Despite this study indicating occurrence of SPLS in Kenya, no study had been conducted to identify the causative pathogen of SPLS infection in Homa Bay and Kisumu Counties.

2.5 Fungal Pathogens Implicated in Leaf Spot Infections

2.5.1 *Fusarium verticillioides*

Fusarium verticillioides is mainly pathogenic to maize where it causes ear rot, and is responsible for production of the toxin fumonisin in poorly dried maize (Fravel *et al*., 2003). However, the fungus has been isolated in many other plant species, such as the Kentucky blue grass, where it caused leaf blight (Lee *et al*., 2013); mango leaf spot (Guo *et al*., 2021) and in date palms leaf spot (Al-Nadabi *et al*., 2020). The infection is influenced by climate, temperature, humidity, insect infestation, pre- and postharvest handling (Fandohan *et al*., 2005). In most cases, *F. verticillioides* occurs at all developmental stages and may be symptomless. The pathogen is culturable on media and has a rapid growth rate (Leslie and Summerell, 2006). However, Ekhuemelo and Nsobondu (2020), reported it as one of the fungi inciting leaf spot disease on sweet potato plants in Nigeria.

2.5.2 *Fusarium oxysporum*

Fusarium oxysporum is a fungal species complex of different strains and consists of soil borne pathogens with more than 150 host-specific forms (Baaayen *et al*., 2000). The pathogen is notorious in plant infection causing several diseases including wilts, blights and spots (Nirmaladevi *et al*., 2016). Most of the strains are saprophytic and are able to grow and survive for long periods on organic matter and in the rhizosphere of many plant species (Fravel *et al*., 2003). In sweet potatoes, *F. oxysporum* has been isolated from reserve roots causing tuber rot during storage (Scattolini, 2020). Infected sweet potato plants can survive and produce storage roots that are infected. If such tubers are used as seed material, they transmit the fungus to the fresh sprouts which may wilt in plant beds (Hegda *et al*., 2012). Yield losses of up to 50 percent occur under warm weather and in dry soils, and most of the infected plants die within a few days after visible symptoms appear on the leaves and vines (Gunua, 2010; Okungbowa and Shittu, 2012). However, there is little information on *F. oxysporum* isolated from sweet potato in western Kenya, although wilting and leaf spot symptoms are relatively common (Low *et al*., 2013).

2.5.3 *Fusarium proliferatum*

Fusarium proliferatum is a mycotoxin producing fungus which is mostly seed-borne pathogen of grain crops such as maize, sorghum and pearl millet (Gaige *et al*., 2020; Vismer *et al*., 2019). The fungus has a worldwide distribution, causing a variety of diseases in economically important plants (Zakaria *et al*, 2016). Punja, (2020) identified *F. proliferatum* as the main cause of root and crown spot in *Cannabis* plants, while Beck *et al*., (2020) isolated the fungus from storage onions. In the *Cannabis* plant, the disease occurs at all stages of growth; from propagation, vegetative growth and flowering (Zamir, 2020). In sweet potatoes, few studies have reported its pathogenicity with da Silva and

Clark (2013) reporting that it causes root spot infection. There has been no study conducted in the sweet potato growing regions of western Kenya to determine the occurrence of *F. proliferatum.*

2.5.4 *Macrophomina phaseolina*

Macrophomina phaseolina is a soil-borne fungus that affects about 500 plant species in more than 100 families. It causes diseases such as stem and root rot, charcoal rot and seedling blight (Ghosh *et al*., 2018). Under high temperatures of between 30–35 °C and low soil moisture of below 60%, it causes substantial yield losses in crops such as soybean and sorghum, impacting incomes of farmers (Kaur *et al*., 2012). Infection of sweet potato by *Macrophomina phaseolina* is accompanied by the occurrence of *Fusarium* spp. Stokes *et al*. (2013) isolated ten *Fusarium species* from Mississippi sweet potatoes in conjunction with *Macrophomina phaseolina*, six species were pathogenic and included *Fusarium oxysporum* and *F. solani* isolates found to be in 70% of the samples isolated from field grown tissue. *Macrophomina phaseolina* causing disease of sweet potato was found to be prevalent and aggressive in Mississippi (Arancibia *et al*., 2013, Burdine, 2008). Ekhuemelo and Nsobondu (2020), also reported it as one of the fungi inciting leaf spot disease on sweet potato plants in Nigeria. However, *Macrophomina phaseolina* is yet to be identified as a pathogen of sweet potatoes in Kenya.

2.5.5 *Trichoderma harzianum*

*Trichoderm*a spp. are fungi that are present in nearly all soils and other diverse habitats (Waghunde *et al*., 2016). In the soil, *Trichoderma harzianum* proliferation is favored by high levels of plant roots, which they colonize readily. Some strains are highly rhizosphere competent, with a capacity to colonize and grow on roots as they develop. Due to this trait,

Trichoderma harzianum has been used as a biocontrol agent (Nusaibah *et al*., 2019). The most strongly rhizosphere competent strains are added to soil or seeds, and once they come into contact with roots, they colonize the root surface or cortex, depending on the strain (Puyam, 2016). However, some species such as *Trichoderma afroharzianum* have been implicated in ear rot infections of maize (Munkvold and White, 2016; Wise *et al*., 2016; Pfordt *et al*., 2020). Little work has been done in Kenya to isolate and characterize *T. haziarnum* pathogen from infected sweet potatoes.

2.6 Effect of Leaf Spot Disease to Sweet Potato Production

Sweet potato is one of the most important food crops in tropical and subtropical countries where both the roots and tender shoots are used as nutrients (FAOSTAT, 2013). In Kenya, sweet potato is widely grown for food security, nutrition, processing and commercialization (Atakos *et al*., 2020). Sweet potato leaves are the main source of assimilate for dry matter production and increase (Chai *et al*., 2014). Since leaf spot causing fungal pathogen are favored by wet, humid conditions with temperature ranges of 25-30^{o}C (Arora and Khurana, 2004), there is a potential for rapid spread in western Kenya, and thereby threaten sweet potato production.

Leaf spot infection reduces the photosynthetic area of the plant and consequently, impair the amount of assimilate that goes into the sink (Bergh *et al*., 2012). The physiological changes that occur in plants under pathogenic invasion include: increased respiration rates and reduced photosynthesis leading to possible yield losses (Lopes and Berger, 2001). Diseased leaves senesce and drop off and severe infection may result in total defoliation and hence crop failure (Alabi and Waliyar, 2004). This reduces the quality and quantity of the leaves used as vegetable for man and fodder for animals (Amienyo and Ataga, 2008). Notably, most of the leaf spot causing fungi are capable of killing the host partially or

totally by direct destruction of the tissues and also by systemic dispersal of the toxic substances far beyond the original region of infection (Arora and Khurana, 2004).

2.7 Management of Sweet Potato Leaf Spot Disease

Sweet potato leaf spot infection becomes visible about one week after infection (Straat *et al*., 2014). The spots first occur on adaxial leaf surfaces and later on the abaxial leaf surfaces as they perforate the leaf lamina (Straat *et al*., 2014). The spots may be few and small but enlarge over time while others merge into blotches (Mattihalli *et al*., 2018). The main control measure for sweet potato fungal leaf spot diseases is to keep the foliage as dry as possible, removal and disposing of spotted leaves on plants that have fallen, and application of fungicides one week after first disease sign to stop its spread from vines (Jain *et al*, 2019).

2.8 Epidemiology of Fungal Sweet Potato Leaf Spot Disease

Pathogenic fungi are responsible for over 30% of all crop diseases (Jain *et al*., 2019). Fungal leaf spot is among the emerging sweet potato fungal diseases. Upon infection by a fungus, leaf spots become visible about 1 week after they first appear on the adaxial leaf surfaces, and later on the abaxial leaf surface ultimately perforating the lamina (Straat *et al*., 2014). The infection becomes visible as white to grayish-white patches on the edges and enclosed by reddish-brown, brownish, or yellowish margins (Sarsaiya *et al*., 2019). Fungal lesions first occur on the younger leaves as few small spots that enlarge over time. Others coalesce or merge into blotches (Mattihali *et al*., 2018). The blotches create holes on the leaves, which may vary in diameter and outline shape or may be identical to the initial spots (Mattihali *et al*., 2018). Spores of the causative fungi are dispersed by water droplets, personnel working with wet infected plants, mites and insects. The spores may

germinate in water or inside the natural leaf openings such as hydathodes, lenticels and stomata (Camo *et al*., 2019). In parts of western Kenya the causative agent is unknown hence the need for this study that determined the fungi.

2.9 Characterization of Fungal Pathogens that Cause Sweet Potato Leaf Spot

Characterization of pathogenic fungal species based on morphological traits is one of the criteria for its identification (Lin *et al*., 2018). Pathogenic fungal species have several morphological characteristics that help in distinct identification. Key microscopic features of identification include variation in shapes and sizes of macro and microconidia (Raghu *et al*., 2016), and chlamydospores which ensure the survival of the pathogen in soil (El Kichaoui *et al*., 2017). Macroscopic identification is usually based on hyphal colony cultural traits and the pigmentations they produce when cultured on media (Leslie *et al*., 2008). However, the sole use of morphological identification is inefficient due to overlapping morphology among closely related species, such as conidia dimension and colony cultural characteristics (Lievens *et al*., 2008). Molecular markers are therefore useful in species delimitation to alleviate morphological variations. This involves molecular analysis through nucleotide sequencing from conserved gene regions such as Internal Transcribed Spacer (ITS) region (Singha *et al*., 2016). Sequence information using ITS region has been immensely used in phylogeny and taxonomy of fungal species since it reveals variation between the species (Schoch *et al*., 2012).

This study characterized the sweet potato leaf spot pathogenic fungi morphologically by culturing to determine the morphological traits, and genetically by employing the ITS region as a marker for gene analysis since it has primer sites that are shared by all fungi, it is of appropriate length for efficient amplification and sequencing, and it has high

interspecific variation but low intraspecific variation (Lindahl *et al*., 2013). The ITS region is composed of two highly variable spacers, ITS1 and ITS2, and the intercalary 5.8S gene. This rDNA operon occurs in multiple copies in genomes, providing up to 100 times more DNA template from the same starting material than for single-copy genes (Herrera *et al*., 2009). Its amplified fragments contain enough variation that is informative at the phylogenetic level, hence the ITS region that does not code for ribosome components is highly variable (Gazis *et al*., 2011), and closely related species can be differentiated based on their difference in DNA sequences using ITS region (Schoch *et al*., 2012). Since there is a dearth of information on the fungi causing sweet potato leaf spot in parts of western Kenya, this study genetically characterized fungal species from sweet potato leaves to establish the actual causative pathogens in parts of western Kenya.

2.10 Management of Fungal Leaf Spot and Resistance Profiles of Sweet Potato Varieties

Adequate plant disease management is a prerequisite for stable and profitable crop production for ascertained food security. Plant fungal diseases such as SPLS is a major problem in the cultivation of sweet potatoes. There is no effective and complete control method against the disease, and the control of fungal diseases remains difficult in subsistence cropping systems (Rukarwa *et al*., 2010). Both chemical and biological control methods are not effective, however several strategies such as cultural practices, phytosanitary measures, control of vectors and deployment of genetic resistance to prevent or limit the extent of damage have been recommended (Maule *et al*., 2007; van den Bosch *et al*., 2007). Some of the cultural practices that effectively contribute to the control of the disease include destroying all sweet potato crop residue soon after harvesting; planting resistant or tolerant sweet potato varieties, planting clean and fungi-free vines, good

sanitation, practice crop rotation and treating seed roots with an appropriate fungicide prior to planting (Gibson *et al*., 2000).

Attempts to eradicate the SPLS has been unsuccessful since the pathogenic fungi is soil-borne and asexual thus infect many plant species around the world (Yang *et al.,* 2018). Alternative techniques to reduce the damage caused by this pathogen requires screening of cultivars to select the best resistant variety and avoid occurrence of the disease (Joshi *et al*., 2012). Therefore, effective control of this disease can be achieved by introducing high levels of resistance into new and local cultivars. In parts of western Kenya, sweet potato is grown primarily as a subsistence crop, hence chemical control is not widely practiced. Frequent planting with pathogen- free stock is not a lasting solution, as warm climates lead to a high re-infection rate.

Developing cultivars resistant to diseases is a viable option that makes both ecological and economic sense. Resistance breeding depends on understanding of the range of the different strains of the pathogen that exist in the area of production. However, there is currently little information on strains of fungal spp. causing SPLS infection in parts of western Kenya. Breeding for fungi resistant sweet potato cultivars has been recommended as the long-term solution to sustainably control SPLS and other fungal diseases (Domola *et al*., 2008). Lack of resistant, high yielding and locally adapted varieties have limited farmers to susceptible varieties or landraces (Gibson *et al*., 2000). The most effective strategy for long term control of fungal diseases is the use of disease tolerant and high yielding sweet potato varieties (Fraile *et al*., 2011). Breakdown of resistance by different strains or highly virulent fungi can make the resistance redundant (Miano *et al*., 2008; Kreuze *et al*., 2009). For instance, resistant cultivars developed in China and other parts of

the world might be of little value in other environments due to presence of different fungal strains. Therefore, the use of local germplasm in breeding for SPLS resistant varieties is of importance than heavily depending on exotic introductions (Gasura *et al*., 2010). Therefore, there is a need to identify and use local germplasm in breeding for SPLS resistant varieties. This study conducted a controlled susceptibility test using selected sweet potato varieties grown in the study area to determine their resistance to SPLS, and recommended to farmers such varieties for improved productivity.

2.11 Farmers Perception and Knowledge of Sweet Potato Leaf Spot Disease

Sweet potato remains a vital crop in overcoming food insecurity for the fast growing population in sub-Saharan Africa because of its high yield in short growing seasons of rain-fed systems (Low *et al*., 2017). Smallholder farmers in parts of western Kenya value sweet potato because it grows in a variety of climates with few inputs and it is drought resistant (Kivuva *et al*., 2014). Sweet potato is grown as a subsistence crop by the majority of farmers on pieces of land of less than 0.4 ha (Adam *et al*., 2015). This has consequences in production levels and food security since relatively small areas of land are being utilized for cultivation of sweet potato (Echodu *et al.,* 2019). Therefore, these smallholder families risk having insufficient food and income if the crop is devoured by diseases such as SPLS. Thus, the need to ascertain farmers' local knowledge

Understanding of farmers' knowledge related to perceptions of crop diseases and their management is also essential for the development of management strategies that could be supported by government and non-governmental organizations, which cater for farmers' needs and have a high probability of being adopted by the farmers (Adam *et al*., 2015). This is because increase in sweet potato productivity is largely dependent on farmers'

knowledge of the type of causative agent responsible for decreased yield and the appropriate disease control measures to mitigate the problem (Ebregt *et al*., 2004).

There has been increasing interest in incorporating farmers' indigenous knowledge into research and development programmes for finding workable solutions to agricultural problems (Isin and Yildirim, 2007; Obopile *et al*., 2008). This is because efforts to improve disease control and management measures for sweet potatoes are likely to be hampered if farmers' knowledge of the diseases and practices of handling are not known and taken into account appropriately. Despite the established critical role of farmers' knowledge in the control and mitigation of pests and diseases, very few studies have focused on SPLS in parts of western Kenya. Yet, according to Heong *et al*., (2002), control measures for pests and diseases are more robust if farmers' knowledge, perception and practices are taken into consideration. This study established the farmer's knowledge and perception on SPLS infection in parts of western Kenya where the crop is grown in relatively large scale for subsistence and commercial purposes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Homa bay and Kisumu Counties in western Kenya. Homa Bay County covers an area of 4,267.1 km^2 and is located in south western Kenya along Lake Victoria shoreline (Fig 1). It borders Kisumu and Siaya counties to the north, Kisii and Nyamira counties to the east and Migori County to the south. The county is divided into 8 sub counties namely: Homa Bay town, Karachuonyo, Ndhiwa, Suba, Kasipul, Kabondo-Kasipul, Rangwe and Mbita. Homa Bay County has semi-arid climatic conditions with daily temperatures ranging between 26°C during the coldest months (April to November) and 34°C during the hottest months (January to March) (Jaetzold *et al*., 2007). The county receives between 250 mm and 1200 mm of rainfall annually, with the average annual rainfall estimated at 1,100 mm. It has two rainy seasons; long rains between March to May and short rains between September and November (Jaetzold *et al*., 2007). The County is divided into two agro-ecological zones: Upper Midlands that receive adequate rainfall thus support crops such as maize, beans and sugarcane, and the Lower Midlands that receive low amount of rainfall and crops such as millet, sorghum and sweet potatoes are grown. The study was specifically carried out in Kabondo-Kasipul and Rangwe Sub Counties (Fig3.1).

Kabondo-Kasipul Sub County is divided into twenty-one sub-locations that is: Kakng'utu West, Kakng'utu East, Lower Kodhoch West, Upper Kodhoch West, Kakumu, Miriu, Kowidi, Kodhoch East, Kodumo East, Kodumo West, Rongo/Pala, Kasewe A, Kasewe B, Kilusi, Kokwanyo West, Kokwanyo East, Kakelo Kamroth, Kakelo Dudi, Kojwach Kamiyoro, Kojwach Kamuga and Kojwach Kawere. In 2014, the Department of

Agriculture in Rachuonyo South District commissioned a survey on the performance of agriculture-based projects in the district and reported that there was a lot of economic potential in the sweet potato projects in Kabondo Kasipul Sub County (Homa bay County Government, 2016).

Rangwe Sub-County is divided into sixteen sub locations: Kaura, Kanam, Kamenya, Kowili, Korayo, Kotieno, Kanyanjwa, Kamagawi, Genga, Kajulu, Koyolo, Kanyaruanda, Gongo, Komenya, Kokoko and Kanyiriema. The two sub counties receive adequate rainfall for sweet potato production unlike the other sub-counties.

Kisumu County covers an area of 2085.9 km^2. It neighbors Siaya County to the West, Vihiga to the North, Nandi County to the North East, Kericho County to the East, Nyamira County to the South and Homa Bay County to the South West. The county has a shoreline on Lake Victoria, occupying northern, western and a part of the southern shores of the Winam Gulf. It has 7 sub counties namely; Kisumu East, Kisumu West, Kisumu Central, Seme, Nyando, Nyakach and Muhoroni. Since it is situated on the equator, it has a hot and humid year-round climate with little annual rainfall of approximately 1,200 mm. The average temperature is 29°C and humidity is high in the morning between 80-90% but falls to 40-50% on a hot day (Jaetzold *et al*., 2007). Kisumu has two rainy seasons from March through June, and November through December. Average rainfall is in the range of 258 to 816 mm per month (Jaetzold *et al*., 2007). Kisumu East Sub-County was selected as study site because of active cultivation of sweet potatoes by farmers in East and West Kajulu. (Fig3.1).

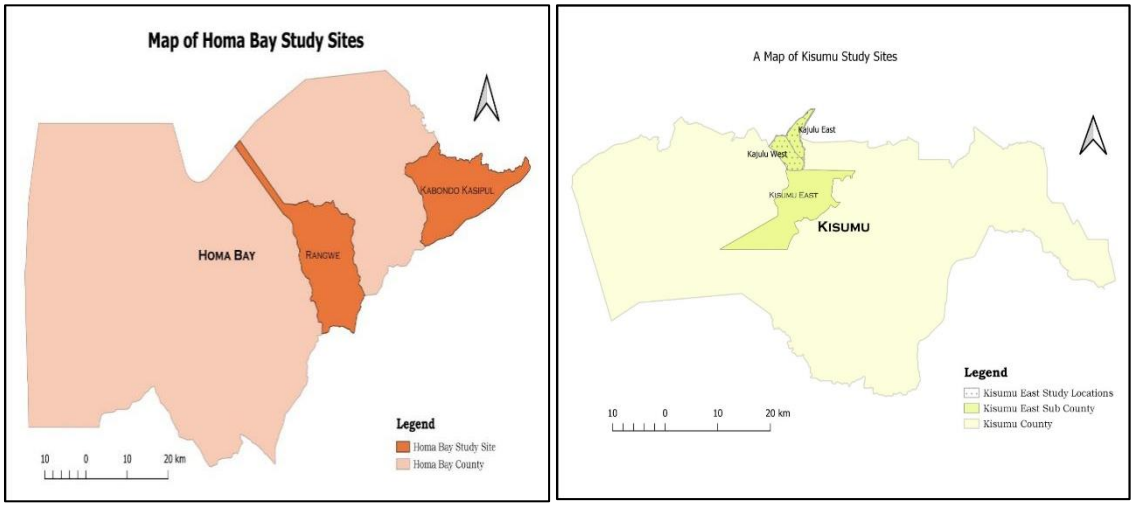

Figure 3.1. A map of Homa-Bay & Kisumu Counties showing sampling Sub-Counties: ,Kabondo-Kasipul, Rangwe & Kisumu East

3.2 Sampling and Experimental Designs

3.2.1 Multi-Stage Sampling Design for Study Site Selection

As a result of the expansive nature of the study area, it was impossible to sample every farm. A multi-stage sampling design was used to select representative villages and farms in each of the sub-counties described in Section 3.1. The sub-counties were initially divided into clusters based on locations with the largest acreage of sweet potato farms. The locations were assigned numbers, and individual sampling villages identified purposively based on the highest acreage of sweet potato farms. A 4×4 m micro plot was established along diagonal transects at equidistance of 5 m for assessment of SPLS occurrence. This provided the population of sweet potatoes needed to obtain a good sample size for the study. In Rangwe Sub-County, the farms surveyed were at Kamollo, Nyakwadha, Nyawita, Asumbi, Kabor and Gem vilages. In Kabondo-Kasipul Sub-County, the farms surveyed were at Kakelo, Kojwach, Kokwanyo, Oriang -1, Kakngutu and Kakumu. In Kisumu East Sub-County, the farms were at Ongadi, Obwolo, Wathorego, Oriang-2, Okok and Rapogi.

