Effects of Major Fungal Diseases on Performance of Sorghum Germplasm under Field Trials in KALRO Kiboko and Ithookwe in Lower Eastern Kenya

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DECLARATION

I understand that plagiarism is an offence and therefore declare that this research thesis is my original work and has not been presented to any other institution for any other award.

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DEDICATION

This project is dedicated to my dad (John Manyonge), mother (Agnes Masinde), aunts (Metrine, Caroline and Bridgit) and siblings (Martin, Sylvia and Mellan). Thank you for your persistent support and encouragement.

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ABBREVIATIONS AND ACRONYMS

AEZs	**:**	Agroecological zones
AMRI	**:**	Agricultural mechanization research institute
ANOVA	**:**	Analysis of variance
ASALs	**:**	Arid and semi-arid lands
BLAST	**:**	Basic alignment search tool
CIMMYT	**:**	The international maize and wheat improvement center.
CTAB	**:**	Cetyltrimethylammonium bromide
DNA	**:**	Deoxyribonucleic acid
EDTA	**:**	Ethylenediaminetetraacetic acid
EtBr	**:**	Ethidium bromide
FDI	**:**	Fungal disease incidence
FDS	**:**	Fungal disease severity
IBPGR	**:**	International board for plant genetic resources
ICRISAT	**:**	International crops research institute for semi- arid tropics
ITS	**:**	Internal transcribed spacer
JgMV	**:**	Johnson grass mosaic virus
KALRO	**:**	Kenya agricultural and livestock research organization
LSD	**:**	Least significant difference
MDMV	**:**	Maize dwarf mosaic virus
Ml	**:**	millilitre
NaCl	**:**	Sodium chloride
PCR	**:**	Polymerase chain reaction
PDA	**:**	Potato dextrose agar
RCBD	**:**	Randomized complete block design
SCMV	**:**	Sugarcane mosaic virus
SDS	**:**	Sodium dodecyl sulphate
SrMV	**:**	Sorghum mosaic virus
TBE	**:**	Tris borate EDTA
µL	**:**	microliter
50% DFL	**:**	Days to 50% flowering

STG	**:**	Stay green
LA	**:**	Leaf area
DBM	**:**	Dry matter yield
GRY	**:**	Grain yield
GRW	**:**	Grain weigh for ten sampled plants

ABSTRACT

Sorghum is a vital cereal crop especially in the ASALs of Kenya with potential of reducing food insecurity. It's uses range from food for human consumption, feeds for livestock and as raw materials in industrial processes. However, its potential production is limited by fungal diseases. Therefore, this study aimed at assessing the effects of major fungal diseases on growth and yield of selected sorghum germplasm through field trials. enhanced management of fungal diseases. Fourteen sorghum germplasm, including eight improved genotypes bred for drought tolerance, yield improvement and grain quality improvement from ICRISAT and six local land races from farmers, were subjected to a two-season field experiment laid out in Randomized Complete Block Design (RCBD) with four replications conducted at sub-centers of KALRO (Kiboko and Ithookwe) located in different AEZs of Kenya's ASALs. Natural infection of sorghum germplasm involved use of fungal-infected spreader rows. Sorghum plants were sampled from the first to the fourth month to record disease incidence and severity, identify major fungal pathogens, and determine their effects on growth (plant height, number of green leaves, leaf area and days to 50% flowering) and yield (biomass yield, grain yield, grain weight) parameters. At soft dough stage, symptomatic leaf samples were collected and taken to the laboratory for isolation and identification of fungal pathogens by both morphological and molecular methods. Major fungal diseases and their severity scores at KALRO, Kiboko were: leaf blight (6.1), anthracnose (5.5), rust (5.3), gray leaf spot (3.1), oval leaf spot (2.6) ladder leaf spot (2.1), downy mildew (2.3) and covered kernel smut (1.1), while at KALRO, Ithookwe, were: 4.8, 5.6, 4.6, 1.8, 1.6, 1.4, 2.1 and 1.1, respectively. Major fungal pathogens isolated included: *Fusariuum spp*, *Curvularia spp*, *Alternaria spp*, *Bipolaris spp*, *Exerohilum spp*, *Colletotrichum spp* and *Epicoccum spp*. Phylogenetic analysis revealed representative isolates as: *Curvularia akaiiensis, Curvularia lunata, Bipolaris secalis, Exserohilum rostratum, Fusarium napiforme, Alternaria alstroemeriae* (two isolates) *and Epicoccum sorghinum*. Two improved genotypes (Makueni local and Kiboko local 2) recorded, higher: plant height, days to 50% flowering, number of green leaves, leaf area and dry biomass compared to other germplasm. Significant ($P \leq 0.001$) negative correlations were observed between FDS and days to 50% DFL ($r = -0.741$), STG ($r = -0.813$) and LA ($r = -0.543$) indicating that fungal diseases inhibited sorghum