3.2.2 Experimental Design

Laboratory experiments on isolation and characterization of the SPLS fungi were arranged in Completely Randomized Design due to the controlled conditions. Experimental treatments included samples of diseased sweet potato leaves, according to the site of collection. Green-house controlled pot experiments involving susceptibility screening of selected sweet potato varieties against leaf spot fungi was done using factorial experiment laid out in randomized complete block design (RCBD). There were two factors, factor A, sweet potato variety at four levels (Variety 1 = Spk004, Variety 2 = Odinga, Variety 3 = Mugande and the control) and factor B, one leaf spot fungal isolate (*Fusarium* sp). The experiment was replicated three times to reduce variability in the results, to increase the significance of the result and the confidence level with which conclusions were drawn. Un-inoculated sweet potato plant for each treatment served as control for the experiment.

3.2.3 Ecological Survey and Sample Size Determination

A farmer-participatory approach involving ecological survey design was used to study farmer perception and indigenous knowledge of SPLS. A questionnaire (Appendix III) was developed which was appropriate for determining demographic factors of production such as source of vines, variety grown, frequency of growing sweet potatoes, farming system used, method of planting, inorganic fertilizer use, knowledge and history of the disease. A total of 66 farmers were selected according to Cochran (1963) based on their history of sweet potato cultivation. Since the population of sweet potato farmers was large, a target population of 180 farmers was purposively selected and used to determine sample size. The Cochran formula was used to obtain an ideal sample size of sweet potato farmers with a desired level of precision and confidence level.

The sample size was calculated as shown below;

$$n = \frac{z^2 pq}{(e)^2}$$

where n = sample size, Z^2 = z score (95%), e = level of precision (0.05%), p = estimated proportion presenting population (0.072) and q = 1-p.

$$n = \frac{1.96^2 \times 0.072 \times 0.928}{(0.05)^2} = 103$$

Reduced sample size:

$$n = \frac{n_o}{1 + \frac{(n_o - 1)}{N}}$$

$$\frac{103}{1 + \frac{(103-1)}{180}} = 66 \text{ farmers}$$

3.3 Data Collection Procedures

3.3.1 Evaluation of Disease Incidence and Severity

Disease incidence and severity was evaluated on farmers' fields with habitable space of diseased plants according to Sseruwagi *et al*. (2004). A quadrat of 1 m × 1 m was randomly thrown in 4 m x 4 m micro plots established in the sampled farms, and disease incidence assessed diagonally along the quadrat. The average of diseased plants was obtained against the total number of plants. Disease incidence was calculated using the formula below derived from Mahantesh *et al.* (2017).

$$\% \text{ Disease incidence} = \frac{\text{Number of infected potatoes}}{\text{Total no. of potatoes assessed}} x100$$

Disease severity assessment was based on visual estimation of percentage leaf area with spot on 20 randomly selected plants per quadrat using a scale of 1 – 5 (CIP, 2017) as shown in Section 3.4. The percent severity index was calculated using the formula below:

$$\text{Percent Disease Severity} = \frac{\text{Number of individual ratings}}{\text{Total number of leaves assessed}} x \frac{100}{\text{Maximum scale}}$$

3.3.2 Characterization of Sweet Potato Leaf Spot Fungi

3.3.2.1 Collection of Diseased Leaf Samples from Farmers Fields

A sterile scalpel was used to cut symptomatic leaf spot parts, wrapped in sterile zip lock bags and labelled using a permanent marker pen. Sample labels consisted of sampling site and collection number. Samples were then packed in cooler boxes and taken to the Botany Research Laboratory at Jaramogi Oginga Odinga University of Science and Technology (JOOUST) for fungal isolation and characterization.

3.3.2.2 Laboratory Isolation of Fungal Cultures

Fungal cultures of the leaf spot pathogen were isolated from diseased leaf samples immediately upon arrival at the laboratory following procedure used by Ogolla *et al.* (2021) for fungal isolation. Cleaned glass petri-dishes were sterilized at 160^{o}C in a hot air oven (Model Memmert, UNB400) for 2 hours. About 25 g of commercial Potato Dextrose Agar (PDA) medium was put in media bottles and sterilized at 121^{o}C and pressure of 15 psi for 15 minutes in an autoclave (Model X280A). The media was then transferred to a water bath for media tempering at 50^{o}C for 30 minutes after which 25 mg/l of Ampiclox was incorporated to inhibit bacteria contaminants. Thin sections of diseased leaves of about 5 mm^2 were cut using a sterile scalpel and surface sterilized with 0.5 % sodium hypochlorite solution for 10 seconds, then rinsed in a series of sterile distilled water. The excised leaves were transferred to a sterilized blotting paper in a petri dish to remove the excess water. Single blot dried leaf-sections were aseptically plated at the centre of PDA media for growth and the plates sealed with parafilm. Inoculated plates were put in an incubator (Memmert TYP INB200) set at 28 ^{o}C for 10 days, according to Emitaro *et al.,* (2017).

After fungal growth, pure isolates were obtained by serial sub-culturing in fresh PDA media. The colonies emerging from sweet potato leaf host were picked with sterile fine tip needle and sub cultured on fresh PDA plates devoid of antibiotics to obtain pure cultures which were identified based on their morphological traits and kept in the fridge at 4 ^{o}C for subsequent characterization.

3.3.3 Morphological Characterization of Fungal Pathogens

Fungal colony morphology was studied macroscopically and microscopically. The isolates were identified according to Ogolla *et al*. (2018) based on cultural characteristics such as top and bottom surface color, rate of growth and colony diameter; and microscopically by observation of conidia type and shape, hyphae septation, and conidiophore structures using stereomicroscope (DE/Axio Imager A1) after staining with lactophenol cotton blue stain and examining under x40 to reveal morphology of spores and mycelium.

3.3.4 Molecular Characterization of Fungal Pathogens

3.3.4.1 Genomic DNA Extraction

Approximately 4 mg of pure mycelium was collected by scraping from the colony surface and used to extract DNA using ZYMO Quick-DNA Fungal/Bacterial Microprep Kit (Catalogue D6007 - Zymo Research, South Africa) according to the manufacturers' specifications (Appendix VII). The **c**oncentration and quality of the DNA was assessed using gel electrophoresis of 1 µl of extracted DNA on a 0.7% (w/v) agarose gel according to Kuhn *et al*., (2018) (Appendix VIII). Quantification of the extracted DNA was done using UV spectrophotometer (Model UV-1800 Shmadzu) followed by normalization to 50 ng/µl.

3.3.4.2 PCR Amplification and Sequencing

Polymerase chain reaction (PCR) amplification of the ITS gene region was performed in a programmable Master cycler thermo-cycler (C1000-BioRad, USA) using the PCR conditions described by Vellinga *et al*., (2003). The primers used for the ITS region were adopted from White *et al*. (1990) and included; forward primer ITS5 (5'-TCCTCCGCTTATTGATATGC-3') and the reverse primer ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and the amplification protocols were as described by Schoch (2012). The PCR was performed in a 25-µl reaction mixture comprising of 2.5 µl input of fungal DNA, of double-distilled water (16.25 µl), 2.5 µl of USB 10× buffer with $MgCl_2$ (10 mM; 1 µl of USB $MgCl_2$ (25 mM), 0.5 µl of deoxynucleoside triphosphate (dNTP) mixture (10 mM each; 0.25 µl AmpliTaq polymerase (5 U/µl; Applied Biosystems, Carlsbad, CA), 0.5 µl of Hotstart-IT DNA Fidelitaq polymerase; Affymetrix), and 1 µl (5 µM) of each primer (Usyk *et al*, 2017). The PCR amplification procedure was initiated at 94°C (4 min), 35 cycles of denaturation at 94°C (1 min), annealing at 56°C (1 min) and extension at 72°C (1 min), with final extension at 72°C (10 min). The amplified products were separated by horizontal gel electrophoresis on 1.5 % (w/v) agarose gel on 0.5X TBE at 70V for 60 min., using nuclease free water as negative control and *Trichoderma* sp. as positive control. The products were visualized under UV after staining with 2 µl GelRed™ (Thermo Scientific) and a total of 10 PCR amplicons were purified using a Thermo Scientific®GeneJET Purification Kit (EU, Lithuania) according to the manufacturer's specifications. The PCR amplicons were evaluated on gel electrophoresis and submitted to Macrogen Europe BV, in England for purification and Sanger sequencing using the same ITS primers (https://www.macrogen-europe.com/services). Sanger

sequencing involve three main steps: DNA sequence for chain terminator PCR, size separation by gel electrophoresis and gel analysis and determination of DNA sequence.

3.3.4.3 Gel Electrophoresis

The chain-terminated oligonucleotides were separated by size through gel electrophoresis (Appendix VIII), where SPLS fungal DNA samples were loaded into one end of the gel matrix, and an electric current of 40 V was applied. Since DNA is negatively charged, oligonucleotides were pulled towards the positive electrode. Because all DNA fragments have the same charge per unit of mass, the speed at which the oligonucleotides moved was determined only by size. The smaller a fragment was, the faster it moved through the gel due to less friction it experienced while moving. The oligonucleotides were thus arranged from the smallest to the largest, reading the gel from bottom to top.

3.3.4.4 Determination of Phylogenetic Relationship of the Fungal Isolates

Ten fungal isolates were successfully sequenced and assembled using CLC Main Workbench (CLC Bio, Version 6.8.3). Assembled multiple sequences of about 500bp were transferred to MEGA Version 6 software and aligned using CLUSTAL W method according to Tamura *et al*., (2014). Sequences with greater than 99% similarity were retrieved for phylogenetic analysis. Evolutionary histories and diversity of the isolates were determined using the Neighbour- Joining method and distances computed using Jukes-Cantor model (Tamura *et al*., 2011). A bootstrap test (1000 replicates) was used to cluster associated taxa and replicate trees with above 50% likelihoods indicated on the branches.

3.3.3 Susceptibility of Sweet Potato Cultivars to Sweet Potato Leaf Spot Disease

Three sweet potato varieties (Odinga which is farmer-held; Mugande and SPK-004 which are improved varieties by KALRO) were planted in pots at JOOUST green house and

arranged in a randomized complete block design to form three rows containing each variety according to Scattolini *et al*. (2020). The growth buckets measuring 25 cm in diameter and 40 cm in length were filled with 7 kg of sterilized soil and planted with vines from sweet potato varieties commonly grown in the study area. The environmental conditions of the greenhouse were; air temperature (30-35^{o}C); relative humidity (50-60 %); and photosynthetic radiation (400-700 nm). Isolate NF4 (Ac.No. OK560339.1) identified as *Fusarium proliferatum* was used to inoculate sweet potatoes in the green house. The isolate above was chosen due to its rapid growing characteristics. A suspension of the fungal isolate was prepared by serial dilution using sterile water and conidia of $1x10^3$ conidia/ml and sprayed to the potted sweet potatoes at 5 true leaves after planting.

Disease score according to the rating on Table 3.1 below was taken weekly after inoculation for 5 weeks, to determine the variety that was more susceptible using percent disease index (PDI) according to Ekhuemelo and Nsobondu, (2020).

Table 3.1 Disease rating scores

Disease Rating	% of leaf surface with spots	Intensity of symptoms
1	0	Free from disease
2	15	Slight symptoms
3	16-30	Moderate symptoms
4	31-45	Slightly severe Symptoms
5	>50	Severe Symptoms

3.3.4 Farmers' Perception and Indigenous Knowledge

A survey of farmers' perception and knowledge of SPLS was conducted using a structured questionnaire as proposed by Neindow *et al*. (2018) (Appendix II1). The questionnaire was administered to 22 farmers per Sub-County through interviews giving a total of 66 farmers. The questionnaire was used to obtain information on the sweet potato variety grown during study period, age of sweet potato at the time of study, source of vines as planting materials, history of growing sweet potatoes, frequency of growing sweet potatoes, sweet potato farming system, sweet potato varieties tolerant to the disease /susceptible to the disease, use of inorganic fertilizers, planting method used, ability to differentiate sweet potato diseases in the farms, age of sweet potato most infected by the SPLS disease and history of the disease.

3.3.4.1 Validation of Survey Instruments

Content validity of the questionnaire used for data collection was done by the supervisors to ensure that the questionnaire gathered the intended information accurately. Consistency of the questionnaires was assessed by administering the questionnaire to different respondents during the pilot study and verifying by the supervisors.

3.3.4.2 Pilot Study

A pilot study was conducted in 15 sweet potato farms in Kibos, Kisumu East Sub County, with similar sweet potato acreages as the study sites, and brown, irregular spots on the sweet potato leaves. From the pilot study, the questions in the questionnaire were too many and administering it would take a much longer time, thus it was adjusted to suit the study period. Some of the questions were too difficult for the respondents, thus were moderated. It also predicted the response patterns of respondents thus suitable changes were made to make the questionnaire valid. The pilot study helped with planning and execution of the actual study in terms of logistics. It ascertained that the research was feasible with in the duration planned and at affordable cost.

3.3.5 Ethical Issues and Considerations

An ethical review permit was sought from Jaramogi Oginga Odinga University of Science and Technology Ethics and Review Committee (Appendix II). During the interviews, farmers were assured that the data collected was principally for research purposes. Data on susceptibility test was conducted within controlled green-house conditions at JOOUST, with a high degree of accuracy following the procedures outlined in the proposal. Consultation on production process, source of vines, suitable cultivars, planting methods, weeding, harvesting, pest and diseases of sweet potatoes was also sought from experts in sweet potato production in KALRO Kisumu in order to obtain reliable and valid information.

3.3.6 Data Analysis

Disease incidence and severity data was presented in percentages and subjected to one-way Analysis of Variance (ANOVA) using General Linear Model and significant means separated

using least significant difference ($p \leq 0.05$) in Scientific Analysis System (SAS) version 9.4. Morphological data was tabulated and analyzed by hierarchical cluster analysis while molecular data were assembled and trimmed on CLC Main Workbench (CLC Bio, Version 6.8.3). Assembled sequences were transferred to MEGA Version 6 and aligned using CLUSTAL W according to Tamura *et al*., (2014). Individual consensus sequences of the ITS gene regions were used to evaluate closely related sequences at the NCBI GenBank (www.ncbi.blast.lnm.nih.gov) using Basic Local Alignment Search Tool (BLAST Query).

Data obtained from the green-house on susceptibility test was presented in percentages and subjected to a one-way ANOVA using Statistical Analysis Software (SAS) version 9.4. Significantly different means were separated using least significant difference ($p \leq 0.05$).

The Statistical Package for Social Scientists (SPSS) version 25 was used to analyze association of responses between male and female farmers using the Chi-Square (χ^2) test.

CHAPTER FOUR

RESULTS

4.1. Incidence and Severity of Sweet Potato Leaf Spot

4.1.1 Incidence of Sweet Potato Leaf Spot

Sweet potato leaf spot incidence was significantly different ($p \leq 0.0001$) in some villages such as Kamollo, Nyakwadha and kakelo respectively (Table 4.1; Appendix IV). The highest incidence of 30.38% was observed in Kamollo village while the lowest incidence (11%) was observed at Kakelo village. The overall mean percentage incidence was 16.45%.

Table 4.1 Incidence of sweet potato leaf spot in different villages of Rangwe, Kisumu-East and Kabondo -Kasipul Sub Counties

Village	**Sub-county**	**Incidence (%)**
Kamollo	Rangwe	30.38[a]
Gem	Rangwe	26.64[ab]
Nyakwadha	Rangwe	22.08[bc]
Wathorego	Kisumu-East	21.59[bcd]
Nyawita	Rangwe	20.49[cb]
Okok	Kisumu-East	18.82[ecd]
Kabor	Rangwe	18.77[ecd]
Oriang_2	Kisumu East	17.43[edf]
Asumbi	Rangwe	15.38[efg]
Kakngutu	Kabondo-Kasipul	15.22[efg]
Ongadi	Kisumu-East	14.63[fgh]
Obwolo	Kisumu- East	14.22[fgh]
Rapogi	Kisumu-East	12.97[ghi]
Kokwanyo	Kabondo-Kasipul	12.81[ghi]
Oriang_1	Kabondo-Kasipul	12.10[hi]
Kakumu	Kabondo-Kasipul	11.96[hi]
Kojwach	Kabondo-Kasipul	11.73[hi]
Kakelo	Kabondo-Kasipul	11.00[i]
Mean		**16.45**
LSD (p≤0.05)		**1.248**
CV (%)		**20.261**

[a]Means followed by the same letters are not significantly different (p≤0.05).

Overall Sub-County SPLS incidence resulted in a significant difference ($p \leq 0.0001$; Appendix IV) between Rangwe, Kabondo -Kasipul and Kisumu-East (Fig 4.1). Rangwe Sub-County had the highest percentage incidence of 21.35%, followed by Kisumu-East Sub-County at 16.9% while lowest incidence of 12.45% was observed in Kabondo-Kasipul Sub-County

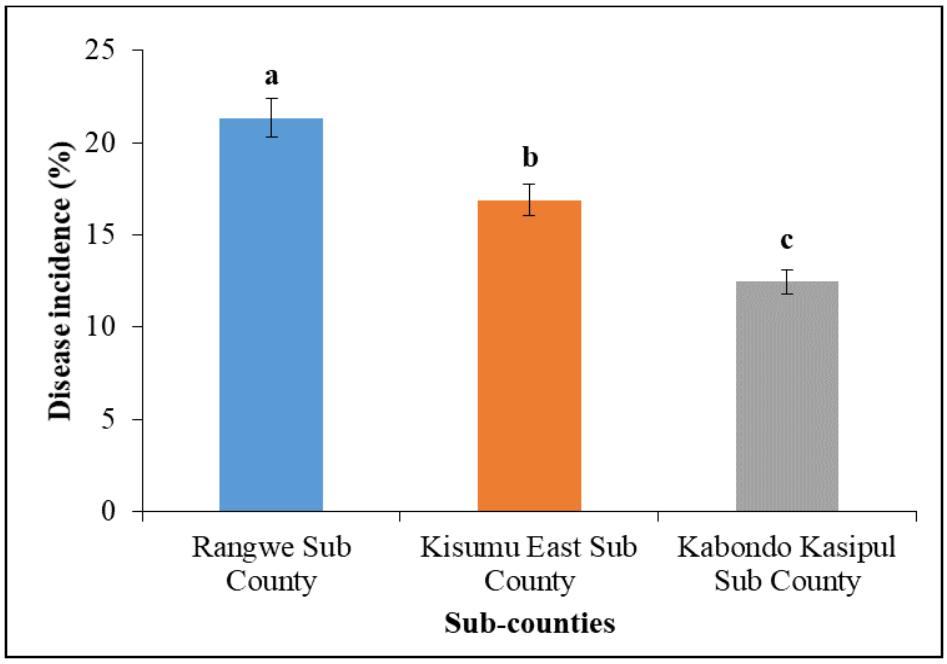

Figure 4.1 Sweet Potato leaf spot incidences in Rangwe, Kisumu East and Kabondo-Kasipul Sub-Counties

4.1.2 Severity of Sweet Potato Leaf Spot

Sweet potato leaf spot severity was significantly different ($p \leq 0.0001$; Appendix V) in some villages such as Kokwanyo, Kakelo, Asumbi, Kabor and Rapogi respectively. A severity index of 28.37% was observed at Kokwanyo village in Kabondo Kasipul Sub-County, which was significantly the highest, compared to the least severity of 15.27% observed in Rapogi village within Kisumu East Sub-County. SPLS severity had an overall mean percentage of 22.794% and a co-efficient variation of 20.02% (Table 4.2).

Table 4.2 Severity of sweet potato leaf spot in different villages of Rangwe, Kisumu-East and Kabondo-Kasipul Sub Counties

Name of Villages	Sub-County	Severity (%)
Kokwanyo	Kabondo-Kasipul	28.37[a]
Kojwach	Kabondo-Kasipul	26.62[a]
Kakelo	Kabondo-Kasipul	23.99[b]
Nyawita	Rangwe	20.49[b]
Okok	Kisumu-East	23.94[b]
Asumbi	Rangwe	23.67[bc]
Obwolo	Kisumu-East	23.51[bc]
Oriang-1	Kabondo-Kasipul	23.17[bc]
Wathorego	Kisumu-East	23.02[bc]
Kakumu	Kabondo-Kasipul	22.99 [bc]
Nyakwadha	Rangwe	22.93[bc]
Gem	Rangwe	22.75[bc]
Oriang_2	Kisumu-East	22.66[bc]
Kabor	Rangwe	22.13[c]
Kamollo	Rangwe	22.10[c]
Kakngutu	Kabondo-Kasipul	18.12[d]
Ongadi	Kisumu-East	17.42[d]
Rapogi	Kisumu-East	15.27[e]
Mean		22.794
LSD ($p \leq 0.05$)		1.766
CV (%)		20.02

[a]Means followed by same letters are not significantly different at ($p \leq 0.05$).

Kabondo-Kasipul and Kisumu-East Sub-Counties did not report significant variation ($p \geq 0.05$; Appendix V) in severity of SPLS. Their percent disease severity was 23.96% and 21.49% respectively (Fig. 4.2). However, the disease severity in Rangwe Sub-County was not significantly different ($p \geq 0.05$) with Kabondo-Kasipul and Kisumu East sub-counties. The least significant difference observed for sub counties was 1.766.

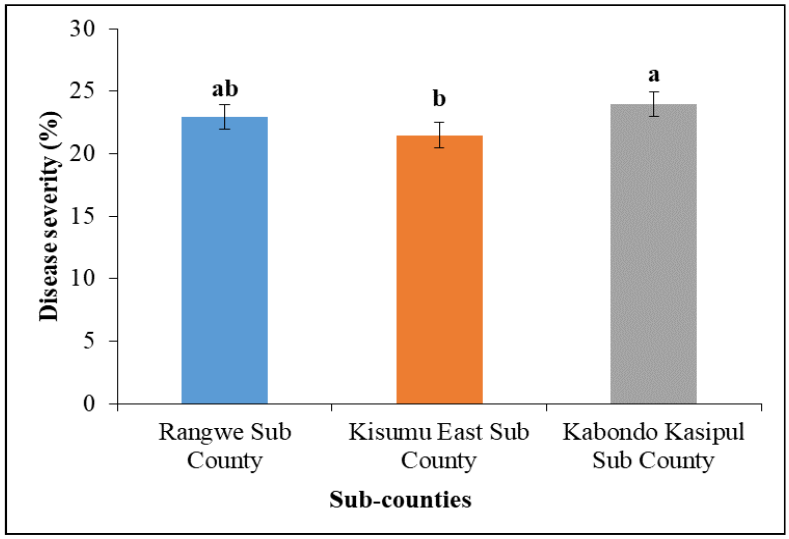

Figure 4.2 Sweet potato leaf spot severity in Rangwe, Kisumu-East and Kabondo-Kasipul Sub Counties. [a]Means followed by same letters are not significantly different at ($p \leq 0.05$).

4.2 Characterization of Sweet Potato Leaf Spot Fungal Pathogens

4.2.1 Morphological Characterization

A total of 12 fungi were isolated in potato dextrose agar (PDA) media after a series of sub culturing to obtain pure isolates. Isolation was based on colour of mycelia, hyphae septation and conidiophore characteristics and were given accession numbers based on source and researcher's name and species abbreviation as NF1-NF12 (Norah Fungi 1-12). Macroscopic and microscopic characteristics of each isolate are illustrated on Table 4.14. Isolate NF1 from Kabondo-Kasipul Sub County had similar phenotypic characteristics as NF12 from Rangwe Sub County. Isolates NF2 and NF6 from Kabondo-Kasipul and Kisumu-East respectively also had similar characteristics. Isolates NF3 and NF4 from Kabondo-Kasipul Sub County were similar to NF5 and NF8 of Kisumu-East Sub County. Isolates NF7 and NF11 each had unique characteristics.

Table 4.3 Phenotypic characteristics of sweet potato leaf spot fungi from Rangwe, Kisumu-East and Kabondo-Kasipul Sub Counties.

Source	Isolate code	Macroscopic characteristics	Microscopic characteristics	Tentative Identification
Kabondo-Kasipul	NF-1	Colonies grew rapidly, reaching a diameter of 5 cm after 10 days. Were dark brown in color initially and later greyish on PDA medium. Front plate grey, back brown.	Septate hyphae with microsclerotia within the hyphae or engrossed in PDA medium. Hyphae formed black colored microsclerotia, which were smooth and oblong.	*Microphomina spp*
	NF-2	Colonies grew slowly, reaching 3 cm after 10 days, formed white floccose mycelium which later turned greyish violet. Front plate on PDA white, back is grey.	Hyphae septate, macroconidia long, slender and straight, while microconidia were short and produced from monophialides.	*Fusarium spp*
	NF-3	Colonies produced sparse white aerial mycelia of 2-3 cm in 10 days. Front plate color on PDA medium is white or purple, while the reverse is cream white.	Hyaline septate hyphae, macroconidia abundant produced on cream sporodochia, Microconidia were oval in shape.	*Fusarium spp*
	NF-4	Colonies produced sparse white aerial mycelia of 2-3 cm in 10 days. Front plate color on PDA medium is white or purple, while the reverse is cream white.	Hyaline septate hyphae, macroconidia abundant produced on cream sporodochia, Microconidia were oval in shape.	*Fusarium spp*
Kisumu East	NF-5	Colonies produced sparse white aerial mycelia of 2-3 cm in 10 days. Front plate color on PDA medium is white or purple, while the reverse is cream white.	Hyaline septate hyphae, macroconidia abundant produced on cream sporodochia, Microconidia were oval in shape.	*Fusarium spp*
	NF-6	Colonies grew slowly, reaching 3 cm after 10 days, formed white floccose mycelium which later turned greyish violet. Front plate on PDA white, back is grey	Hyphae septate, macroconidia long, slender and straight, while microconidia were short and produced from monophialides.	*Fusarium spp*

Source	Isolate code	Macroscopic characteristics	Microscopic characteristics	Tentative Identification
	NF-7	Grew slowly to produce cream and woolly colonies of 2-3 cm in 10 days. Front plate color on PDA medium is cream or yellow, while the reverse is brown.	Hyaline septate hyphae, conidiophores, phialides, macroconidia and microconidia present. Macroconidia have a distinct basal foot.	*Fusarium spp*
	NF-8	Colonies produced sparse white aerial mycelia of 2-3 cm in 10 days. Front plate color on PDA medium is white or purple, while the reverse is cream white.	Hyaline septate hyphae, macroconidia abundant produced on cream sporodochia, Microconidia were oval in shape.	*Fusarium spp*
Rangwe	NF-9	Produced white colonies with a diameter of 3 cm in 10 days, and a light purple pigment. Front plate color on PDA medium is white, while the reverse is purplish.	Hyphae septate, macroconidia long, slender and straight, while microconidia were short and produced from monophialides.	*Fusarium spp*
	NF-10	Produced white colonies with a diameter of 3 cm in 10 days, and a light purple pigment. Front plate color on PDA medium is white, while the reverse is purplish.	Hyaline septate hyphae, slender macroconidia and microconidia present. Macroconidia have a distinct basal foot.	*Fusarium spp*
	NF-11	Colonies grew rapidly reaching a diameter of 4-5 cm in 10 days. On PDA medium, colonies initially white and wooly, later compact in time. Front plate mycelia white in color, later yellow-green with concentric rings.	Septate hyaline hyphae, conidiophores present aseptate and pyramidal. Phialides solitary or in clusters (mono or multi-phialides).	*Trichoderma spp*
	NF-12	Colonies grew rapidly, reaching a diameter of 5 cm after 10 days. Were dark brown in color initially and later greyish on PDA medium. Front plate grey, back brown.	Septate hyphae with microsclerotia within the hyphae or engrossed in PDA medium. Hyphae formed black colored microsclerotia, which were smooth and oblong.	*Microphomina spp*

Some colonies NF1, NF11 and NF12 grew rapidly reaching a diameter of 5cm after 10 days while others grew slowly reaching a diameter of 2-3cm within the growth period. Colonies surface color ranged from grey, white, cream- yellow and brown while reverse side color was brown, grey, cream white, purple and yellow-green as shown in sample plates 4.1.

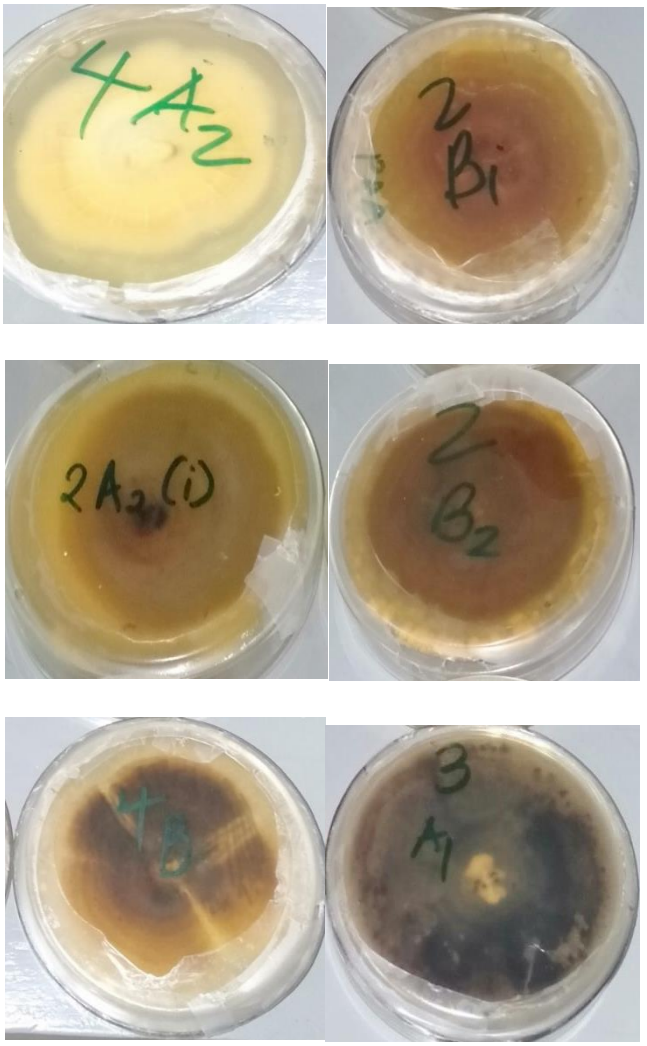

Plate 4.1 Selected cultures of SPLS fungal mycelia on PDA plates

Upon staining with lactophenol cotton blue stain and observation through the stereomicroscope, all isolates had septate hyaline hyphae, macroconidia and microconidia of different shapes also formed on conidiophores as shown in plates 4.2 and 4.3 respectively.

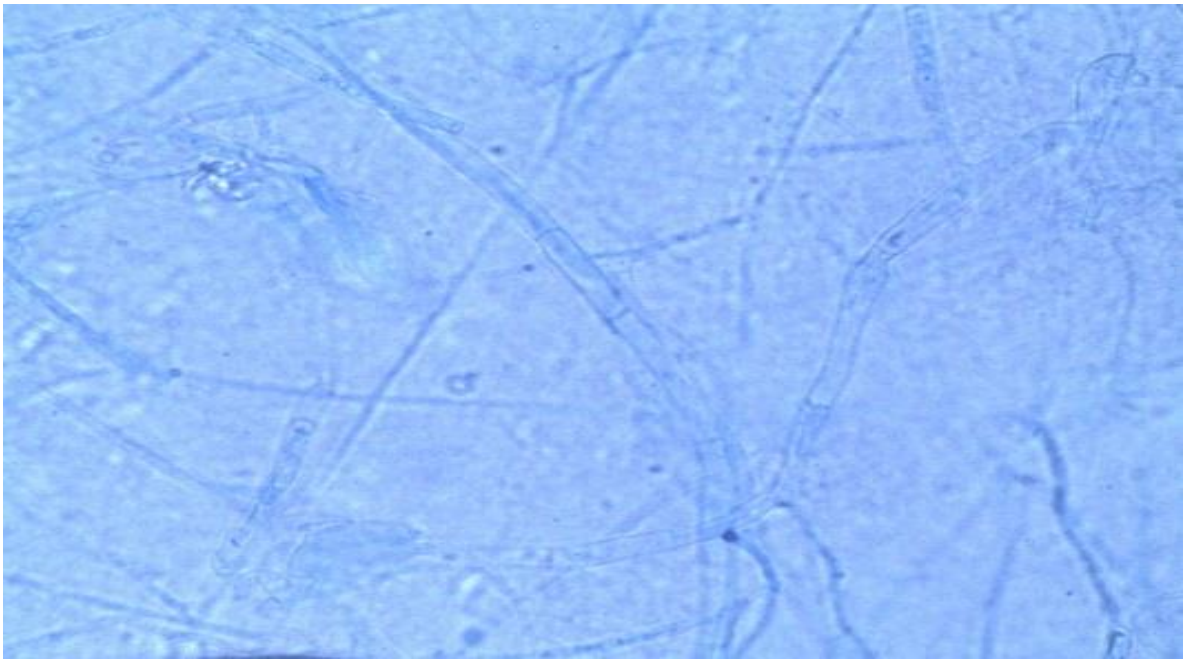

Plate 4.2 Microscopic analysis of SPLS fungal mycelia showing hyphae septation (MGX400)

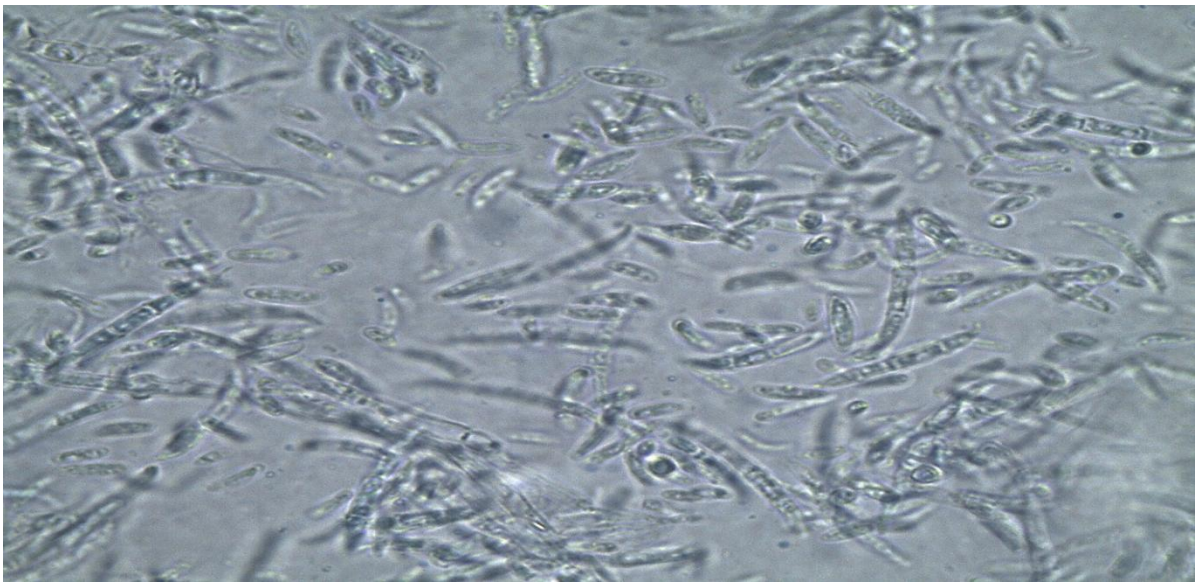

Plate 4.3 Mycelia of SPLS fungal isolate showing micro and macroconidia (MG X400)

When the phenotypic characteristics of the fungal isolates were analyzed by hierarchical cluster analysis, a dendogram was formed (Figure 4.3). The isolates separated into two main clusters A and B at 75% similarity level. Cluster A had only one fungal isolate - NF11 whose colony was white and woolly, but later turned yellow-green with concentric rings on the front surface. The hyphae were septate and hyaline, conidiophores were aseptate and pyramidal

with phialides. It was tentatively identified as *Trichoderma* sp. Cluster B consisted of many isolates with two sub clusters, 1 and 2. Sub cluster 1comprised of NF1 and NF12 that grew rapidly on PDA media and formed grey mycelia on the front surface. The hyphae were septate with microsclerotia which were smooth and oblong. The isolates were tentatively identified as *Macrophomina* sp. Sub cluster 2 consisted of two further groups, I and II. Cluster B, sub cluster 2, group I consisted of NF7 that was cream in color and had macroconidia with distinct basal foot. It was tentatively identified as *Fusarium* sp. Cluster B, sub cluster 2, group II comprised of many mixed isolates, NF2, NF3, NF4, NF5, NF6, NF8, NF9 and NF10. The colonies were mainly white, had hyaline septate hyphae but with different conidia and conidiophore characteristics. They were tentatively described as *Fusarium* sp. The similarity matrix showed that the values for different sweet potato leaf spot isolates ranged from 0.00 to 6.251 (Table 4.4).

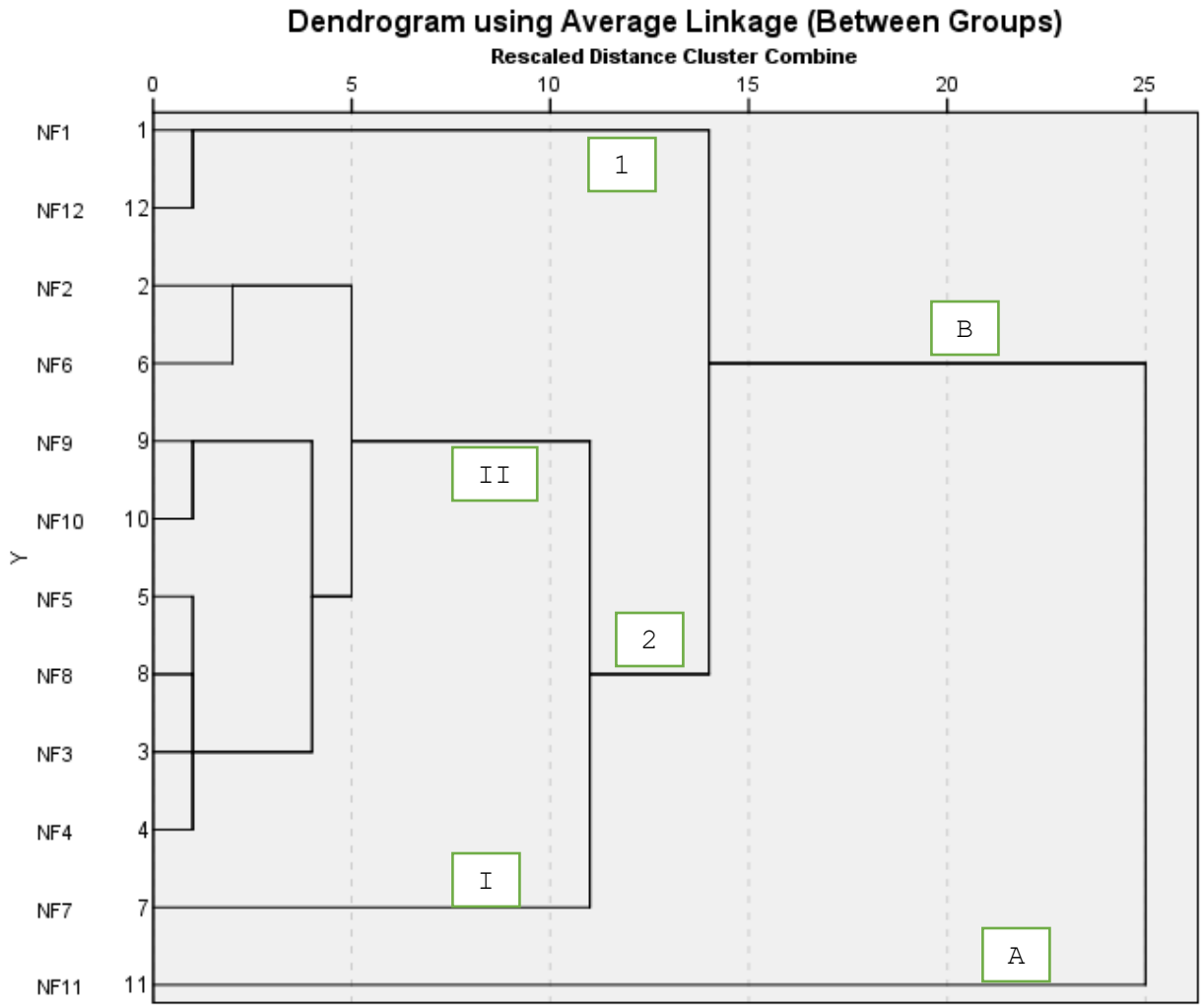

Figure 4.3 A cluster dendrogram showing microscopic and macroscopic characteristics of the sweet potato leaf spot fungal pathogens isolated from Kabondo-Kasipul, Rangwe and Kisumu East-Sub Counties

Table 4.4. Phenotypic Similarity Matrix for the sweet potato leaf spot fungal pathogen isolated from Kabondo-Kasipul, Rangwe and Kisumu-East Sub Counties

	NF1	NF2	NF3	NF4	NF5	NF6	NF7	NF8	NF9	NF10	NF11	NF12
NF1	0.00											
NF2	3.980	0.00										
NF3	4.803	2.533	0.00									
NF4	4.803	2.533	0.00	0.00								
NF5	4.803	2.533	0.00	0.00	0.00							
NF6	4.236	0.838	2.391	2.391	2.391	0.00						
NF7	6.251	3.506	3.291	3.291	3.291	3.292	0.00					
NF8	4.803	2.533	0.000	0.000	0.000	2.391	2.391	0.00				
NF9	4.468	2.031	1.514	1.514	1.514	2.197	2.858	1.514	0.00			
NF10	5.796	3.235	2.263	2.263	2.263	2.891	3.098	2.263	2.518	0.00		
NF11	6.162	5.460	4.864	4.864	4.864	5.129	5.054	4.864	5.069	3.474	0.00	
NF12	0.000	3.980	4.803	4.803	4.803	4.236	6.251	4.803	4.468	5.796	6.162	0.00

4.2.2 Molecular Characterization

The molecular weight of genomic DNA of SPLS fungal isolates was confirmed on 0.7% (w/v) agarose gel stained with SYBR green. It was approximately 500 base pairs (bp). Out of the 12 SPLS fungal isolates, 10 were successfully amplified as shown in plate 4.4 below.