growth. Correlations between FDS and yield data (DBM, GRY and GRW) were also negative but insignificant (P>0.05). Seven improved genotypes (Makueni Local, Kiboko Local 2, IESV 24029 SH, Marcia, KARII Mtama 1, Serena and Seredo) showed the least mean FDS scores across several diseases and had significantly higher GRY (1.9 - 2.8 t/ha) compared to local varieties that showed higher FDS and lower yield (<1.9 t/ha). Future studies need to: focus on evaluating and developing biocontrol methods to manage fungal pathogens infecting sorghum crops in lower Eastern Kenya. Improved varieties from ICRISAT that are superior to local landraces should be tested in other agro-ecological zones of Arid and semi-arid lands to confirm their tolerance stability.
Key words*: Sorghum; fungal diseases; pathogens; growth; yield; tolerance; ASALs.*

CHAPTER ONE: INTRODUCTION

1.1 Background information

Sorghum (*Sorghum bicolor* L. Moench.) originated from the tropical area of Africa with oldest records of cultivation dating back to 300 B.C. in Egypt (Mwadalu and Mwangi, 2013). Globally, it is grown throughout the tropics, semi-tropics and arid regions (Mwadalu and Mwangi, 2013). It is ranked world's fifth most important cereal after maize, rice, barley and wheat (Taylor, 2003), and third in Sub-Saharan Africa after maize and rice (Mengistu *et al*., 2018). Globally, it is grown in nearly 40.67 million hectares with estimated production of 57.60 million tones. Majority of areas growing sorghum are in Africa (66.6%), Asia (18.5%) followed by Americas (13.3%) (Das *et al.*, 2020). In Eastern and central Africa, it is approximated to be grown in 10 million hectares with Sudan accounting for 21.4%, Ethiopia 7.3%, Tanzania 3.5%, Uganda 2%, Rwanda 0.8% and Kenya 0.6% (Mitaru *et al*., 2012).

Sorghum is the third most important cereal in Kenya after maize and wheat in terms of production (Mwadalu and Mwangi, 2013), with main production areas concentrated in Nyanza, Western and Eastern regions (Oyier *et al*., 2016). It has the potential of alleviating both poverty and malnutrition in Arid and Semi-Arid lands (ASALs) due to possessing various nutritional attributes and its adaptability to low-moisture and fertile soils (International Fund for Agricultural Development, 2018). Some of its uses include: feeds for livestock, food for human consumption and industrial uses in baking, brewing and ethanol processing due to its diversity (Oyier *et al.*, 2016). It also not only contains sufficient concentrations of minerals namely: phosphorus, potassium, calcium, zinc, magnesium, iron, and sodium, but also vitamins like; A, B, D, E, K and β-carotene (Kazungu *et al*., 2023).

Although the estimated annual national sorghum production in 2022/2023 seasons was at 0.8 t/ha (United States Department of Agriculture, 2023), this is far below the crop's production potential which is estimated between 2 - 5 t/ha (Kilambya and Witwer, 2013). This is as a result of abiotic as well as biotic stresses such as striga weed, pests and diseases (Mitaru *et al*., 2012). Anthropogenic activities and climate variability have

proliferated the development of more virulent pathogens and new fungal diseases infecting sorghum (Dania and Adedoyin, 2020). The major diseases of sorghum caused by pathogenic fungi include: anthracnose (*Colletotrichum sublineolum* Henn), leaf blight (*Exserohilum turcicum*), smuts: covered kernel smut (*Sporisorium sorghi* Ehrenberg (Link), loose smut (*Sphacelotheca cruenta* (Kuhn), Head smut (*Sporisrium reilianum* (Kuhn) and long smuts (*Tolyposporium entrenbargii* (Kuhn)), downy mildew *Peronoscleropora sorghi*), rusts (*Puccinia purpurea*), Ergot (*Claviceps africana*), grain mold and charcoal rot (Mengistu *et al*., 2018; Teferi and Wubshet, 2015; Thakur *et al*., 2007b; Ngugi *et al*., 2002). These diseases can occur in combinations or as single, causing significant damage and high losses annually (Thakur *et al*., 2007b).