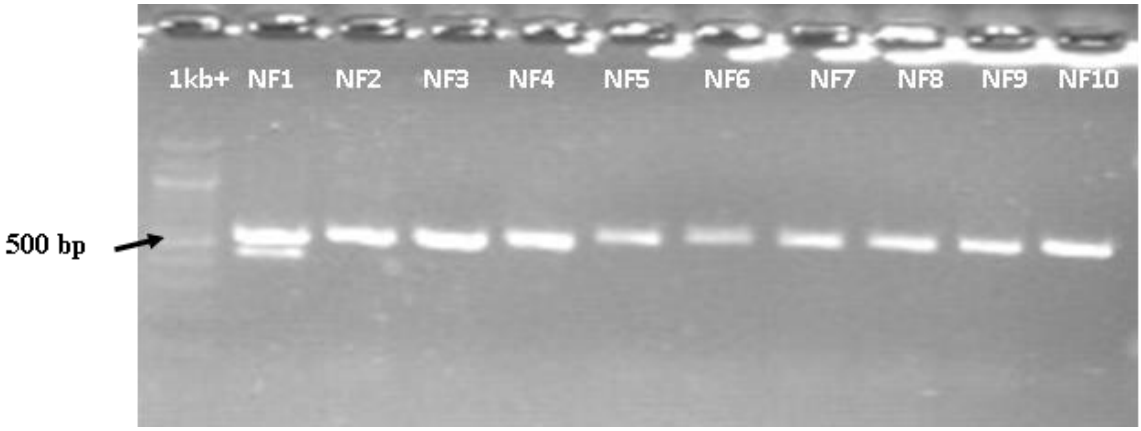

Plate 4.4 Agarose gel image of PCR products of ITS genes of ten fungal species isolated from infected sweet potatoes leaves from parts of western Kenya.

Sequencing of the ITS rDNA gene for fungi indicated that sequences of the SPLS isolates were different in the arrangement of the nucleotide base pairs. Fungal sequences submitted to National Centre of Biotechnology Information (NCBI) for similarity search showed >99.00% match identity to those already deposited in the Genebank. The isolate sequences were deposited to NCBI GeneBank and given accession numbers ranging from OK560336.1 to OK560345.1 (Table 4.5). Based on Basic local Alignment Search Tool (BLAST) searches, fungal isolates were found to belong to Genus *Fusarium* with three distinct species: *Fusarium chlamydosporum, Fusarium proliferatum* and *Fusarium verticillioides.*

Table 4.5 Identities of 10 fungal isolates obtained from infected sweet potato leaves in three western Kenya Sub-Counties, based on the ITS gene sequences and closely related sequences obtained from NCBI

Isolate Identity	NCBI Acc. No. (this study)	Site of isolation	Similarity (%)	Closely Related Species	Similar NCBI Acc. No.	Country of Origin
NF-1	OK560336.1	Kabondo-Kasipul	99.54	*Fusarium chlamydosporium*	KT824431.1	India
NF-2	OK560337.1	Kabondo-Kasipul	100	*Fusarium proliferatum*	ON365805.1	Mexico
NF-3	OK560338.1	Kabondo-Kasipul	100	*Fusarium proliferatum*	ON365805.1	Mexico
NF-4	OK560339.1	Kabondo-Kasipul	100	*Fusarium proliferatum*	ON329681.1	Mexico
NF-5	OK560340.1	Kisumu East	100	*Fusarium proliferatum*	ON365805.1	Mexico
NF-6	OK560341.1	Kisumu East	100	*Fusarium proliferatum*	ON365805.1	Mexico
NF-7	OK560342.1	Kisumu East	100	*Fusarium proliferatum*	ON365805.1	Mexico
NF-8	OK560343.1	Kisumu East	100	*Fusarium proliferatum*	ON365805.1	Mexico
NF-9	OK560344.1	Rangwe	100	*Fusarium verticillioides*	ON003558.1	India
NF-10	OK560345.1	Rangwe	99.81	*Fusarium verticillioides*.	MW928602.1	China

NOTE: BLASTN analysis of the sequences indicated that the sequences from the present study matched with the reliable reference sequences from Gen Bank. BLASTN search of all the sequences of the isolates showed above 99% sequence similarity to the internal-transcribed spacer (ITS) genes.

4.2.2.1 Evolutionary relationships of SPLS fungal isolates

The evolutionary profile of the SPLS fungal isolates conducted in Molecular Evolutionary Genetic Analysis (MEGA 6) software clustered the isolates into two clades A and B (Figure 4.5). Clade A comprised of 9 isolates with accession numbers OK560337 to OK560345. Two isolates in clade A clustered with *Fusarium verticillioides* at 100% bootstrap support value, that is NF9 (Acc No.OK560344) and NF10 (Acc No. OK560345). Seven isolates in clade A clustered with *Fusarium proliferatum* at 100% bootstrap support value that is OK560337 to OK560343.

Clade B clustered at 99% bootstrap support value with *Fusarium chlamydosporum* in which isolate NF1 (Acc No. OK560336) of this study occurred. The ITS gene for *Auricularia delicata* was used as a rooter.

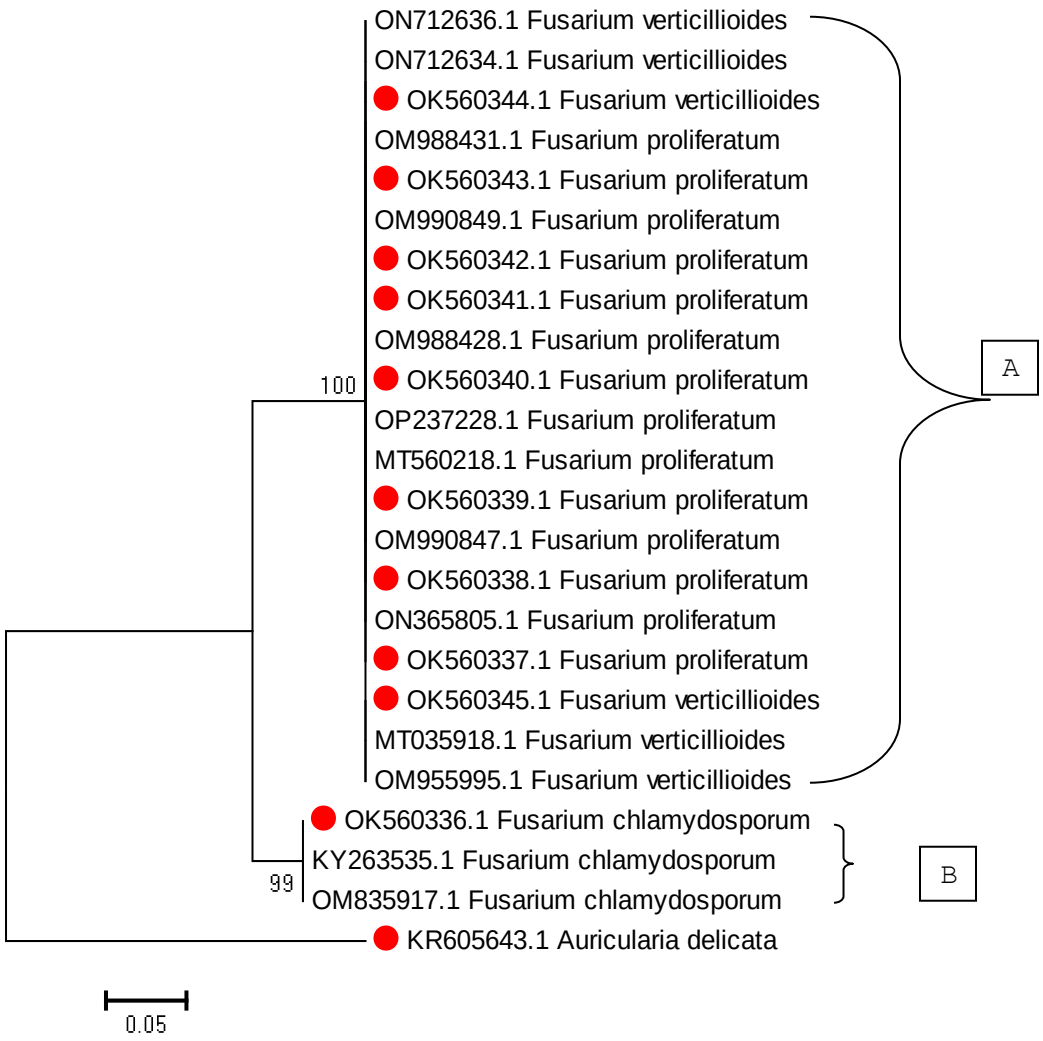

Figure 4.4 A Neighbour-Joining phylogenetic tree built from the 10 ITS sequences (with dots) of fungal pathogens which cause sweet potato leaf spot and related sequences (100% similarity) retrieved from the NCBI portal (https://blast.ncbi.nlm.nih.gov). **NOTE:** The evolutionary history was inferred using the Neighbor-Joining method (Saitou *et al*., 1987), and percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) shown next to the branches (Felsenstein, 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004). Evolutionary analyses were conducted in MEGA 6 (Tamura *et al*., 2021)

4.3 Susceptibility of Sweet Potato Varieties to SPLS Pathogen

4.3.1 Susceptibility Tests

The three sweet potato varieties SPK004, Odinga and Mugande significantly responded differently ($p<0.0001$ Appendix VI) to inoculation with the SPLS fungal pathogen (Plate 4.5).Variety 2 (Odinga) developed more spots, while Variety 3 developed the least number of spots during the study period.

Plate 4.5 Selected images of sweet potato varieties used for the greenhouse experiment

From the disease score analysis, Variety 2 (Odinga) was more susceptible at 33.18% while variety 3 (Mugande) had lowest susceptibility at 17.33% **(**Table 4.6; Figure 4.5)**.**

Table 4.6 Weekly susceptibility (%) of sweet potato varieties to SPLS fungi (*Fusarium proliferatum*) after inoculation

Sweet potato variety	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
SPK004	18.83[a]	15.60[a]	41.40[a]	35.43[b]	27.92[b]
Odinga	25.93[a]	20.00[a]	40.37[a]	42.73[a]	36.87[a]
Mugande	6.53[b]	5.27[b]	12.97[b]	26.33[c]	35.57[a]
p-value	0.0001	0.0001	0.0001	0.0001	0.0001
Mean	14.504	11.54	26.93	33.638	32.892
LSD	1.673	1.8	2.357	1.695	1.577
CV (%)	8.493	10.606	11.483	6.624	5.747

[a]Means followed by the same letters are not siginificantly different (p≤0.05)

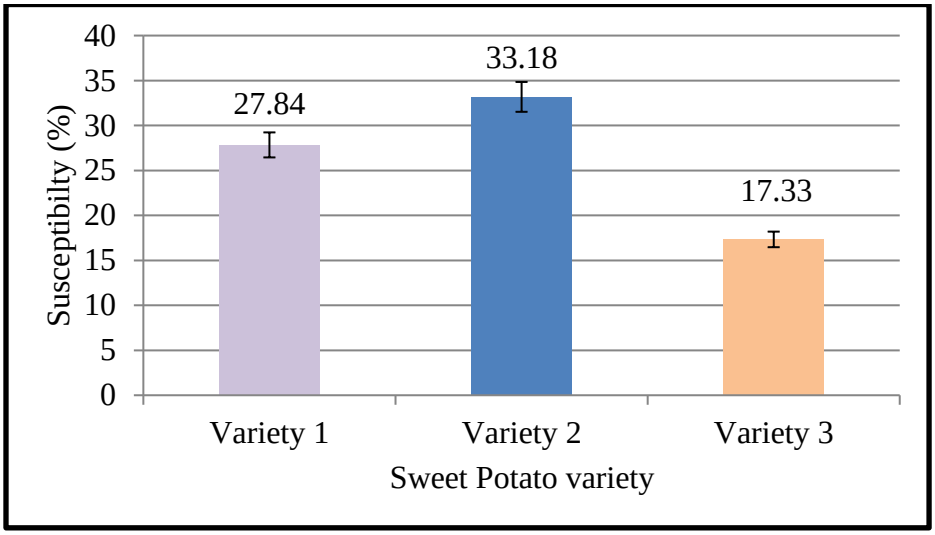

Figure 4.5 Susceptibility of three sweet potato varieties under greenhouse experiment. Variety 1 =SPK004, Variety 2= Odinga, Variety 3= Mugande

4.3.2. Growth Stage Responses

Susceptibility of sweet potatoes to fungal pathogen inoculum at different stages of growth in the greenhouse experiment differed significantly (p≤0.0001). At week four the sweet potato leaves were found to be more susceptible at 34.83% while week one had lowest susceptibility of 17.1% (Table

4.7). The mean difference between week 3, week 4 and week 5 were not statistically significant. Mean difference between week 1 and week 2 when compared to those of week three, four and five were statistically significant ($p < 0.05$; Table 4.7).

Table 4.7 Susceptibility of sweet potato leaves to fungal pathogen isolate at different growth stages under greenhouse experiment

Age	Susceptibility (%)
Week 1	17.1[b]
Week 2	23.62[b]
Week 3	31.58[a]
Week 4	34.83[a]
Week 5	33.45[a]
Mean	26.116
LSD ($p<0.05$)	7.022
CV (%)	27.843

[a]Means followed by the same letters are not significantly different ($p \leq 0.05$).

4.4: Farmers Perception and Indigenous Knowledge of Sweet Potato Leaf Spot

4.4.1 Sweet Potato Variety Grown During the Study

The sweet potato variety grown during the study was only significantly different in Kabondo - Kasipul Sub County [(χ^2 = 4.7743 (df2, N = 22), p < 0.0248)] and Kisumu-East Sub-Counties [(χ^2=8.294 (df2, N=22), p < 0.004)]. Averagely, 50% of female farmers in Kabondo-Kasipul reported to have planted variety1 (SPK004) while 4.55% had planted Variety2 (Odinga). Equal number of male and female farmers (22.73%) had planted both variety 1 and 2. In Kisumu-East Sub-County, 86% of the farmers grew variety1, while only 13.64% grew variety2 (Table 4.8).

Table 4.8 Variety of sweet potato grown during the study in Kabondo-Kasipul, Rangwe, and Kisumu-East Sub Counties

Factor	Sub-County	Farmer response	Sex of respondents			*Chi* Square	*p* value
			Male (%)	Female (%)	Total %		
Variety of sweet potato grown during study.	Rangwe	V1	18.18	63.64	81.82	1.2731	**.2592**
		V2	9.09	9.09	18.18		
	Kabondo-Kasipul	V1	22.73	50.0	72.73	4.7743	**.0248**
		V2	22.73	4.55	27.27		
	Kisumu-East	V1	4.55	8.82	86.36	8.294	**.004**
		V2	9.09	4.55	13.64		

4.4.2 Age of Sweet Potato on Farm during the Study

The age of sweet potato on-farm at the time of study was only significantly different in Rangwe Sub County [(χ^2 (df2, N=22) =1.3941, p < 0.04427)]. An equal number of female farmers (31.82%) reported having sweet potatoes of age 2-4 months and 4-6 months while most male farmers (18.18%) had sweet potatoes of age 4-6 months. Averagely, 50% of farmers had sweet potatoes of age 4-6 months (Table 4.9).

Table 4.9 Age of sweet potato grown during the study in Kabondo-Kasipul Rangwe and Kisumu-East Sub Counties

Factor	Sub-County	Farmer response	Sex of respondents			*Chi* Square	*p* value
			Male (%)	Female (%)	Total %		
What is the age of sweet potato currently grown in your farm?	Rangwe	0-2 months	4.55	9.09	3.64		
		2-4 months	4.55	31.82	36.36	1.3941	**.04427**
		4-6 months	18.18	31.82	50.00		
	Kabondo-Kasipul	0-2 months	13.64	4.55	18.18		
		2-4 months	18.18	22.73	40.91	1.9454	.3781
		4-6 months	13.64	27.27	40.91		
	Kisumu-East	0-2 months	0.00	27.27	27.27		
		2-4 months	13.64	27.27	40.91	5.0175	.0814
		4-6 months	0.00	31.82	31.82		

4.4.3 Source of sweet Potato Vines for Planting

The relationship between source of sweet potato vine planting materials and occurrence of SPLS was not signifcant in all the three sub counties [χ^2 (df 2, N = 66, $p \geq 0.071 - 0.659$)]. However, the highest number of famers who obtained sweet potato vines from friends (50%) were females from Rangwe Sub-County. Farmers who used last season's vines as planting materials were females from Kisumu-East Sub-County with a value of 88.89%. The highest value of vines obtained from friends and the last planting season was by female farmers in Kisumu-East Sub-County at 83.33% (Table 4.10).

Table 4.10 Source of sweet potato planting vines used by farmers in Kabondo-Kasipul Rangwe and Kisumu-East Sub Counties

Factor	Farmer response	Sub-County	Response	Sex of respondents			*Chi* Square	*p* value
				Male (%)	Female (%)	Total %		
	Friends	Rangwe	Yes	33.33	50.00	83.33	1.20	0.273
			No	16.67	0.00	16.67		
		Kabondo-Kasipul	Yes	16.67	66.67	83.33	0.24	0.624
			No	0.00	16.67	16.67		
		Kisumu-East	Yes	0.00	50.00	50.00	3.00	0.083
			No	33.33	16.67	50.00		
Where you obtained the last sweet potato vine?	Last season	Rangwe	Yes	16.67	83.33	100.00	1.20	0.094
			No	0.00	0.00	0.00		
		Kabondo-Kasipul	Yes	60.00	40.00	100.00	0.84	0.63
			No	0.00	0.00	0.00		
		Kisumu-East	Yes	0.00	88.89	88.89	0.92	0.071
			No	11.11	0.00	11.11		
	Friends and last season	Rangwe	Yes	10.00	60.00	70.00	0.47	0.490
			No	10.00	20.00	30.00		
		Kabondo-Kasipul	Yes	50.00	33.33	83.33	1.20	0.273
			No	0.00	16.67	16.67		
		Kisumu-East	Yes	0.00	14.29	14.29	0.19	0.659
			No	16.67	**83.33**	85.71		

4.4.4 History of Growing Sweet Potatoes

The duration (History) in which the farmers grew sweet potatoes in the farms studied, was only significantly different in Rangwe Sub County [(χ^2 (df2, N=22) = 1.023, p<0.04996)]. In the Sub County 36.36% of farmers had grown sweet potatoes for less than two years, while 31.82% and 27.27% of farmers had grown sweet potatoes for a period of 2-4 years and for more than 5 years respectively. More female farmers had grown sweet potatoes for 2, 2 – 4, and >5 years with values of 22.73%, 22.73% and 27.27% respectively (Table 4.11).

Table 4.11 History of growing sweet potato in studied farms in Kabondo-Kasipul, Rangwe and Kisumu-East Sub Counties

Factor	**Sub-County**	**Farmer response**	**Sex of respondents**			***Chi* Square**	***p* value**
			Male (%)	**Female (%)**	**Total %**		
For how long have you been growing potatoes in the same farms?	Rangwe	< 2 years	13.64	22.73	36.36	1.0231	**.04996**
		2-4 years	9.09	22.73	31.82		
		> 5 yeas	4.55	27.27	31.82		
	Kabondo-Kasipul	< 2 years	13.64	22.73	36.36	0.6089	.7371
		2-4 years	13.64	18.18	31.82		
		> 5 yeas	18.18	13.64	31.82		
	Kisumu-East	< 2 years	13.64	22.73	36.36	6.0789	.0818
		2-4 years	0.00	31.82	31.82		
		> 5 yeas	0.00	31.82	31.82		

4.4.5 Frequency of Growing Sweet Potatoes

Frequency of sweet potato production in the same farm was not significantly different in the three Sub Counties [(χ^2 (df2, N=66, p=0.3384-0.868)]. However, more female farmers (50.0%) in Kisumu-East grew sweet potatoes continuously in the same farm, as compared to female farmers in Rangwe (40.91%) and Kabondo-Kasipul (36.36%) Sub Counties. More male farmers (13.64%) grew sweet potatoes continuously in Kabondo-Kasipul Sub County as compared to Rangwe (9.09%) and

Kisumu-East (4.55%). More female farmers grew sweet potatoes continuously as compared to males (Table 4.12).