Incidences of these fungal diseases may change with agro-ecological zones due to variations in climatic, soil factors, physical geography and farming practices (Ngugi *et al*., 2002). For example; Ngugi *et al*. (2002) reported high level of anthracnose and ladder leaf spot in humid areas with elevated humidity and rainfall, oval leaf spot in lower rainfall zones while rust was not influenced by rainfall and humidity, hence was widespread in all areas surveyed. There is need for monitoring of diseases in sorghum production systems due to annual variation in weather conditions and dynamics of plant diseases (Anitha *et al*., 2020). Preliminary studies have majorly explored the distribution, incidence, severity of sorghum diseases (Ogolla *et al*., 2019; Teferi and Wubshet, 2015; Ngugi *et al*., 2002) and significance of fungal diseases in different agro-ecologies (Tsedaley *et al*., 2016). However, they fall short of providing extensive and quantitative data on effects of major fungal diseases on growth and yield of various sorghum varieties under field trials. This is especially true for sorghum genotypes improved for various traits, but not evaluated for fungal resistance in Kenya. Broadly, the aim of this study was to evaluate the effects of major fungal diseases on performance of selected sorghum varieties in two agro-ecological zones of lower eastern Kenya.

1.2 Statement of the problem

Over the years, low sorghum yields have been realized within the dry lower Eastern region of the country (Thuranira, 2015). This has been attributed biotic stresses, such as

diseases caused by fungi, bacteria and viruses which compromise both the quantity and quality of both grain and fodder (Anitha *et al*., 2020). Some of the fungal diseases which infect sorghum include; anthracnose, leaf blight, smuts, grain mold, rust, charcoal rot, downy mildew, and ergot (Mengistu *et al*., 2018; Njoroge *et al*., 2018; Teferi and Wubshet, 2015; Ngugi *et al*., 2002). Anthracnose causes yield loss of up to 67% in varieties that are susceptible under conditions of high temperature and humidity, while leaf blight leads to losses up to 50% (Mengistu *et al*., 2018). Grain mold is associated with yield losses ranging between 30-100% depending on variety, flowering stage and conditions of weather during flowering to harvesting period (Thakur *et al*., 2006).

Fungal disease incidences are propelled by farmers in marginal lands who grow sorghum continuously on the same land season after season (Tsedaley *et al*., 2016). This practice leads to rapid buildup and potential survival of inoculum, which enhances spread of diseases to crops that are establishing in the field (Njoroge *et al*., 2018). Pathogens infecting sorghum can also undergo mutation resulting in emergence of new pathotypes or races within a limited time span, hence limiting host plant resistance in plants (Anitha *et al*., 2020 and Little and Perumal, 2019). Moreover, Mofokeng *et al*. (2017) also noted that high levels of variation within a specific species of pathogens also enhance quick adaptation resulting in breaking of host resistance within a short period of time.

Although chemical control using of fungicides as a strategy for management fungal diseases has been used for prolonged time (Wagari, 2019), it's not only uneconomical but may also have negative effects on environment (Mofokeng *et al*., 2017). Therefore, an alternative approach of utilizing host plant resistance which is effective, affordable, eco-friendly and sustainable can be adopted enhancing crop disease control (Mengistu *et al.*, 2018 and Mofokeng *et al*., 2017).

1.3 Broad Objective

To improve sorghum productivity through assessing the effects of major fungal diseases on performance of selected sorghum germplasm under field trials in KALRO, Kiboko and Ithookwe in lower eastern Kenya

1.4 Specific Objectives

i. To identify major fungal diseases affecting sorghum under field conditions in KALRO, Kiboko and Ithookwe in lower eastern Kenya.
ii. To determine the effect of major fungal diseases on performance of selected sorghum varieties in KALRO, Kiboko and Ithookwe in lower eastern Kenya.

1.5 Hypotheses

i. There are no major fungal diseases affecting sorghum under field conditions in KALRO, Kiboko and Ithookwe in lower eastern Kenya.
ii. The effect of major fungal diseases on performance of selected sorghum varieties in KALRO, Kiboko and Ithookwe in lower eastern Kenya is insignificant.