Table 4.12 Frequency of growing sweet potato continuously in Kabondo-Kasipul Rangwe and Kisumu East Sub Counties

Factor	**Sub-County**	**Farmer response**	**Sex of respondents**			***Chi* Square**	***p* value**
			Male (%)	**Female (%)**	**Total %**		
Do you grow sweet potatoes continuously?	Rangwe	Yes	**9.09**	**40.91**	50.00	0.6351	.3384
		No	18.18	31.82	50.00		
	Kabondo-Kasipul	Yes	**13.64**	**36.36**	50.00	2.9333	.868
		No	31.82	18.18	50.00		
	Kisumu-East	Yes	4.55	**50.0**	54.55	0.6304	.4272
		No	9.09	36.36	45.45		

4.4.6 Sweet Potato Farming System in the Study Area

The system of farming practiced in the study area, whether subsistence or commercial was only significant in Kabondo-Kasipul Sub-County [χ^2 (df. 2, N = 22) = 10.476, $p < 0.0017$). Within this sub county, 63.64% of the farmers practiced commercial farming out of which 45.45% were male farmers while 36.36% were subsistence female farmers. Overally, the highest number of farmers practicing subsistence farming were females from Kisumu-East at 77.27%, while 0.0% of male farmers practiced subsistence farming in Kabondo-Kasipul Sub-County (Table 4.13).

Table 4.13 Farming systems practiced by sweet potato farmers in Kabondo-Kasipul Rangwe and Kisumu-East-Sub Counties

Factor	Sub-County	Farmer response	Sex of respondents			*Chi* Square	*p* value
			Male (%)	Female (%)	Total (%)		
Scale of farming practiced by farmer	Rangwe	Subsistence	18.18	54.55	72.73	0.153	.6958
		Commercial	9.09	18.18	27.27		
	Kabondo-Kasipul	Subsistence	**0.00**	**36.36**	36.36	10.476	**.0017**
		Commercial	**45.45**	18.18	63.64		
	Kisumu-East	Subsistence	4.55	**77.27**	81.82	5.4893	.0727
		Commercial	9.09	9.09	18.18		

4.4.7 Planting Method of Sweet Potato Vines

The planting method used by farmers was only significantly different in Kabondo-Kasipul Sub County [(χ^2 df2, N=22) =4.455, p< 0.0348)]. About 54.55% of farmers used ridges to plant the sweet potatoes while 45.45% used Mounds. Most male farmers (18.18%) used mounds while most female farmers (31.82%) planted using ridges (Table 4.14).

Table 4.14 Planting method used to cultivate sweet potatoes in Kabondo-Kasipul, Rangwe and Kisumu-East Sub Counties

Factor	**Sub-County**	**Farmer response**	**Sex of respondents**			***Chi* Square**	***p* value**
			Male (%)	**Female (%)**	**Total %**		
Planting method used	Rangwe	Ridge	4.55	18.18	22.73		
		Mound	22.73	54.55	77.27	0.1725	.6779
	Kabondo-Kasipul	Ridge	13.64	**31.82**	**54.55**		
		Mound	18.18	**22.73**	**45.45**	4.455	**0.0348**
	Kisumu-East	Ridge	4.55	22.73	27.27		
		Mound	9.09	63.64	72.73	0.0643	.7998

4.4.8 Inorganic Fertilizer Use in Sweet Potato Production

The use of inorganic fertilizer in sweet potato farming was only significantly different in Kabondo-Kasipul Sub County [(χ^2 (df2, N=22) = 1.691, p< 0.01935)]. Within the sub county, 27.73% of farmers used fertilizers in sweet potato production. More female farmers (18.18%) used inorganic fertilizers than the male farmers at 4.55% (Table 4.15). However, most farmers in the study area do not use inorganic fertilizer in sweet potato production.

Table 4.15 Use of inorganic fertilizers by sweet potato farmers in Kabondo-Kasipul Rangwe and Kisumu East Sub Counties

Factor	Sub-County	Farmer response	Sex of respondents			*Chi* Square	*p* value
			Male (%)	Female (%)	Total %		
Do you use fertilizer for growing potatoes?	Rangwe	Yes	0	27	27	3.0938	.60786
		No	27.27	45.45	72.73		
	Kabondo-Kasipul	Yes	**4.55**	**18.18**	**27.73**	1.691	**.01935**
		No	40.91	36.36	77.27		
	Kisumu East	Yes	0	13.64	13.64	0.5485	.4589
		No	13.64	72.73	86.36		

4.4.9 Disease Occurrence in Sweet Potato Farms

Disease occurrence was not significant in all the three sub counties [χ^2 df2, N=66) p=0.1757-0.3121)]. However, in Kabondo-Kasipul and Rangwe Sub Counties, farmers reported presence of diseases in their sweet potato farms at 90.91% and 81.82% respectively. Low disease occurrence of 22.73% was reported in Kisumu-East Sub County. Most female farmers reported disease occurrence in Rangwe Sub County at 63.64% while the male farmers in Kisumu-East Sub County reported no disease occurrence. Equal number of male and female farmers (45.45%) in Kabondo-Kasipul Sub County reported disease occurrence (Table 4.16).

Table 4.16 Disease occurrence in sweet potato farms in Kabondo-Kasipul, Rangwe, and Kisumu-East Sub Counties

Factor	Sub-County	Farmer response	Sex of respondents			*Chi* Square	*p* value
			Male (%)	Female (%)	Total %		
Have you experienced diseases in your sweet potato farm?	Rangwe	Yes	18.18	**63.64**	**81.82**	1.2731	0.2592
		No	9.09	9.09	18.18		
	Kabondo-Kasipul	Yes	**45.45**	**45.45**	**90.91**	1.8333	0.1757
		No	0.00	9.00	9.09		
	Kisumu-East	Yes	0.00	22.73	**22.73**	1.0217	0.3121
		No	13.62	63.64	77.27		

4.4.10 Identification of different diseases in sweet potato farms

Ability to differentiate the diseases in sweet potato farms was insignificant in the three sub counties [(χ^2 df2, N=66) p=0.5556-0.6959)]. However, sweet potato farmers in Kabondo-Kasipul and Kisumu-East Sub Counties were able to differentiate the diseases in their farms at 90.91% each, while 72.73% of sweet potato farmers in Rangwe Sub County were not able to differentiate the sweet potato diseases on their farms. Most female farmers (77.27%) in Kisumu-East Sub County were able to differentiate the sweet potato diseases as compared to those in Kabondo-Kasipul at 45.45%, while in Rangwe 54.55% of female farmers reported inability to differentiate sweet potato diseases occurring on their farms. Overally, female farmers were able to differentiate the sweet potato diseases except in Kabondo-Kasipul where 40.91% of male farmers were able to differentiate the sweet potato diseases (Table 4.17).

Table 4.17. Identification of sweet potato diseases by farmers in Kabondo-Kasipul, Rangwe and Kisumu- East Sub Counties

Factor	Sub-County	Farmer response	Sex of respondents			*Chi* Square	*p* value
			Male (%)	Female (%)	Total %		
Are you able to differentiate between the diseases in your farm?	Rangwe	Yes	9.09	18.18	27.27	0.1528	.6959
		No	4.55	54.55	**72.73**		
	Kabondo-Kasipul	Yes	**40.91**	45.45	**90.91**	0.2058	.6500
		No	0	13.64	9.09		
	Kisumu-East	Yes	13.64	77.27	**90.91**	0.3474	.5556
		No	0	9.09	9.09		

4.4.11 Disease History

The SPLS disease history was only significant in Rangwe Sub County [(χ^2 df2, N=22) =10.1161, p<0.0064)], where 13.64% of male farmers reported that it was an old disease while 22.73% of female farmers reported that it was a new disease. However, 63.64% of farmers in the sub county

did not know the history of the disease. Overally, it was considered an unknown disease in Kabondo-Kasipul and Kisumu- East sub counties at 68.18% and 86.36% respectively (Table 4.18).

Table 4.18 History of SPLS disease in farmers' fields in Kabondo-Kasipul, Rangwe and Kisumu-East Sub Counties

Factor	Sub-County	Farmer response	Sex of respondents			*Chi* Square	*p* value
			Male (%)	Female (%)	Total %		
How long has your sweet potato suffered from the disease?	Rangwe	**New**	0.00	**22.73**	22.73	10.1161	**.0064**
		Old	**13.64**	0.00	13.64		
		Don't know	13.64	50.0	**63.64**		
	Kabondo-Kasipul	New	9.09	13.64	22.73	0.0856	.09581
		Old	4.55	4.55	9.09		
		Don't know	31.82	36.35	**68.18**		
	Kisumu-East	New	4.55	4.55	9.09	2.5596	.2781
		Old	0.00	4.55	4.55		
		Don't know	9.09	77.27	**86.36**		

CHAPTER FIVE

DISCUSSION

5.1 Incidence and Severity of Sweet Potato Leaf Spot Disease

Sweet potato leaf spot incidences and severity differed significantly in some of the villages surveyed. Disease incidence ranged from as low as 11% to 30.38% at Kakelo and Kamollo villages respectively. The incidence range observed were comparable to a report by Osiru *et al.* (2007) with a range of 0 to 49.2% in Uganda. However, this study findings differed with those of Bashaasha *et al*. (1995b) where above 50% incidence rate was reported. Incidences of <50% have earlier been reported in Kirinyaga, Muranga, and Kiambu (Skoglund *et al.*, 1993; Skoglund and Smit, 1994). Variation in SPLS incidence in this study may partly be attributed to climatic and soil conditions which affect disease development. Further, individual plant defense mechanisms or susceptibility to infections vary by species and environmental patterns (Kandolo *et al.,* 2016). Cool humid weather conditions favour leaf spot development and foliar destruction (Arene and Nwankiti, 1978; Ilondu, 2013). On the other hand, disease incidence may be influenced by the specific sweet potato variety cultivated (Thompson *et al.*, 2011). Pathogen factors such as virulence, dispersal rate, and farm practices such as sources of vine and variety may also influence disease incidence and severity (Osiru *et al.*, 2007; Sseruwu *et al.*, 2016). The implication of SPLS in yield reduction is due to its negative effect on plant physiology. The plants' photosynthesis capacity and translocation of sugars, water and minerals may be hampered drastically due to killing of leaf areas infested by leaf spot (Parry, 1990; Ilondu, 2013). Infected plants experience impaired physiological activities such as increased respiration rates and reduced photosynthesis that lead to yield losses (Bilgrami and Dube, 1976; Amienyo and Ataga, 2008).

5.2 Characterization of Sweet Potato Leaf Spot Pathogen Isolates

The 12 pure isolates obtained in this study were different in their cultural characteristics. The spores of isolates were brownish, whitish or greyish in colour. The spore colours compared well to those observed by other studies (Gabrekiristos *et al*., 2020; Ekhuemelo and Nsobundu, 2020). The fungal isolates had profuse mycelia growth on PDA media. The mycelia were hyaline, septate, and irregularly branched. These features of spores and mycelia are similar to those reported for *Fusarium sp.* in other studies (Nagrale *et al.,* 2013). Variation in colony growth characteristics may point at possible existence of different pathogen strains that utilise different components of culture media.

The observed cultural and microscopic characteristics of the fungal leaf spot pathogen exhibit plasticity in terms of mycelial colour and pigmentation on culture media. Morphological characterization of fungal pathogens in culture did not give clear difference between isolates. Thus, molecular characterization was carried out using Internal Transcribed Spacer (ITS) regions of fungal ribosomal DNA (rDNA) for pathogen isolates characterization. Analysis of the ITS sequences through BLAST and MEGA analysis revealed ≥ 99% similarity with different species of *Fusarium* which included *Fusarium chlamydosporium* (Isolate NF1), *Fusarium proliferatum* (NF2-NF8) and *Fusarium verticillioides* (Isolate NF9 and NF10). The findings of this study contrast those of Maharachchikumbura *et al.* (2016) who reported *Neopestalotiopsis ellipsospora* as the causative agent of SPLS in China. In South Africa *Fusarium chlamydosporium* has been isolated from the inflorescence of Myrtacease tree where it causes misshapen inflorescence that do not bear fruits (Mkandawire *et al*., 2022).

The *Fusarium sp*. pathogens observed in this study were genetically identical to previously studied species which were implicated in blight, wilt and leaf spot diseases of sweet potatoes and other crops (Imazaki and Kadota, 2015; Han *et al*., 2015; Omar *et al.,*2018, Zakaria, 2018; Sun *et al.,* 2018;

(Ekhuemelo and Nsobundu, 2020); Jabłońska *et al.,* 2020). On the other hand, species of *Fusarium proliferatum* and *Fusarium verticillioides* have been associated with leaf spot lesions (Zhang and Ma, 2017; Omar *et al.,* 2018; Ekhuemelo and Nsobundu, 2020). However, *F. oxysporum* f. sp. batatas had also been isolated from sweet potato foliar (Thanaa *et al.,* 2018). In India, *Fusarium verticillioides* has been implicated in maize stalk rot (Borah *et al.,* 2016). Infection by *Fusarium proliferatum* disrupts photosynthetic activity of the plants thus reducing growth, and making plants susceptible to infection by opportunistic pathogens. It is also associated with root rot in *Codonopsis lanceolate* plant, a substitute for *Panax ginseng* with immunomodulatory and anti-inflammatory properties in China (Gao *et al*., 2017). *Fusarium verticillioides* have been reported to primarily affect maize as well as causing leaf spot disease of sweet potato (Ekhuemelo and Nsobundu, 2020). It was also found to be pathogenic in leaf spot disease of sweet potato in this present study. This corroborates with the findings of (Ekhuemelo and Nsobundu, 2020) which reported *Fusarium* species as being able to cause leaf spot infection under favourable temperature and humidity conditions. Ilondu (2013), also agreed that *Fusarium spp* could incite leaf spot disease in a variety of plants. In Egypt it is associated with pepper wilting (Ismail *et al.,* 2017).

5.3 Susceptibility of Sweet Potato Varieties to Leaf Spot Pathogen Isolates

This study indicated that sweet potato variety grown significantly influenced development of SPLS pathogen. Variety 2 (Odinga) was found to be more susceptible at 33.18% while variety 3 (Mugande), had lowestq susceptibility. These findings corroborate with those of Scattolini *et al.* (2020) that reported that SPLS susceptibility differs depending on variety. None of the varieties tested was found to be resistant to the leaf spot disease. Lack of varieties with resistance or tolerance to diseases has been pointed out to be a major constraint in sweet potato production (Bashasha *et al*., 1995). Thus, there is need to continue evaluating available sweet potato varieties for their resistance

to fungal leaf spot in the study area to identify varieties that may be used to breed for resistant vines. Further, integrated crop management should be considered together with growing of sweet potato cultivars with lower susceptibility to fungal pathogens.

Susceptibility of sweet potato varieties to fungal leaf spot pathogens at different ages under greenhouse experiment differed significantly. Four weeks old sweet potatoes were found to be more susceptible at 34.83%. Susceptibility test was found to align to farmers' response on sweet potato age susceptibility. In the contrary, higher percentages of respondents reported that sweet potatoes were more susceptible to fungal leaf spot pathogen at the age of 4-6 months. Whereas studies have reported effect of sweet potato age to their susceptibility to other fungal diseases (Osiru *et al.*, 2007), I did not find such information regarding fungal leaf spot. However, greenhouse experiment results agreed with those of Osiru *et al.* (2007) that seedlings or younger vines are less susceptible to leaf spot disease when compared to mature vines. Thus, it may be concluded that sweet potatoes grown in the study area are more susceptible to fungal leaf spot at older age than when younger. Susceptibility of old vines as compared to young vines may also be attributed to occurrence of phytochemicals such as phenol oxidases, phenolic compounds and phytoalexins in the younger leaves that confer resistance to infection by fungi and other microorganisms.

5.4 Farmers Perception and Knowledge of SPLS Infection

5.4.1 Variety Grown, Source of Vines, Farming History and Farming Frequency

Sweet potato varieties cultivated by farmers during this study significantly differed between Kabondo-Kasipul and Kisumu- East Sub-Counties, where most farmers grew variety2 (Odinga). Variation in sweet potato variety grown is supported by Bashaasha *et al.* (1995a) and Sindi and Wambugu (2012) who reported that certain varieties of sweet potatoes were most preferred by the

farmers compared to others. Varietal preference may be attributed to high yields, good root qualities, good performance in different types of soil and sweet potato colour.

Majority of farmers reported to have obtained sweet potato planting materials from the last season's vines in all locations of study, with Rangwe and Kabondo-Kasipul scoring 100% and Kisumu East having 88.89%. However, source of sweet potato planting material was not significantly associated with the sub counties of study. The findings on source of sweet potato planting material reported in this study agrees with the findings of Sseruwu *et al*., (2015) and Aldow (2017) who reported that the majority of sweet potato farmers obtains planting materials from last season crop. Out of 37.88% of farmers who obtained vines from last season sweet potato farm, 25.76% reported occurrence of diseases as compared to 12.12% who did not report occurrence of sweet potato fungal leaf spot. The relationship between source of sweet potato vines and occurrence of SPLS was thus significant. This implies that the major source of SPLS transmission in the study area is recycling of sweet potato vines among farmers.

The duration in which the farmers grew sweet potatoes in the farms studied was only significantly different in Rangwe Sub County. More female farmers grew sweet potatoes continuously as compared to male farmers. Continuous cultivation of sweet potatoes by farmers had earlier been reported by Bashaasha *et al.* (1995a). Continuous cultivation of sweet potato by farmers may be attributed to the fact that it is a food security, drought and pest tolerant crop that can be produced all year round. Thus, sweet potato ensures continuous food supply where other crops are faced with risk of failing (Wambui, 2020).

5.4.2 Farming Systems, Cultivation Method and Inorganic Fertilizer Use

The system of farming practiced in the study region was both subsistence and commercial. It was only significant in Kabondo-Kasipul Sub-County, where up to 63.64% of farmers reported to grow sweet potatoes for commercial purposes. This finding agrees with the report of Ezin *et al.* (2018) that sweet potato is grown mainly for the fresh market since the sweet potato tubers are sold in the surrounding markets for income. Most male farmers (45.45%) planted sweet potatoes for commercial use while the female farmers (36.36%) grew the crop for subsistence. This may be attributed to the fact that women generally own less land and the land they have is often of lower quality than the land owned by men. Bach and Andersen (2008), reported that women in Africa only own 1 per cent of the land and they have to contend with limited access to financial and technical resources.

The planting method used by farmers was only significantly different in Kabondo-Kasipul Sub County. Most farmers preferred planting sweet potatoes in mounds as opposed to ridges. The finding on use of mounds as sweet potato planting method differs with the report of Sindi and Wambugu (2012) that ridges planting method was most popular compared to mounds. Nonetheless, the findings of this study concurs with those of Bashaasha (1995b) who reported use of mounds to be the most preferred method of planting sweet potatoes.

The use of inorganic fertilizer in sweet potato farming was only significantly different in Kabondo-Kasipul Sub County. More female farmers (18.18%) used inorganic fertilizers than male farmers (4.55%). Only 27.73% of farmers used inorganic fertilizers in sweet potato production. Use of inorganic fertilizer in cultivation of sweet potato reported in this study differs to the report by Bashaasha (1995b) who observed that though farmers do apply manure, use of inorganic fertilizer is rare in sweet potato farming. Soil nutrition changes caused by appropriate fertilization leads to an

overall improvement in crop productivity since continuous cultivation depletes soil organic matter hence the need for fertilization during sweet potato production (Mcharo *et al.,* 2013). Growth and yield of sweet potato is affected by plant population and nutrient supply thus lower yields could be attributed to poor soil nutrients (Otoo *et al.,* 2000). Organic and inorganic fertilizers are thus required to improve the yield and growth rate of sweet potatoes. Dapaah *et al.* (2004) emphasized that application of nitrogen, phosphorus and potassium fertilizers influence growth and yield of sweet potatoes. Potassium and nitrogen are required by sweet potatoes for high yield since Potassium increases water uptake, efficient use of nitrogen, translocation of assimilates, photosynthesis, drought and diseases resistance. Potassium also enhances sweet potato tuber taste, shape, size, colour and texture. (Nedunchezhiyan *et al*., 2012).

5.4.3 Occurrence of Sweet Potato Leaf Spot Disease

Sweet potato leaf spot disease occurrence was not significant in the three sub counties. Most sweet potato farmers in Kabondo-Kasipul and Rangwe Sub Counties reported high disease prevalence while farmers in Kisumu-East Sub-County reported lowest disease prevalence. This finding is supported by those of Sseruwu *et al.* (2015) who reported variation of farmers' responses on occurrence of diseases in different areas. Factors such are varied environmental factors and farming practices may be attributed to these differences

CHAPTER SIX

CONCLUSSION AND RECOMMENDATIONS

6.1 Conclusion

The incidence and severity of SPLS was significant in some villages in the three sub counties. The SPLS incidence was highest in Rangwe Sub County and lowest in Kabondo-Kasipul Sub County while SPLS severity was highest in Kabondo-Kasipul Sub County and lowest in Kisumu-East Sub County.

From cultural isolation different fungal isolates were obtained. The isolates produced brown, white and grey spores. The myecilia were hyaline, septate and produced micro and macroconidia. From molecular characterization, different strains of *Fusarium spp* were the agent of leaf spot disease of sweet potato in the study area. The fungal pathogens included; *Fusarium chlamydosporum, Fusarium proliferatum* and *Fusarium verticillioides*. *Fusarium proliferatum* was the most common pathogen in my isolates.

Sweet potato variety grown influenced development of sweet potato leaf spot infection. Variety 2 (Odinga) was found to be more susceptible while variety 3 (Mugande), had lowest susceptibility. Susceptibility of sweet potato cultivars to SPLS pathogen was also influenced by the age of sweet potato vines. Older vines were found to be more susceptible than younger vines.

Variety 1(SPK004) and variety 2(Odinga) were preferred in the study area. Variety 1 is orange fleshed thus rich in beta-carotene, a precursor for vitamin A thus necessary to alleviate malnutrition in younger children, while variety 2 is high yielding with tasty tubers. The source of sweet potato vines for planting was mainly recycling the last season crop and borrowing from friends. More female farmers grew sweet potatoes continuously as compared to the male farmers in all the three

sub counties. Most male farmers grew sweet potatoes for commercial purposes while female farmers mainly practiced subsistence farming. The planting method involved both mounds and ridges, while inorganic fertilizer use was minimum and only by few female farmers. Most farmers reported occurrence of sweet potato diseases in their farms, however, the female farmers were more able to differentiate sweet potato diseases as compared to the male farmers. Overally, sweet potato leaf spot disease was considered an unknown disease in the study area.

6.2 Recommendations

1. SPLS disease incidence and severity was varied in the three Sub Counties of Kabondo-Kasipul, Rangwe and Kisumu-East. Similar study is recommended in other Sub Counties within Homabay and Kisumu Counties to establish SPLS occurrence in those regions.
2. Since different strains of *Fusarium spp* were found to cause SPLS, it is recommended that the effect of each species on sweet potato production be established.
3. Sweet potato variety Odinga was more susceptible compared to Mugande and Spk004 varieties. Therefore, Mugande and Spk004 varieties are recommended for further evaluation and adoption by farmers.
4. The farmers in the study area considered SPLS an unknown disease. It is recommended that agricultural extension officers create awareness on the existence of SPLS disease and sensitise farmers on the various diseases that affect their sweet potato crop including SPLS that is known in other parts of the world.

6.3 Suggestions for Further Research

1. Other sweet potato producing counties should be investigated to establish the incidence and severity of SPLS disease.
2. A social study on the economic significance and impact of SPLS fungi on the income of sweet potato farmers is recommended.
3. Further studies should be done to establish the effect of SPLS on other sweet potato varieties not evaluated in this study.
4. The perception and knowledge of farmers in other parts of the country on SPLS occurrence and significance should be studied.

REFERENCES

Abass, J. (2018). Women grow 70% of Africa's food. But have few rights over the land they tend. World Economic Forum articles. Retrieved from https://www.weforum.org/agenda/2018/03/women-farmers-food-production-land-rights.

Abdallah, B., Githiri, S. M., Kariuki, W. and Saha, H. M. (2021). Evaluation of different methods of multiplying sweet potato planting material in coastal lowland Kenya. *African Journal of Agricultura Research, 17*(2), 208-214.

Abong', G., Ndanyi, V., Kaaya, A., Shibairo, S., O. M., Lamuka, P., Sopade, P. (2016). Current Research in Nutrition and Food Science. *Current Research in Nutrition and Food Science, 4*(3), 162-181.

Adam R.I., Kirimi S., Lone B. (2015). Farmers' knowledge, perceptions and management of diseases affecting sweet potatoes in the Lake Victoria Zone region, Tanzania,Crop Protection,Volume 72,Pages 97-107.

Adhikari, A., Ibrahim, A. M., Rudd, J. C., Baenziger, P. S., Easterly, A., Garst, N., and Sarazin, J. B. (2020). Supplementing selection decisions in a hybrid wheat breeding program by using F 2 yield as a proxy of F 1 performance. *Euphytica*, *216*(8), 1-18.

Afzal, N., Afionis, S., Stringer, L. C., Favretto, N., Sakai, M., & Sakai, P. (2021). Benefits and trade-offs of smallholder sweet potato cultivation as a pathway toward achieving the sustainable development goals. *Sustainability*, *13*(2), 552.

Alajo, A. (2009). Distribution and characterization of sweetpotato *Alternaria* blight isolates in Uganda. *Msc. Makerere University*.

Alakonya, A. E. (2016). Ear rot and fumonisin B1 levels in self-selected maize varieties at different harvest intervals in Western Kenya (Master's thesis). Kenyatta University, Nairobi, Kenya. Retrieved from http://irlibrary.ku.ac.ke/handle/123456789/2038

Aldow, A. M. (2017). Factors affecting sweet potato production in crop–livestock farming systems in Ethiopia. *Master of Science in Agroecology, Norwagian University of Life Science*.

Al-Nadabi, H., Maharachchikumbura, S. S., Al-Gahaffi, Z. S., Al-Hasani, A. S., Velazhahan, R., & Al-Sadi, A. M. (2020). Molecular identification of fungal pathogens associated with leaf spot disease of date palms (Phoenix dactylifera). *All Life*, *13*(1), 587-597.

Altizer S, Ostfeld RS, Johnson PT, Kutz S, Harvell CD. (2013). Climate change and infectious diseases: from evidence to a predictive framework. *Science*; 341:514–519.

Amienyo, C. A. and Ataga, A. (2008). Survey of fungi assocated with diseased sweet potato (Ipomoea batatas (L) Lam) leaves in some farms in Rivers State, Nigeria. *Nigeria Journal of Botany, 21*(2), 336 – 341.

Anginyah, T. J., Narla, R. D., Carey, E. E., & Njeru, R. (2001). Etiology, Effect of Soil pH and Sweetpotato Varietal Reaction to Alternaria leaf Petiole and Stem Blight in Kenya. African Crop Science Journal, 9(1), 287-282.

Anyan, F. Y. (2018). Farmers Perceptions and Attitudes Towards the Use of Agricultural Indigenous Knowledge in Farming. *Journal of Agriculture and Crops*, *4*(6), 63-67.

Aoki, T. (2009). Taxonomic system of the genus Fusarium. *Microbiol Cult Coll, 25,* 1-12

Arancibia, R. A., Main, J. L., & Clark, C. A. (2013). Sweetpotato tip rot incidence is increased by preharvest applications of ethephon and reduced by curing. *HortTechnology*, *23*(3), 288-293.

Arene, O. A. (1978). Sweet potato diseases in Nigeria. *PANS*, *24*(3), 294 - 305.

Arie, T. (2019). Fusarium diseases of cultivated plants, control, diagnosis, and molecular and genetic studies. *Journal of Pest Science*, *44*, 275–281.

Arora, R. K., & Khurana, S. P. (2004). Major fungal and bacterial diseases of potato and their management. In *Fruit and vegetable diseases* (pp. 189-231). Springer, Dordrecht.

Atakos V, Joyce Maru, Dinah Borus and Nathan (2020). https://www.standardmedia.co.ke/farmkenya/crop/article/2001376606/how-nutritious-sweet-potatoes-and-potatoes-are-shaping-kenyas-food-security.

Baayen, R. P., O'Donnell, K., Bonants, P. J., Cigelnik, E., Kroon, L. P., Roebroeck, E. J., & Waalwijk, C. (2000). Gene genealogies and AFLP analyses in the Fusarium oxysporum complex identify monophyletic and nonmonophyletic formae speciales causing wilt and rot disease. *Phytopathology*, *90*(8), 891-900.

Babu, A.N, Jogaiah S. and Ito S. I. (2015). Improvement of growth, fruit weight and early blight disease protection of tomato plants by rhizosphere bacteria is correlated with their beneficial traits and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase. *Plant Science*. 2; 231:62–73

Bach C.F and Per Pinstrup-Andersen. (2008). Agriculture, growth and employment in Africa, Civil Society input to the Africa Commission on Effective Development Cooperation with Africa. April 2008.

Bashaasha, B., Mwanga, R. and p'Obwoya, C. O. (1995a). *Sweetpotato in the Farming and Food Systems of Uganda: A Farm Survey Report.* Kampala, Uganda: International Potato Center (CIP).

Bashaasha, B., Mwanga, R. O., p'Obwoya, C. O. and Ewell, P. T. (1995b). Sweetpotato in the farming and food systems: A farm survey report. Nairobi/ Kampala: International Potato Centre (CIP)/ National Agricultural Research Organization (NARO).

Bashaasha, B., Mwanga, R., P'Obwoya, C. and Ewell, P. (1995b). Sweetpotato in the farming and food systems of Uganda. *Proceedings of the 5th Triennial Symposium of International Society of Tropical Root Crops Africa Branch, November 22-28, 1995*, (pp. 225-228). Kampala, Uganda.

Bergh K, Orozco P, Gugerty MK and Anderson CL. (2012). Sweet Potato Value Chain: Nigeria. Evans School Policy Analysis Research Brief No. 220.

Beyer M, Verreet J A, Ragab W S. (2005). Effect of relative humidity on germination of ascospores and macroconidia of *Gibberella zeae* and deoxynivalenol production. *International Journal of Food Microbiology*; 98:233–240.

Bilgrami, K. and Dube, H. (1976). *A textbook of modern plant pathology.* New Delhi: Vikas Publishing House, PVT Ltd.

Bodah E.T. (2017). Root rot diseases in plants: a review of common causal agents and management strategies. *Agriculture Research and. Technology*; 5(3):555661.

Borah, Siddhartha & Deka, Suresh & Sarma, Hridip. (2016). First Report of Fusarium verticillioides Causing Stalk Rot of Maize in Assam, India. Plant Disease. 100. 10.1094/PDIS-01-16-0074-PDN.

Buko, D. H., Gedebo, A., Spetz, C. and Hvoslef-Eide, A. K. (2020). An update of sweet potato viral disease incidence and spread in Ethiopia. *African Journals of Agricultural Research, 16*(8), 1116-1126.

Camó C, Bonaterra A, Badosa E, et al. Antimicrobial peptide KSL-W and analogues: promising agents to control plant diseases. *Peptide*. 2019; 112:85–95.

Chai, A.-L., Du, G.-F., Shi, Y.-X., Xie, X.-W. and Li, B.-J. (2014). Leaf Spot on Sweet Potato (Ipomoea batatas) Caused by *Stemphylium solani*, a New Disease in China. *Journal of Phytopathology, 163*, 1046–1049.

Chalwe, M. J., Adebola, P. and Pillay, M. (2017). Assessing the Genetic Diversity of Alternaria Bataticola in South Africa using Molecular Markers. *Asian Journal of Applied Sciences, 5*(2), 280-290.

Chandrasekara, A., & Josheph Kumar, T. (2016). Roots and tuber crops as functional foods: a review on phytochemical constituents and their potential health benefits. *International journal of food science, 2016*.

Claessens, L., Stoorvogel, J. and Antle, J. (2008). Exante assessment of dual-purpose sweet potato in the crop–livestock system of western Kenya: A minimum-data approach. *Agricultural Systems, 99*(1), 13-22.

Clark, C. A., Ferrin, D., Smith T. and Holmes, G. (2013). Compedium of sweet potato diseases, pests and disorders. American Phytopathological Society, St. Paul, MN.

Clark, C. and Moyer, J. (1988). *Compendium of Sweet potato Diseases.* U.S.A.: APS Press, The America Phytopathological Society.

Clark, S. M., Di Leo, R., Van Cauwenberghe, O. R., Mullen, R. T., & Shelp, B. J. (2009). Subcellular localization and expression of multiple tomato γ-aminobutyrate transaminases that utilize both pyruvate and glyoxylate. *Journal of experimental botany*, *60*(11), 3255-3267.

Clarkson JP, Fawcett L, Anthony SG, Young C. A model for *Sclerotinia sclerotiorum* infection and disease development in lettuce, based on the effects of temperature, relative humidity and ascospore density. *PLoS ONE.* 2014;9:e94049.

Cochran, W.G. (1963) Sampling Techniques, Wiley, New York.

Compendium, I. S. Ipomoea batatas (sweet potato).

Cotten, T.K.; Munkvold, G.P. Survival of *Fusarium moniliforme*, *F. proliferatum*, and *F. subglutinans* in maize stalk residue. *Phytopathology* 1998, *88*, 550–555.

Degebasa, A. C. (2019). Review of Potato Research and Development in Ethiopia: Achievements and Future Prospects.

da Silva, W. L., & Clark, C. A. (2013). Infection of sweetpotato by Fusarium solani and Macrophomina phaseolina prior to harvest. *Plant disease*, *97*(12), 1636-1644.

Dapaah, H., S.A. Ennin, O. Safo-Kanatanka, M.M. Buri, A.. Dankyi, J.A. Otoo (2004).Sweet potato production guide: a resource and reference manual IFAD/MOFA root and tuber improvement programme publication RTIP, MOFA, Kumasi, Ghana.

Desjardins AE. 2006. *Fusarium mycotoxins: chemistry, genetics, and biology*. APS Press, St. Paul, MN.

Dhingra O. D., Sinclair J. B. (1978). *Biology and Pathology of Macrophomina Phaseolina.* Minas Gerais: Universidade Federal de Viçosa: Impresa Universitaria

Domola, M. J., Thompson, G. J., Aveling, T. A. S., Laurie, S. M., Strydom, H., & Van den Berg, A. A. (2008). Sweet potato viruses in South Africa and the effect of viral infection on storage root yield. *African Plant Protection*, *14*(1), 15-23.

Echodu, R., Edema, H., Wokorach, G., Zawedde, C., Otim, G., Luambano, N., . . . Asiimwe, T. (2019). Farmers' practices and their knowledge of biotic constraints to sweetpotato production in East Africa. *Physiological and Molecular Plant Pathology*, *105*, 3-16.

Ekhuemelo, C. and Mariagoretti, C. N. (2020). Pathogenicity of fungi associated with leaf spot disease of sweet potato (Ipomea batatas L. (Lam) in Makurdi, Benue State, Nigeria. *GSC Biological and Pharmaceutical Sciences, 11*(02), 250–256.

El Kichaoui, A. Y., Bara'a, A., & El Hindi, M. (2017). Isolation, molecular identification and under lab evaluation of the entomopathogenic fungi M. anisopliae and B. bassiana against the red palm weevil R. ferrugineus in Gaza Strip. *Advances in Microbiology*, *7*(01).

Emitaro W.O., Musyimi D.M., Otiato D.A. and Onyango B. (2017). Morphological, Biochemical and Molecular Characterization of Xanthomonas campestris pv vesicatoria 130 of African nightshades (Solanum scabrum). International Journal of Biosciences,11(1): 190-197.

Epeju, W. F. and Rukundo, P. M. (2018). Food Security and Income through Sweet Potato Production in Teso, Uganda. *Sustainable Agriculture Research, 1*(7), 146-155.

Ezin, V., Quenum, F., Bodjrenou, R.H. *et al.* Assessment of production and marketing constraints and value chain of sweet potato in the municipalities of Dangbo and Bonou. *Agric & Food Secur* **7,** 15 (2018). https://doi.org/10.1186/s40066-018-0164-6

Fandohan, P., Gnonlonfin, B., Hell, K., Marasas, W. F. O., & Wingfield, M. J. (2005). Natural occurrence of Fusarium and subsequent fumonisin contamination in preharvest and stored maize in Benin, West Africa. *International Journal of Food Microbiology*, *99*(2), 173-183.

FAO, IFAD, UNICEF, WFP & WHO. (2020). The State of Food Security and Nutrition in the World 2020. Transforming food systems for affordable healthy diets. Rome, FAO. 320 pp.https://doi.org/10.4060/ca9692en

FAOSTAT (2013). http://faostat3.fao.org/home/index.html

farmlink_kenya. (2017). (S. P. Kenya, Producer) Retrieved 02 26, 2020, from http://www.farmlinkkenya.com/sweet-potato-farming/:

Felsenstein J. (**1985**). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**:783-791.

Fernandes, M.R.; Huber, D.; Basnyat, P.; Zentner, R.P. Impact of agronomic practices on populations of *Fusarium* and other fungi in cereal and noncereal crop residues on the Canadian Prairies. *Soil Tillage Res.* **2008**, *100*, 60–71.

Fraile, A., Pagán, I., Anastasio, G., Sáez, E., & García-Arenal, F. (2011). Rapid genetic diversification and high fitness penalties associated with pathogenicity evolution in a plant virus. *Molecular Biology and Evolution*, *28*(4), 1425-1437.

Fravel, D., Olivain, C., & Alabouvette, C. (2003). Fusarium oxysporum and its biocontrol. *New phytologist*, *157*(3), 493-502.

Fry WE, Birch PR, Judelson HS, Grünwald NJ, Danies G, Everts KL, Gevens AJ, Gugino BK, Johnson DA, Johnson SB, et al. Five reasons to consider *Phytophthora infestans* a reemerging pathogen. *Phytopathology.* 2015;105:966–981.Fumonisin contamination of maize (Zea mays) in aflatoxin hot zones

Gabrekiristos, E., Teshome, D. and Ayana, G. (2020). Cultural, Morphological and Pathogenic Variability among Isolates of Fusarium oxysporum f. sp. capsici Causing Wilt of Hot Pepper in Central Rift Valley, Ethiopia. *Journal of Plant Pathology and Microbiology*, *11*(6), 499.

Gaige, A. R., Todd, T., & Stack, J. P. (2020). Interspecific competition for colonization of maize plants between Fusarium proliferatum and Fusarium verticillioides. *Plant Disease*, *104*(8), 2102-2110.

Gao, J., Wang, J., Yang, C., Wang, Y., Lu, B. H., & Yang, L. N. (2017). Fusarium proliferatum, a new pathogen causing Codonopsis lanceolata root rot in China. *Plant Disease*, *101*(9), 1679-1679.

Gasura, E., & Mukasa, S. B. (2010). Prevalence and implications of sweetpotato recovery from sweet potato virus disease in Uganda. *African Crop Science Journal*, *18*(4).

Gazis, R., Rehner, S., & Chaverri, P. (2011). Species delimitation in fungal endophyte diversity studies and its implications in ecological and biogeographic inferences. *Molecular ecology*, *20*(14), 3001-3013.

Ghosh T., Biswas M. K., Guin C., Roy P. (2018). A review on characterization, therapeutic approaches and pathogenesis of *Macrophomina phaseolina*. *Plant Cell Biotechnol. Mol. Biol.* 19 72–84.

Gibson R. W. , Jeremiah S. C. , Aritua V, Msabaha R. P. , Mpembe I, Ndunguru J. (2000). Sweet potato virus disease in Sub Saharan Africa: Evidence that neglect of seedlings in the traditional farming system hinders the development of superior resistant landraces. J. Phytopathol. 148:441-447.

Gibson, R. W., & Kreuze, J. F. (2015). Degeneration in sweetpotato due to viruses, virus-cleaned planting material and reversion: a review. *Plant Pathology*, *64*(1), 1-15.

Glenna, L., Borlu, Y., Gill, T., Larson, J., Ricciardi, V. and Adam, R. (2017). Food security, sweet potato production,and proximity to markets in northernGhana. *FACETS*, *2*, 919–936.

Goldberg NP. (2006). Fusarium leaf spot and Crown Root rot. New Mexico State University Extension Guide, 2.

Gordon T.R. (2017) *Fusarium oxysporum* and the *Fusarium* wilt syndrome. *Ann. Rev. Phytopathology.*; 55:23–39.

Grüneberg, W. J., Abidin, E., Ndolo, P., Pereira, C. A., & Hermann, M. (2004). Variance component estimations and allocation of resources for breeding sweetpotato under East African conditions. *Plant Breeding*, *123*(4), 311-315.

Gunua, T. G. (2010). A review of sweet potato scab and studies on Fusarium wilt of sweet potato (*Ipomoea batatas* (L.) Lam.) and approaches to its management.

Han, K.-S., Park, J.-H., Back, C.-G. and Mi-Jeong. (2015). First Report of Fusarium subglutinans Causing Leaf Spot Disease on Cymbidium Orchids in Korea. *Mycobiology, 43*(3), 343–346.