1.6 Research justification

Sorghum is a food security crop mostly grown in arid and semi-arid lands (ASALs) in Africa and Asia (Chepng'etich *et al*., 2014). It is a drought tolerant crop with potential of offering farmers an alternative to mitigate adverse effects arising from global warming and climate variability as compared to other crops such as maize and wheat (CGIAR, 2022). Kazungu *et al.* (2023) also noted that it's an important food source that can be used to manage obesity and diabetes due to its grains containing low levels of starch and proteins that easily digested, compared to other cereals. The crop potential to provide cover against food insecurity is under threat due to fungal pathogens, which cause diseases that reduce its yields (Teferi and Wubshet, 2015).

Some of the fungal pathogens that have been reported to infect sorghum in Kenya include: *Colletotrichum sublineolum* (anthracnose), *Exserohilum turcicum* (leaf blight), *Cercospora sorghi* (Grey leaf spot), *Puccinia purpurea, Ramulispora sorghicola* (oval leaf spot), *Cercospora fusimaculans* (ladder leaf spot), *Gloeocercospora sorghi* (zonate leaf spot), *Sporisoriumholci-sorghi* (Head smut) and *Sporisorium sorghi* (Covered kernel smut) (Ngugi *et al*., 2002). Isolation and identification of fungal pathogens is of

importance in assessing not only their effects but also establishing best control strategies (Alsohaili and Ban-Hassan, 2018).

Majorly drought tolerance and grain yield among sorghum genotypes has been evaluated in the Semi-arid lands of Kenya (Njaimwe *et al*., 2017). The present study involves screening for fungal diseases and pathogens, assessing their effects on growth and productivity of selected sorghum varieties and level of tolerance of the selected varieties especially under field experiments. Tolerant varieties identified from this study will boost sorghum production thereby improving sustainable food security and incomes among farmers. This also conforms with the Agricultural Transformation and Growth Strategy (ASTGS) policy of 2019-2029 which is anchored towards: increasing incomes small of small holder farmers, improving agricultural output and value addition, and boosting food security among households. Information obtained will also benefit sorghum breeders, extension service providers and other stakeholders and form a basis for further research not only in lower eastern Kenya but also in other regions.

CHAPTER TWO: LITERATURE REVIEW

2.1 Classification and morphology of sorghum

Sorghum (*Sorghum bicolor* L. Moench.) belongs to *Poacea* family, genus Sorghum Moench, tribe *Andropogoneae*, and subtribe *Sorghinae* (Dahlberg, 2000). It is a C4, short day and self-pollinating crop with wider adaptations of growing in different environmental conditions (Mengistu *et al*., 2018). Although sorghum's origin is in the tropics, it can grow in temperate regions and other regions where other C4 plants cannot survive (Tari *et al*., 2013). Sorghum possesses a significant variety of morphological features, which include; plant height varies between 0.5-6m (Bosire, 2019). This is not only due to difference in internode number and length, but also in peduncle and panicle length (Tari *et al*., 2013). The stem's waxy internodes improve tolerance to drought and limits water loss through the atmosphere (Mwadalu and Mwangi, 2013). Sorghum roots are composed of primary and secondary rooting system, with the latter, which grows from root crowns, takes over the functions of the first due to their finite growth (Tari *et al*., 2013; Mwadalu and Mwangi, 2013).

The secondary roots usually establish themselves to permanent roots and can reach up to a depth of 2m with 1m sideways during early stages of plant growth (Mwadalu and Mwangi, 2013). The secondary roots are important in extracting of water and nutrients from the soil due to being fine and branching twice as in rooting system of maize plant (Mwadalu and Mwangi, 2013). Therefore, sorghum's root system and distribution enhance survival under drought conditions. Tillers grow from the nodes located on lower parts of the plant while lateral branches grow at higher parts of the stem when moisture is sufficient (Tari *et al*., 2013). Sorghum leaves have a small surface area compared to maize, that are waxed, consisting rows of motor cells arranged along the midrib which enables rolling of leaves during moisture deficiency (Mwadalu and Mwangi, 2013).

The number sorghum leaves vary between 8-22 depending on conditions of environment with the stem being solid, dry and succulent (Mwadalu and Mwangi 2013). A fully developed leaf consists of a ligule, which attaches the leaf sheath and leaf blade. The final leaf from the top or flag leaf is usually smaller in area when compared with the

preceding leaves. The leaf area expansion reaches its peak at booting stage, which is characterized by an increase in demand of water while at the same time there is efficient maximum use of water absorbed (Rao *et al*., 2004).

2.2 Importance of sorghum and farming in Kenya

Sorghum is among the five main crop species grown because of its potential of being utilized as food, fuel, feeds, fibre, and fertilizer and in fermentation (Tari *et al*., 2013). Both grains and stover have different uses. Sorghum grains are consumed by human mainly through boiling of flour to make uji, sadza, ugali and sometimes fermented to prepare injera, traditional bread (Wortmann *et al*., 2009). According to Karanja *et al*. (2006), 15% sorghum flour can be mixed with 85% flour from wheat to make bread. Bosire (2019) reviewed that sorghum grains can be recommended in the dietary uptake due to grains consisting high percentages of carbohydrates, proteins fats, fiber and ash in the order 70-80%, 11-13%, 2-5%, 1-3% and 1-2%. Wortmann *et al*. (2009) further states that the stalks are used as sources of fuel for cooking, fodder for livestock, building material and less used mulch. Moreover, most sorghum stalks are used as livestock feeds in Kenya, Uganda and Zimbabwe compared to other regions (Wortmann *et al*., 2009). In industrial use, sorghum is used as a raw material in beer and non- alcoholic malt beverages production (Taylor, 2003). Sorghum fibers can be used as raw materials for manufacture of environmentally friendly material, wallboard, solvents and fences (Bosire, 2019).

Sorghum farming in Kenya is dominated by smallholder farmers who are estimated to be around 240,000 with farms ranging between 1-1.5 acres, growing it for main purpose of subsistence (Njagi *et al*., 2019). Mwadalu and Mwangi (2013) approximates that of 104,041 hectares of land under sorghum farming, 50.2% lie in Eastern region. The introduction of Gadam variety of sorghum in eastern Kenya by KARI in 2009 as a strategy to boost farmer incomes and food security has paid back with its grains characterized by elevated starch, used as food and reduced proteins makes it fit for industrial malting processes (Mwadalu and Mwangi, 2013). Therefore, it has high potential of not only stimulating economic development but also improving food security

status and offering employment opportunities in the ASALs of Kenya (Mwadalu and Mwangi, 2013; Njagi *et al*., 2019).

2.3 Production constraints of sorghum

Sorghum production is limited by abiotic factors such as inadequate soil nutrients and toxicity, drought stress, temperature stress and waterlogging (Tari *et al*., 2013) and biotic factors such as diseases, striga weed and pests (Mengistu *et al*., 2018; Mitaru *et al*., 2012).

2.3.1 Abiotic stresses affecting sorghum

Moisture stress due to high soil temperatures, drought or high concentrations of salts in the soil affects various stages of sorghum growth (Assefa *et al*., 2010). A study by Bayu *et al*. (2005) reported a reduction in germination rate, percentage and emergence as evidenced by reduction in coleoptiles, mesocotyls, radicle elongation, seedling shoot, root area and length with increasing water stress. Both leaf area and plant height are reduced during water stress (Assefa *et al*., 2010). Khaton *et al*. (2016) noted a significant reduction in plant height, number of grains per panicle, grain weight of 1000 seeds and number of filled grains per hill due to water stress in different sorghum varieties.

Sorghum's reproductive stages are more sensitive to drought stress compared with vegetative stages (Assefa *et al*., 2010). Mengistu *et al*. (2018) reviewed that the approximated yield loss range in sorghum due to drought during the vegetative stage is 50-60% while between booting and anthesis stages is 87%. A study by Dorcas *et al*. (2019) in eastern Kenya noted all farmers acknowledged that sorghum production was majorly hindered by inadequate rainfall. This is also worsened by estimated high levels of evaporation up to 50% of the total rainfall in semi-arid of Kenya (Kinama *et al*., 2005).

Low temperature limits germination of seeds, emergence, plant growth and both rate and capacity of photosynthesis in sorghum (Tari *et al*., 2013). A study by Rutayisire *et al*. (2020) revealed that cool weather conditions significantly delayed days to 50% flowering and maturity due to reduction in growth rate during the vegetative stage and longer period

of grain filling respectively, with susceptible varieties dying at early stages of development. Elevated temperatures above optimal reduces enzymatic activities, chlorophyll content and rate of photosynthesis (Tari *et al*., 2013).