Hegda, V.; Misra, R.S. and Jeeva, M.L. 2012. Sweet potato diseases: diagnosis and management. Fruit, Vegetable and Cereal Science and Biotechnology, 6 (Special Issue 1): 65-78

Heong, K. L., Escalada, M. M., Sengsoulivong, V., & Schiller, J. (2002). Insect management beliefs and practices of rice farmers in Laos. *Agriculture, ecosystems & environment*, *92*(2-3), 137-145.

Herrera, C. M., & Pellmyr, O. (Eds.). (2009). Plant animal interactions: an evolutionary approach. John Wiley & Sons.

https://cipotato.org/sweetpotato/sweetpotato-facts-and-figures/

https://www.cabi.org/isc/datasheet/28783

http://www.all-about-sweet-potatoes.com/history-origin-sweet-potato.html).

Ilondu, E. (2013). Etiology and assessment of leaf spot disease of sweet potato (Ipomoea batatas (L.) Lam) in selected farms in Delta State, Nigeria. *Agriculture and Biology Journal of North America*, *4*(4), 476-484.

Imazaki, I. and Kadota, I. (2015). Molecular phylogeny and diversity of Fusarium endophytes isolated from tomato stems . *FEMS Microbiology Ecology*, FEMS Microbiology Ecology in Eastern Province of Kenya. African Journal Health Sciences in Eastern Province of Kenya. *African Journal Health Sciences*.

Infonet-biovision. (2020). *Sweet potato*. Retrieved 02 26, 2020, from https://infonet-biovision.org/PlantHealth/Crops/Sweet-potato

Iqbal, Z, Khan, M. A, Sharif, M, (2018). An automated detection and classification of citrus plant diseases using image processing techniques: A review. Computer Electron Agric. 2018; 153:12–32.

Isin, S., & Yildirim, I. (2007). Fruit-growers' perceptions on the harmful effects of pesticides and their reflection on practices: The case of Kemalpasa, Turkey. *Crop protection*, *26*(7), 917-922.

Ismail, M. A., Moubasher, A., El-Eraky, A. M., El-Shaer, A. H., & Gouda, H. A. (2017). Virulence of wilt pathogens against pepper cultivars in Egypt. *International Journal of Technical Research & Science*, *1*(10), 304-314.

Jabłońska, E., Piątek, K., Wit, M., Mirzwa-Mróz, E. and Wakuliński, W. (2020). Molecular diversity of the Fusarium fujikuroi species complex from maize. *European Journal of Plant Pathology, 150*, 859–877.

Jaetzold R, Schmit H, Hornetz B, Shisanya B (2007). Farm management Handbook of Kenya Vol II. Second edition. PART C East Kenya. Nairobi.

Joshi, M.; Srivastava, R.; Sharma, A.K.; Prakash A. (2012). Screening of resistant cultivars and antagonistic *Fusarium oxysporum* for biocontrol of Fusarium wilt of chilli. *Plant Pathology and Microbiology, 3*, 134.

Juroszek P, von Tiedemann A (2011) Potential strategies and future requirements for plant disease management under a changing climate. *Plant Pathology*; 60:100–112.

Kalagatur, N.K., Kamasani, J.R. and Mudili V. (2018). Assessment of detoxification efficacy of irradiation on zearalenonemycotoxin in various fruit juices by response surface methodology and elucidation of its *in-vitro* toxicity. *Frontiers in Microbiology*, 9: 2937.

Kandolo, S., Aveling, T., van der Waals, J., Truter, M. and Laurie, S. (2018). Effects of wetness duration, inoculum concentration and temperature on the development of Alternaria blight on transplants of sweet potato. *Acta Hortic., 1204*, 211-218.

Kandolo, S., Thompson, A., Callitz, F., Laurie, S., Truter, M. and Aelin, T. (2016). Field tolerance of selected varieties to fungicide efficacy against Alternaria blight of sweet potato. *African Crop Science Journal*, *24*(3), 331 - 339.

Kansiime, C., Rutebemberwa, E., Asiimwe, B. B., Makumbi, F., Bazira, J., & Mugisha, A. (2015). Annual trends of human brucellosis in pastoralist communities of south-western Uganda: a retrospective ten-year study. *Infectious diseases of poverty*, *4*(1), 1-8.

Karyeija, R. F., Gibson, R. W. and Valkonen, J. P. (1998). The Significance of Sweet Potato Feathery Mottle Virus in Subsistence Sweet Potato Production in Africa. *Plant Disease, 82*(1), 4-15.

Kaur S., Dhillon G. S., Brar S. K., Vallad G. E., Chand R., Chauhan V. B. (2012). Biology, economic importance and current diagnostic trends. *Crit. Rev. Microbiol.* 38 136–151.

Kilui Wabwile, V. (2016). *Effect of Improved Sweet Potato Varieties on Household Food Security in Bungoma County, Kenya* (No. 634-2016-41507).

Kivuva, B. M., Musembi, F. J., Githiri, S. M., Yencho, C. G., & Sibiya, J. (2014). Assessment of production constraints and farmers' preferences for sweet potato genotypes. *Journal of Plant Breeding and Genetics*, *2*(1), 15-29.

Kokaeva, L. Y., Kudryavtzeva, N. N., Pobedinskaya, M. A., Statsyuk, N. V., Zaitchik, B. T. and Elansky, S. N. (2015). *Virulence of Alternaria strains toward potato and tomato cultivars.* Romania.

Kreuze, J. F., Perez, A., Untiveros, M., Quispe, D., Fuentes, S., Barker, I., & Simon, R. (2009). Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: a generic method for diagnosis, discovery and sequencing of viruses. *Virology*, *388*(1), 1-7.

Kumar K.N., Venkataramana M., Allen J.A., Chandranayaka S., Murali H.S., Batra H.V. (2016). Role of *Curcuma longa* L. essential oil in controlling the growth and zearalenone production of *Fusarium graminearum*. *LWT-Food Science and Technology*, 69:522–528

Lazarovits, G, Turnbull A., Johnston-Monje, D. (2014). Plant health management: biological control of plant pathogens. In: Van Alfen NK , editor. Encyclopedia of agriculture and food systems. New York, NY: Academic Press; p. 388–399.

Lebot, V. (2010). Sweet potato. In *Root and tuber crops* (pp. 97-125). Springer, New York, NY.

Lese, V., Holland, E., Wairiu, M., Havea, R., Patolo, S., Nishi, M. and Hoponoa, T. (2018). Facing food security risks: The rise and rise of the sweet potato in the Pacific Islands. *Global Food Security*, *18*, 48-56.

Leslie, J. F., & Summerell, B. A. (2006). Fusarium laboratory workshops—a recent history. *Mycotoxin Research*, *22*(2), 73-74.

Leslie, J. F., & Summerell, B. A. (2008). *The Fusarium laboratory manual*. John Wiley & Sons.

Lievens, B., Rep, M., & Thomma, B. P. (2008). Recent developments in the molecular discrimination of formae speciales of Fusarium oxysporum. *Pest Management Science: formerly Pesticide Science*, *64*(8), 781-788.

Liljeroth E, Lankinen A, Wiik L, (2016). Potassium phosphite combined with reduced doses of fungicides provides efficient protection against potato late blight in large-scale field trials. *Crop Prot*ection, 86:42–55.

Lindahl, B. D., Nilsson, R. H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjøller, R. & Kauserud, H. (2013). Fungal community analysis by high-throughput sequencing of amplified markers–a user's guide. *New phytologist*, *199*(1), 288-299.

Low J. W. (1997) Potato in southwest Uganda: threats to sustainable production. Afr Crop Sci J 5:395–412

Low, J. W. et al. 2009. Sweetpotato in Sub-Saharan Africa. In: Loebenstein, G.and Thottappilly, G. (eds.). The Sweet potato. Dordrecht: Springer Science + Business Media B.V

Low, J., Ball, A., Magezi, S., Njoku, J., Mwanga, R., Andrade, M. & Van Mourik, T. (2017). Sweet potato development and delivery in sub-Saharan Africa. *African Journal of Food, Agriculture, Nutrition and Development*, *17*(2), 11955-11972.

Magarey, R. D., Sutton, T. B., Thayer, C. L. (2012). A simple generic infection model for foliar fungal plant pathogens. *Phytopathology.* 95:92–100.

Mahantesh, S., Karegowda, C., Kavitha, S., Kavita, T. and Punith, N. (2017). In vitro evaluation of fungicides, bio agents and natural plant extracts against early blight caused by A. solani. *International Journal of Chemical Studies, 5*(5), 1346-1350.

Maharachchikumbura, S. S., Wu, S. P., Hyde, K. D., Al-Sadi, A. M. and Liu, Z. Y. (2016). First report of sweet potato leaf spot caused by Neopestalotiopsis ellipsospora in Guizhou Province, China. *Journal of Plant Pathology*, *98*(3), 686.

Maina, S. (2014). Detection, distribution and genetic diversity of sweetpotato leaf curl virus from western, coast and central regions of Kenya. *M.Sc. thesis, Kenyatta University, Nairobi, Kenya*.

Maina, S., Miano, D. W., Mbogo, E., Amimo, J. O., Irungu, J. and Njiruh, P. N. (2017). Occurrence and genetic variability of partial coat protein gene of Sweet potato leaf curl virus (SPLCV) in Kenya. *African Journal of Biotechnology, 16*, 2112-2120.

Makini, F., Mose, L., Kamau, G., Salasya, B., Mulinge, W., Ongala, J., . . . Oluwole, F. A. (2018). *Innovation Opportunities in Sweet Potato production in Kenya.* Accra Ghana: Forum for Agricultural Research in Africa (FARA) .

Martin, W. J. (2002). Control, Sweet Potato Diseases and their. *Root crop symposium*, 1-9.

Mattihalli C, Gedefaye E, Endalamaw F, (20118). Plant leaf diseases detection and auto-medicine. *IoT*, 1–2:67–73.

Mattihalli, C., Gedefaye, E., Endalamaw, F., & Necho, A. (2018). Plant leaf diseases detection and auto-medicine. *Internet of Things*, *1*, 67-73.

Maule, A. J., Caranta, C., & Boulton, M. I. (2007). Sources of natural resistance to plant viruses: status and prospects. *Molecular plant pathology*, *8*(2), 223-231.

Mcharo M., P. Ndolo, (2013) Root-yield performance of pre-release sweet potato genotypes in Kenya. J. Appl. Biosci., 65 (2013), pp. 4914 4921, 10.4314/jab.v65i0.89576.

Megan R.W. (2017, Health Benefits of Carrots: Medical News Today Newsletter 2(1) Pp2– 4

Miano, D. W., LaBonte, D. R., & Clark, C. A. (2008). Identification of molecular markers associated with sweet potato resistance to sweet potato virus disease in Kenya. *Euphytica*, *160*(1), 15-24.

Mkandawire, R. I., Yilmaz, N., Steenkamp, E. T., Kvas, M., Wingfield, M. J., & Fourie, G. (2022). Diversity of Fusarium species associated with healthy and malformed Syzygium cordatum inflorescences in South Africa. *European Journal of Plant Pathology*, 1-20.

Moretti A, Logrieco A, Bottalico A, Ritieni A, Randazzo G, Corda P. (1995). Beauveric in production by *Fusarium subglutinans* from different geographical areas. *Mycology Research*, 99:282–286. doi:10.1016/S0953-7562(09)80899.

Motsa, Nozipho M., Modi, Albert T. and Mabhaudhi, Tafadzwanashe. (2015). Sweet potato (Ipomoea batatas L.) as a drought tolerant and food security crop. *South African Journal of Science*, *111*(11-12), 1-8. https://dx.doi.org/10.17159/sajs.2015/20140252

Mukasa, S. B., Rubaihayo, P. R., & Valkonen, J. P. (2003). Incidence of viruses and virus like diseases of sweetpotato in Uganda. *Plant disease*, *87*(4), 329-335.

Mukhopadhyay, S.K., Chattopadhyay, A., Chakraborty, I. *et al.*,(2011). Crops that feed the world 5. Sweetpotato. Sweetpotatoes for income and food security. *Food Sec.* **3**, 283 (2011). https://doi.org/10.1007/s12571-011-0134-3

Munkvold GP. 2017. *Fusarium* species and their associated mycotoxins, p 51–106. *In* Moretti A, Susca A (ed), *Mycotoxigenic fungi*. Humana Press, New York, NY.

Munkvold, G. P., & White, D. G. (Eds.). (2016). *Compendium of corn diseases* (Vol. 165). St. Paul, MN: APS Press.

Musembi, K. B., Githiri, M. S., Yencho, C. G. and Sibiya, J. (2015). Combining ability and heterosis for yield and drought tolerance traits under managed drought stress in sweet potato. *Euphytica, 201*(3), 423–44.

Mwihia, T., Straetmans, M., Ibrahims, A., Njau, J., Muhenje, O., Nagrale, D. T., Gaikwad, A. P. and Sharma, L. (2013). Morphological and cultural characterization of Alternaria alternata (Fr.) Keissler blight of gerbera (Gerbera jamesonii H. Bolus ex J.D. Hook). *Journal of Applied and Natural Science, 5* (1), 171-178.

Nagrale, D. T., Gaikwad, A. P. and Sharma, L. (2013). Morphological and cultural characterization of Alternaria alternata (Fr.) Keissler blight of gerbera (Gerbera jamesonii H. Bolus ex J.D. Hook). *Journal of Applied and Natural Science, 5* (1), 171-178.

Nalini S, Parthasarathi R. Optimization of rhamnolipid biosurfactant production from *Serratia rubidaea* SNAU02 under solid-state fermentation and its biocontrol efficacy against Fusarium wilt of eggplant. *Ann Agric Sci*. 2018;16(2):108–115

Nedunchezhiyan M, Byju G (2005) Effect of planting season on growthand yield of sweet potato (*Ipomoea batatas* L.) varieties. Journal of Root Crops 31 (2), 111-114.

Nedunchezhiyan M., G. Byju, R.C. Ray, M. Nedunchezhiyan, G. Byju, R.C. Ray, Neela, S., & Fanta, S. W. (2019). Review on nutritional composition of orange-fleshed sweet potato and its role in management of vitamin A deficiency. *Food science & nutrition, 7*(6), 1920-1945.

Neindow, M., Sowely, E. N. K., & Abubakari, A. H. (2018). Farmers' knowledge and perceptions of leaf spot disease of groundnut and its management in Northern Region of Ghana. *Journal of Agricultural Biotechnology and Sustainable Development, 10*(9), 170-177.

Nilsson, R. H., Kristiansson, E., Ryberg, M., Hallenberg, N., & Larsson, K.-H. (2008). Intraspecific ITS variability in the kingdom fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evolutionary Bioinformatics*, **4**, EBO.S653.

Nirmaladevi, D., Venkataramana, M., Srivastava, R. K., Uppalapati, S. R., Gupta, V. K., Yli-Mattila, T., ... & Chandra, N. S. (2016). Molecular phylogeny, pathogenicity and toxigenicity of Fusarium oxysporum f. sp. lycopersici. *Scientific reports*, *6*(1), 1-14.

Nusaibah, S. A., & Musa, H. A. (2019). Review report on the mechanism of Trichoderma spp. as biological control agent of the Basal Stem Rot (BSR) disease of Elaeis guineensis. *Trichoderma-The Most Widely Used Fungicide*, 79.

Nwankwo, I. I. (2015). Evaluation of sweet potato seedlings for growth pattern and susceptibility to major leaf diseases of sweet potatoes. *Journal of Agricultural and Crop Research, 3*(5), 73-79.

Obopile, M., Munthali, D. C., & Matilo, B. (2008). Farmers' knowledge, perceptions and management of vegetable pests and diseases in Botswana. *Crop Protection, 27*(8), 1220-1224.

Ochieng, J., Afari-Sefa, V., Lukumay, P. J., & Dubois, T. (2017). Determinants of dietary diversity and the potential role of men in improving household nutrition in Tanzania. *PloS one, 12*(12), e0189022.

Ogolla, F. O., Onyango, B. O., Muraya, M. and Mulambula, S. (2018). Morphological Characterization of Sorghum *E. turcicum* Isolate from Different Areas of Tharaka Nithi County, Kenya. *The Journal of Scientific and Engineering Research, 5*(12), 218-226.

Ogolla, F., Nyakinywa, R., Chabari, S. and Onyango, B. (2021). In-vitro Evaluation of Fungicide Sensitivity of Tomato Leaf Blight Pathogens. *Jurnal Pertanian Tropik, 8*(1), 11-25.

Okoth, S., Rose, L., Ouko, A., Netshifhefhe, N., Sila, H. and Viljoen, A. (2017). Assessing Genotype-By-Environment Interactions in Aspergillus Ear Rot and Pre-Harvest Aflatoxin Accumulation in Maize Inbred Lines. *Agronomy*, *7*(4), 86. MDPI AG. Retrieved from http://dx.doi.org/10.3390/agronomy7040086

Okungbowa, F. I., & Shittu, H. O. (2012). Fusarium wilts: An overview. *Environ. Res. J*, *6*(2), 83-102.

Omar, H. N., Mohd, M., Izham, N. M., Nor, M. and Zakaria, L. (2018). Characterization and pathogenicity of Fusarium species associated with leaf spot of mango (Mangifera indica L.). *Microbial Pathogenesis, 114*, 362-368.

Onditi, J., Nyongesa, M. and van der Vlugt, R. (2020). Prevalence, distribution and control of six major potato viruses in Kenya. *Tropical plant pathology*. doi:10.1007/s40858-020-00409-x

Osiru, M., Adipala, E., Olanya, O. M., Lemaga, B., & Kapinga, R. (2007). Occurrence and distribution of Alternaria leaf petiole and stem blight on sweetpotato in Uganda. *Plant Pathology Journal*, *6*(2), 112-119.

Otoo, J.A., Missah, A., Osei, C., Manu-Aduening, J., Carson, A.G., Oduro, I., Okai, D.B., Marfo, K.A., 2000. The release of the first sweet potato varieties in Ghana, in: Proceedings of the Triennial Symposium of ISTRC. Japan.

Ouko, A., Okoth, S., Amugune, N. and Vesa, J. (2018). Field assessment of agronomic performance resistance to aflatoxin and fumonisin accumulation in selected maize inbred lines in Kenya. Agricultural Forests and Fisheries, 7,94-100.

Roullier C., Benoit L., McKey D.B., Lebot V(2013). Historical collections reveal patterns of diffusion of sweet potato in Oceania obscured by modern plant movements and recombination. *Proc. Natl. Acad. Sci. USA.* 110:2205–2210.

Parry, D. W. (1990). *Plant pathology in Agriculture.* New York: Cambridge University Press.

Paul, N.C.; Park, W.; Lee, S.; Chung, M.N.; Lee, H.-U.; Yang, J.-W. Occurrence of Sweetpotato (*Ipomoea batatas*) Wilt and Surface Rot Disease and Determining Resistance of Selected Varieties to the Pathogen in Korea. *Plants* **2020**, *9*, 497.

Pegg, K. G., Coates, L. M., O'Neill, W. T. and Turner, D. W. (2019). The Epidemiology of Fusarium Wilt of Banana. (M. Dita, Ed.) *Frontier in Plant Sciences , 10*, 1395.

Pfordt, A., Ramos Romero, L., Schiwek, S., Karlovsky, P. and von Tiedemann, A. (2020). Impact of Environmental Conditions and Agronomic Practices on the Prevalence of *Fusarium* Species Associated with Ear- and Stalk Rot in Maize. *Pathogens (Basel, Switzerland)*, *9*(3), 236. https://doi.org/10.3390/pathogens9030236.

Quisumbing, A. R. (1995). *Gender differences in agricultural productivity: A survey of empirical evidence. FCND DISCUSSION PAPER NO. 5.* Retrieved from https://core.ac.uk/download/pdf/6289465.pdf

Rowlandson T, Gleason M, Sentelhas P, Gillespie T, Thomas C, Hornbuckle B. (2015). Reconsidering leaf wetness duration determination for plant disease management. *Plant Diseases*, 99:310– 319.

Rukarwa, R. J., Mashingaidze, A. B., Kyamanywa, S., & Mukasa, S. B. (2010). Detection and elimination of sweetpotato viruses. *African Crop Science Journal*, *18*(4).

Ruto, A. C., Gachene, C., Gicheru, P., Mburu, D., & Khalif, Z. (2017). Crop Yields along the Toposequence of Terraced Andosols in Narok, Kenya. *Tropical and Subtropical Agroecosystems*, *20*(1).

Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406-425.

Salazar-Cerezo, S., Meneses-Sánchez, M. d., Martínez-Contreras, R. D. and Martínez-Montiel, N. (2020). Unraveling the Fungal Community Associated. *Microorganisms, 8*, 459.

Saqib, M., Khalid, M.F., Hussain, S., Anjum, M.A. (2017). Effect of water stress and planting system on growth, yield and quality of sweet potato. Acta Sci. Pol. Hortorum Cultus, 16(6), 201–210. DOI: 10.24326/asphc.2017.6.18

Sarsaiya S, Archana Jain, Qin Wu, Yuanfu Lu & Jingshan Shi (2019) A review of plant leaf fungal diseases and its environment speciation, Bioengineered, 10:1, 409-424.