In the tropics and sub-tropics, sorghum plants are affected by either periodic or permanent waterlogging caused by overflooding, storms and continuous rains which not only has negative impacts on the texture of soils but also chemical processes in plants reducing growth and biomass production (Tari *et al*., 2013). A study by Promkhambut *et al*. (2010) recorded a significant reduction in shoot growth (71%), leaf area (69%), plant height (30%) and dry matter accumulation in both leaf and culm by 72% and 68% respectively due to waterlogging. Limited shoot growth was associated with reduction in plant height, which was consistent with reduction in youngest leaf expansion rate. The study also noted an increase in the number of senescent leaves, reduction in nodal root number per plant, longest root length and root dry weight.

Inadequate levels of nitrogen in soil between sowing and flowering reduces the size of the panicle, number of both primary and secondary branches, and florets at panicle emergence stage. When low nitrogen levels coincide with low moisture levels in soil, leaf area reduces, leading to reduction in trapping of solar radiation and lower yields (Rao, 2004). Salt stress is caused by over-accumulation of different ions e.g., calcium, magnesium, chloride, sulphate and sodium in soil, with sodium chloride being the greatest threat to growth and development processes reducing photosynthesis in mature leaves and leading to chlorosis (Tari *et al*., 2013).

2.3.2 Biotic stresses affecting sorghum

Mengistu *et al*. (2018) reviewed that biotic factors which include; insect pests, diseases and weeds contribute up to 40% yield reduction in sorghum varieties that are less tolerant. Of the biotic factors, striga weed is a threat with potential of causing yield loss up to 100% where monocropping is majorly practiced in soils with low fertility (Mitaru *et al*., 2012). Esilaba (2006), also approximated that yield losses in Kenya due to striga weed is 2-3kgha^{-1}. Yield losses due to striga weed correlate with systems of land use,

fertility of soils, striga density, patterns of rainfall and the susceptibility of the plants (Atera *et al*., 2012). The main striga weed species that cause losses in sorghum are *Striga asiatica* (L.) Kuntze and *Striga hermonthica* (Del.) Benth (Atera *et al*., 2013). Striga weed control is challenging due to the fact that before it emerges on the soil surface, it already has caused damage to the crop (Esilaba, 2006). However, integrated weed control has been recommended as the best option to counter the parasitic weed infestations since it encompasses biological, cultural, chemical and mechanical methods. Important insect pests such as stalk borer complex (*Sesamia calamistis* (Hampson), *Busseola fusca* (Fuller), and *Chilo partellus* (Swinhoe.), shoot fly (*Atherigona soccata*), and termites cause significant losses in sorghum (Wortmann *et al*., 2009). Wortmann *et al*. (2009) approximated that sorghum yield losses caused by the species of stalk borer are above 1.3 million metric tons per year (Mg y^{-1}) and also birds for example, Quelea (*Quelea quelea*) contribute to sorghum yield losses in Kenya. Major viruses that attack sorghum plants; Sorghum mosaic virus (SrMV), Sugarcane mosaic virus (SCMV), Johnson grass mosaic virus (JgMV) and Maize dwarf mosaic virus (MDMV) can cause yield loss of between 2-5% yearly (Mofokeng *et al*., 2017). The key host for Maize dwarf mosaic virus and Johnson grass mosaic virus is Johnson grass, while some aphid species such as *Rhopalosiphum maidis* (Fitch) and *Schizaphis graminum* (Rondani) act as vectors for the spread of potyviruses; sorghum mosaic virus and maize dwarf mosaic virus (Little and Perumal, 2019).

Bacterial diseases that cause insignificant effects on sorghum yields include bacterial leaf streak, bacterial leaf spot and Bacterial top and stalk rot (TeBeest *et al*., 2004; Spurlock *et al*., 2002). Fungal diseases contribute majorly in reduced sorghum production in Eastern and Southern Africa (Wortmann *et al*., 2009). Thakur *et al*. (2007b) listed some of the fungal diseases of economic importance in the Semi- Arid tropics causing losses annually are: smuts, charcoal/ stalk rot, ergot, leaf blight, grain mold, rusts, anthracnose and downy mildew.

2.4 Major fungal diseases of sorghum

Sorghum is infected by a wide range of diseases which compromise its productivity (Anitha *et al*., 2020). These diseases can not only be categorized based on the part of the plant which they infect such as foliar diseases, stalk diseases, leaf sheath diseases and panicle and grain diseases but also with the symptoms they produce for example leaf spots, leaf blight, rusts, smuts, downy mildew and grain mold (Thakur *et al*., 2007). Some of the major fungal diseases are discussed below.