Sarsaiya S, Shi J, Chen J. A comprehensive review on fungal endophytes and its dynamics on Orchidaceae plants: current research, challenges, and future possibilities. *Bioengineered*. 2019; 10(1):316–334.

Scattolini, A., Hernández, R. L. and González, I. H. (2020). Identification of Fusarium oxysporum causing sweet potato rot (Ipomoea batatas L & Agrociencia Lamb) in Uruguay. *Agrociencia Uruguay*, *24*(1), 124.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A. ... & Fungal Barcoding Consortium. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences*, *109*(16), 6241-6246.

Schreinemachers, P., Balasubramaniam, S., Boopathi, N. M., Ha, C. V., Kenyon, L., Praneetvatakul, S. & Wu, M. H. (2015). Farmers' perceptions and management of plant viruses in vegetables and legumes in tropical and subtropical Asia. *Crop Protection*, *75*, 115-123.

Scruggs, A. C., and Quesada-Ocampo, L. M. 2016. Etiology and epidemiological conditions promoting Fusarium root rot in sweetpotato. Phytopathology 106:909-919.

Shahbandeh, M. (2021). Global sweet potato production by country 2019. Retrieved 02 26, 2021, from https://www.statista.com/statistics/812359/global-sweet-potato-production-by-country/

Sharma, M., Deep, S., Bhati, D. S., Chowdappa, P., Selvamani, R. and Sharma, P. (2013). Morphological, cultural, pathogenic and molecular studies of Alternaria brassicae infecting cauliflower and mustard in India. *African Journal of Microbiology Research, 7*(26), 3351-3363.

Shiferaw, B., Prasanna, B. M., Hellin, J., & Bänziger, M. (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food security*, *3*(3), 307-327.

Sindi, K. and Wambugu, S. (2012). *Going to scale with sweet potato vines.* Nairobi: International Potato Center.

Singha, M., Wu, B., & Zhang, M. (2016). An object-based paddy rice classification using multi-spectral data and crop phenology in Assam, Northeast India. *Remote Sensing*, *8*(6), 479.

Skoglund, L. and Smit, N. (1994). Major Diseases and Pests of sweetpotato in East Africa. *CIP*, 67-69.

Skoglund, L., Gatumbi, R. and Kihurani, A. (1993). Non-viral foliar pathogens and disorders of sweetpotato in Kenya. *International Journal of Pest Management, 39*, 452-458.

Sseruwagi, P., Rey, M. E. C., Brown, J. K., & Legg, J. P. (2004). The cassava mosaic geminiviruses occurring in Uganda following the 1990s epidemic of severe cassava mosaic disease. *Annals of Applied Biology*, *145*(1), 113-121.

Sseruwu, G., Shanahan, P., Melis, R. and Ssemakula, G. (2015). Farmers awareness and perceptions of alternaria leaf petiole and stem blight and their preferred sweetpotato traits in Uganda. *Journal of Plant Breeding and Genetics, 03*(02), 25-37.

Sseruwu, G., Shanahan, P., Melis, R. and Ssemakula, G. (2016). Genetic analysis of resistance to Alternaria leaf petiole and stem blight of sweetpotato in Uganda. *Euphytica, 3*, 393-404.

Stathers, T., Low, J., Mwanga, R., Carey, T., McEwan, M., David., S., Gibson, R., Namanda, S., McEwan, M., Malinga, J., Ackatia-Armah, R., Benjamin, M., Katcher, H., Blakenship, J., Andrade, M., Agili, S., Njoku, J., Sindi, K., Mulongo, G., Tumwegamire, S., Njoku, A., Abidin, E., Mbabu, A., Mkumbira, J., Ogero, K., Rajendran, S., Okello, J., Bechoff, A., Ndyetabula, D., Grant, F., Maru, J., Munyua, H., Mudege, N., Muzhingi, T. (2018). Everything You Ever Wanted to Know About Sweet potato: Reaching Agents of Change ToT Manual. International Potato Center, Lima, Perú. https://hdl.handle.net/10568/98334 12 vols. 664 p.

Stokes, C. E., R. A. Arancibia and R. E. Baird. (2013). Diversity and density indices of the pathogenic microbial community present in tip/end rot disease of sweetpotato. HortScience

Sun, S., Lui, Q., Han, L., Ma, Q., He, S., Li, X., . . . Liya, W. (2018). Identification and Characterization of Fusarium proliferatum, a New Species of Fungi that Cause Fungal Keratitis. *Scientific Reports, 8*, 4859.

Taber, K. S. (2018). The use of Cronbach's alpha when developing and reporting.

Tabu, A. J. (2000). Reaction Of Ipomoea batatas (L) Lam Lines To Alternaria Leaf And Stem Blight And Effects Of Soil Ph On Disease Severity. *Msc_ Thesis, University of Nairobi*.

Tai H.M., Peng G.L., Jaspreet S.(2019. Sweet potato: origin and production,Academic Press,Pages 5-25,ISBN 9780128136379, https://doi.org/10.1016/B978-0-12-813637-9.00002-8.

Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (*USA*) 101:11030-11035.

Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* https://doi.org/10.1093/molbev/msab120.

Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **2013**, *30*, 2725–2729.

Taylor, J. W., Jacobson, D. J., Kroken, S., Kasuga, T., Geiser, D. M., Hibbett, D. S., & Fisher, M. C. (2000). Phylogenetic species recognition and species concepts in fungi. *Fungal genetics and biology*, *31*(1), 21-32.

Thanaa, M. A., Farag, M. and Hanaa, G. A. (2018). Fusarium Wilt of Sweet Potato Caused by Fusarium oxysporum f. sp. batatas in Egypt. *Egypt. J. Phytopathol., 46*(1), 21-35.

Thompson, A., Narayanin, C. and Smith, M. M. (2011). A disease survey of Fusarium wilt and Alternaria blight on sweet potato in South Africa. *Crop Protection, 30*(11), 1409-1413.

Truong, V., Avula, R. Y., Pecota, K. V. and Yencho, G. C. (2018). Sweetpotato Production, Processing, and Nutritional Quality. In M. Siddiq and M. A. Uebersax (Eds.), *Handbook of Vegetables and Vegetable Processing, Volume II, Second Edition. Edited by Muhammad Siddiq and Mark A. Uebersax* (Second Edition ed., Vol. II, pp. 811-836). John Wiley & Sons Ltd. Retrieved from https://www.ars.usda.gov/ARSUserFiles/60701000/Sweetpotato%20Publications/s158.pdf

Tugume, A. K., Mukasa, S. B. and Valkonen, J. P. (2016). Mixed Infections of Four Viruses, the Incidence and Phylogenetic Relationships of Sweet Potato Chlorotic Fleck Virus (Betaflexiviridae) Isolates in Wild Species and Sweet potatoes in Uganda and Evidence of Distinct Isolates in East Africa. *PLoS ONE, 11*(12), e0167769.

Van den Bosch, F., Jeger, M. J., & Gilligan, C. A. (2007). Disease control and its selection for damaging plant virus strains in vegetatively propagated staple food crops; a theoretical assessment. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1606), 11-18.

Van Jaarsveld, P. J., Faber, M., Tanumihardjo, S. A., Nestel, P., Lombard, C. J., & Benadé, A. J. S. (2005). β-Carotene–rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. *The American journal of clinical nutrition*, *81*(5), 1080-1087.

Vellinga, E. C., de Kok, R. P., & Bruns, T. D. (2003). Phylogeny and taxonomy of Macrolepiota (Agaricaceae). *Mycologia*, *95*(3), 442-456.

Vismer, H. F., Shephard, G. S., Van der Westhuizen, L., Mngqawa, P., Bushula-Njah, V., & Leslie, J. F. (2019). Mycotoxins produced by Fusarium proliferatum and F. pseudonygamai on maize, sorghum and pearl millet grains in vitro. *International journal of food microbiology*, *296*,

W. Low, R.O.M. Mwanga, M. Andrade, E. Carey, A.-M. Ball.Tackling vitamin A deficiency with biofortified sweetpotato in sub-Saharan Africa Glob. Food Sec, 14 (2017), pp. 23-30.https://doi.org/10.1016/j.gfs.2017.01.004

Waghunde, R. R., Shelake, R. M., & Sabalpara, A. N. (2016). Trichoderma: A significant fungus for agriculture and environment. *African journal of agricultural research*, *11*(22), 1952-1965.

Wambui, C. (2020). Kenyan farmers find sweet spot with nutritious, drought-hardy crop. Retrieved 07/03/2021.

Wanjala, B. W., Ateka, E. M., Miano, D. W., Low, J. W. and Kreuze, J. F. (2020). Storage root yield of sweetpotato as influenced by sweetpotato leaf curl virus and its interaction with sweetpotato feathery mottle virus, and sweetpotato chlorotic stunt virus in Kenya. *104*(5), 1477-1486. Retrieved from https://www.biorxiv.org/content/10.1101/795518v1.full.pdf

White, T. J., Bruns, T., Lee, S. J. W. T., and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*, *18*(1), 315-322.

Wokorach, G., Edema, H., Muhanguzi, D. and Echodu, R. (2019). Prevalence of sweetpotato viruses in Acholi sub-region, northern Uganda. *Current Plant Biology, 17*, 42-47.

Yang, Z.; Lin, Y.; Chen, H.; Zou, W.; Wang, S.; Guo, Q.; Chen, X. A rapid seedling assay for determining sweetpotato resistance to Fusarium wilt. *Crop Sci.* 2018, *58*, 1558–1565.

Zakaria, Z. Z., Benslimane, F. M., Nasrallah, G. K., Shurbaji, S., Younes, N. N., Mraiche, F. ... & Yalcin, H. C. (2018). Using zebrafish for investigating the molecular mechanisms of drug-induced cardiotoxicity. *BioMed research international*, *2018*.

Zamir K. Punja (2020) First report of *Fusarium proliferatum* causing crown and stem rot, and pith necrosis, in cannabis (*Cannabis sativa* L., marijuana) plants, Canadian Journal of Plant Pathology, DOI: 10.1080/07060661.2020.1793222.

Zhang, Y. and Ma, L.-J. (2017). Deciphering Pathogenicity of Fusarium oxysporum From a Phylogenomics Perspective. *Advances in Genetics*, *100*, 179-202.

APPENDICES
APPENDIX 1: BOARD POST GRADUATE LETTER

JARAMOGI OGINGA ODINGA UNIVERSITY OF SCIENCE & TECHNOLOGY

BOARD OF POSTGRADUATE STUDIES
Office of the Director

Tel. 057-2501804
Email: bps@jooust.ac.ke

P.O. BOX 210 - 40601
BONDO

Our Ref: S151/4161/2017

Date: 23rd March 2021

TO WHOM IT MAY CONCERN

RE: ADERA NORAH – S151/4161/2017

The above person is a bonafide postgraduate student of Jaramogi Oginga Odinga University of Science and Technology in the School of Biological, Physical, Mathematics and Actuarial Sciences pursuing Master of Science in Microbiology. She has been authorized by the University to undertake research on the topic: ***"Occurrence and Characterization of Fungal Leaf Spot Disease of Sweet Potato (Ipomoea batatas) and Varietal Susceptibility to the Infection in Parts of Western Kenya".***

Any assistance accorded her shall be appreciated.

Thank you.

JARAMOGI OGINGA ODINGA UNIVERSITY OF SCIENCE & TECHNOLOGY
DIRECTOR BOARD OF POST GRADUATE STUDIES
OCT 2022
P.O. BOX 210 - 40601 BONDO, KENYA

Prof. Dennis Ochuodho
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

APPENDIX II: Ethics and Review Committee Letter

JARAMOGI OGINGA ODINGA
UNIVERSITY OF SCIENCE AND TECHNOLOGY
DIVISION OF RESEARCH, INNOVATION AND OUTREACH
JOOUST-ETHICS REVIEW OFFICE

Tel. 057-2501804 P.O. BOX 210 - 40601
Email: erc@jooust.ac.ke **BONDO**
Website: www.jooust.ac.ke

OUR REF: JOOUST/DVC-RIO/ERC/E4 **1st November, 2022**

Norah Adera
S152/4161/2017
JOOUST

Dear Mr. Adera,

RE: APPROVAL TO CONDUCT RESEARCH TITLED "OCCURRENCE AND CHARACTERIZATION OF FUNGAL LEAF SPOT DISEASE OF SWEET POTATO *(ipomoea batatas)* AND VARIETAL SUSCEPTIBILITY TO THE INFECTION IN PARTS OF WESTERN KENYA"

This is to inform you that JOOUST ERC has reviewed and approved your above research proposal. Your application approval number is **ERC 34/11/22-07/03.**The approval period is from 1st November, 2022– 31st October, 2023.

This approval is subject to compliance with the following requirements:

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations and violations) are submitted for review and approval by JOOUST IERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to NACOSTI IERC within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks of affected safety or welfare of study participants and others or affect the integrity of the research must be reported to NACOSTI IERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to JOOUST IERC.

Prior to commencing your study, you will be expected to obtain a research permit from National Commission for Science, Technology and Innovation (NACOSTI) https:oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

For
Prof. Francis Anga'wa
Chairman, JOOUST ERC

Copy to: Deputy Vice-Chancellor, RIO Director, BPS DEAN, SBPMAS/dm

APPENDIX III: Farmers Questionnaire

My name is Norah Aderah. I am a student of Jaramogi Oginga Odinga University of Science and Technology carrying out a research on **Characterization of Fungal Leaf spot disease and Varietal Susceptibility in sweet potato (*Ipomoea batatas*) in Western Kenya**

I have identified you as one of the respondents to give me information regarding this study. The information will be strictly used for this study and is confidential.

Part A: personal Questions

Level of farming ☐ Subsistence ☐ Commercial ☐

Part B: Questions on Production

1. Where do you obtain the sweet potato vines for planting?

   Grocery ☐ Last season crop ☐ Froma friend ☐

2. Do you use inorganic fertilizers in sweet potato farming?

   Yes ☐ No ☐

3. For how long have you been growing sweet potatoes on your farm? .....

4. Do you grow sweet potatoes continuously? Yes ☐ No ☐

   a) Which varieties have you been growing? .....

   b) Which one did you grow this season? .....

   c) What is the age of your sweet potato crop……

   d) What planting system did you use……

5. Have you experienced any diseases in your sweet potato farm?

   a. Yes ☐ b. No ☐

   a) If yes what are the symptoms? (Interrogate on symptoms development)

6. Are you able to differentiate the sweet potato diseases in your farm?

Not at all ☐ Some diseases ☐ All the diseases ☐

   a) Which ones do you know……..

   b) What are the symptoms……

   c) What is the indigenous name of the diseases……..

6. Do you know about sweet potato fungal leaf spot disease? a) Yes ☐

   b) No ☐

7. What are the symptoms of the disease?

8. At what age is the sweet potato affected by the infection most.

a) Which variety is more susceptible to the disease?

b) Which variety is least susceptible to the disease?

9. a) What is the history of the disease?

b) How long has your sweet potato suffered from the disease.

c) What is the effect(s) of the disease on sweet potato leaves, vines and tubers?

Thank you for your participation in this research.

Appendix IV: Analysis of Variance Table for Incidence

Source	DF	Sum of Squares	Mean Square	F Value	p-value
Model	19	8098.22287	426.22226	20.48	<.0001
Error	970	20191.36788	20.81584		
Corrected Total	989	28289.59075			

Source	DF	Type III SS	Mean Square	F Value	p-value
Sub Counties	2	1018.2016	509.100838	24.46	<.0001
Replication	2	220.623062	110.311531	5.30	0.0051
Village	15	6859.3981	457.293209	21.97	<.0001

R-Square	Coeff Var	Root MSE	Log_Incidence Mean
0.286262	20.01564	4.562438	22.79436

Appendix V: Analysis of Variance Table for Severity

Source	DF	Sum of Squares	Mean Square	F Value	p-value
Model	19	15.96376391	0.84019810	13.84	<.0001
Error	966	58.66009979	0.06072474		
Corrected Total	985	74.62386370			

Source	DF	Type III SS	Mean Square	F Value	p-value
Region	2	9.01244036	4.50622018	74.21	<.0001
Replication	2	1.34259815	0.67129907	11.05	<.0001
Village	15	5.60872541	0.37391503	6.16	<.0001

R-Squar	Coeff Var	Root MSE	Log_Incidence Mean
0.213923	20.26079	0.246424	1.216260

Appendix VI: Analysis of Variance Table for Susceptibility Screening

Source		Sum of Squares	Mean Square	F Value	p-value
Model	8	5550.116178	693.764522	9.77	<.0001
Error	36	2557.375987	71.038222		
Corrected Total	44	8107.492164			
Source	DF	Type III SS	Mean Square	F Value	p-value
Week	4	3573.412609	893.353152	12.58	<.0001
Replication	2	26.518738	13.259369	0.19	0.8305
Variety	2	1950.184831	975.092416	13.73	<.0001
R-Squar	Coeff Var		Root MSE		Log_Incidence Mean
0.684566	32.27190		8.428418		26.11689

Appendix VII: Zymo Research Fungal/Bacterial DNA MiniPrep ™ Short Protocol

The extraction of DNA was carried out according to the proctocal recommended by the manufacturer (Zymo Research cooperation)

1. Add 50 – 100 mg (wet weight) fungal cells that have been re suspended in up to 200 µl of water or isotonic buffer (e.g., PBS) or up to 200 mg of tissue to a ZR BashingBead™ Lysis Tube (0.1 mm & 0.5 mm). Add 750 µl Lysis Solution to the tube.
2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for 5 minutes.
3. Centrifuge the ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm) in a micro centrifuge at 10,000 x g for 1 minute.
4. Transfer up to 400 µl supernatant to a Zymo-Spin™ IV Spin Filter (Orange Top) in a Collection Tube and centrifuge at 7,000 x g for 1 minute.
5. Add 1,200 µl of Genomic Lysis Buffer to the filtrate in the Collection Tube from Step 4.
6. Transfer 800 µl of the mixture from Step 5 to a Zymo-Spin™ IIC Column in a Collection Tube and centrifuge at 10,000 x g for 1 minute.
7. Discard the flow through from the Collection Tube and repeat Step 6.
8. Add 200 µl DNA Pre-Wash Buffer to the Zymo-Spin™ IIC Column in a new Collection Tube and centrifuge at 10,000 x g for 1 minute. 9.
9. Add 500 µl g-DNA Wash Buffer to the Zymo-Spin™ IIC Colum n and centrifuge at 10,000 x g for 1 minute.
10. Transfer the Zymo-Spin™ IIC Column to a clean 1.5 ml micro centrifuge tube and add 100 µl (35 µl minimum) DNA Elution Buffer directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA. The eluted DNA was stored at -20ºC for further molecular assessments.

Appendix VIII: Gel electrophoresis protocol

Separation of the DNA material was done according to Kuhn *et al*., (2018) as follows:

Preparation of the Gel

1. Weigh out the appropriate mass (0.7%) of agarose into an Erlenmeyer flask.

2. Add running buffer to the agarose-containing flask. Swirl to mix. The most common gel running buffers are TAE (40 mM Tris-acetate, 1 Mm EDTA) and TBE (45 mM Trisborate, 1 mM EDTA).

3. Melt the agarose/buffer mixture over a Bunsen flame. At 30 s intervals, remove the flask and swirl the contents to mix well. Repeat until the agarose has completely dissolved.

4. Add ethidium bromide (EtBr) to a concentration of 0.5 μg.

5. Allow the agarose to cool either on the bench top or by incubation in a 65 °C water bath. Failure to do so will warp the gel tray.

6. Place the gel tray into the casting apparatus and place an appropriate comb into the gel mold to create the wells.

7. Pour the molten agarose into the gel mold. Allow the agarose to set at room temperature. Remove the comb and place the gel in the gel box.

Setting up of Gel Apparatus and Separation of DNA Fragments

1. Add loading dye to the DNA samples to be separated. Gel loading dye is typically made at 6X concentration (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol). 161 Loading dye helps to track how far your DNA sample has traveled, and also allows the sample to sink into the gel.

2. Program the power supply to desired voltage (1-5V/cm between electrodes).

3. Add enough running buffer to cover the surface of the gel. It is important to use the same running buffer as the one used to prepare the gel.

4. Attach the leads of the gel box to the power supply. Turn on the power supply and verify that both gel box and power supply are working.

5. Remove the lid slowly and carefully load the DNA sample(s) into the gel. An appropriate DNA size marker should always be loaded along with experimental samples.

6. Replace the lid to the gel box. The cathode (black leads) should be closer the wells than the anode (red leads). Double check that the electrodes are plugged into the correct slots in the power supply.

7. Turn on the power. Run the gel until the dye has migrated to an appropriate distance.

Observing Separated DNA fragments

1. When electrophoresis has completed, turn off the power supply and remove the lid of the gel box.

2. Remove gel from the gel box. Drain off excess buffer from the surface of the gel. Place the gel tray on paper towels to absorb any extra running buffer.

3. Remove the gel from the gel tray and expose the gel to uv light. This is most commonly done using a gel documentation system.