2.4.1 Anthracnose

Anthracnose in sorghum is caused by fungus called *Colletotrichum sublineolum* (Tesso *et al*., 2012; TeBeest *et al*., 2004). This pathogen degrades both the quality and quantity of grains and stover by infecting leaves, stalk, grains and panicle (Tesso *et al*., 2012). The fungus can survive for an estimated period of 18 months as microsclerotia, conidia and mycelium in plant residues or on the soil surface and as mycelium in infected seeds (Tesso *et al*., 2012). The prevalence of sorghum anthracnose is usually high in warm and humid climatic conditions especially the tropics, which exhibits favorable conditions for disease development and spread (Mofokeng *et al*., 2017). The degree of direct yield loss due to anthracnose varies with prevailing climatic conditions, sorghum variety and geographic conditions (Marley *et al*., 2005). According to Mengistu *et al*. (2018) and Thakur and Mathur (2007), the approximated yield loss due to anthracnose is up to 67% and above 50% respectively under elevated temperature and humid conditions.

Symptoms of anthracnose disease are clearly visible during booting stage with small, circular (<5mm) to elliptical spots developing on foliage and midribs of susceptible sorghum plants (Mengistu *et al*., 2018; TeBeest *et al*., 2004). These spots are straw in color at the center with elongated margins, which are red, orange, purple, or tan and usually increase in number, as the disease develops to cover a wide area of the leaf surface (TeBeest *et al*., 2004). Black fruiting bodies (acervuli) consisting of small, black, hair like structures (setae) develop at spot centers and these symptoms are easily recognizable on older leaves mid veins (TeBeest *et al*., 2004).

Anthracnose has four separate phases (seedling root rot, foliar or leaf, stalk rot and seed mold) which can both occur on a single growing season causing severe reduction in yields (Mofokeng *et al*., 2017; TeBeest *et al*., 2004). Seed rot arises from infected germinating seeds in soil that is already infected or through sowing of seeds that are infected while the foliar phase appears during the growth stage between 30-40 days after seedling emergence yields (Mofokeng *et al*., 2017). Stalk anthracnose is developed from spores generated in foliar or leaf phase which when spread into the leaf sheaths by wind, rain or dew germinate causing the interior of the stalk to rot and also infecting the stalk above the uppermost foliage (TeBeest *et al*., 2004). According to Mofokeng *et al*. (2017) and TeBeest *et al*. (2004), when the length of the stalk and head are split, a banded pattern ranging from dark red to purple lesions is interspersed with white pith tissue viewed. This phase is particularly common when stem borers in the plant create wounds, which serve as entry points for the fungus to penetrate into the stem (TeBeest *et al*., 2004).

Under favorable conditions, continuous stem and leaf infections leads to panicle invasion and seeds. However, grain filling can be hindered when conditions become severe (TeBeest *et al*., 2004). The stalk phase determines yield because destroying of the stalk tissue reduces nutrient movement to the grains that are developing (Mofokeng *et al*., 2017; TeBeest *et al*., 2004). The heads of plants infected with anthracnose are smaller, lighter and mature earlier compared to uninfected plants (TeBeest *et al*., 2004).

2.4.2 Leaf blight

Leaf blight caused by *Exserohilum turcicum* develops under elevated humidity and temperatures ranging between 18-27°C during the growing season (Mathur *et al*., 2007; TeBeest *et al*., 2004). The fungus survives in crop residues in the tropical areas as conidia and mycelia while chlamydospores in temperate regions with the main mode of spread being by air (Mathur *et al*., 2007). The disease incidence reduces with dry conditions during sorghum growth stages; however, it can cause up to 50% yield loss in susceptible cultivars when it emerges before panicle initiation (TeBeest *et al*., 2004).

Symptoms of leaf blight include; small, red spots which when they enlarge and combine lead to wilting of leaves that are still young (Mathur *et al*., 2007). On mature crops, small tan or reddish spots which can progress to long elliptical reddish purple (12mm wide by 2.5-12cm long) emerge on leaves before developing to upper foliage and stem (Mathur *et al*., 2007; TeBeest *et al*., 2004). When the fungus sporulates on the spots or lesions, a dark gray color appears on the sorghum leaf surface (TeBeest *et al*., 2004).

2.4.3 Rusts

Rust caused by fungus *Puccinia purpurea* Cook, infects sorghum plants through generating urediniospores, which live on not only planted sorghum and ratoons but also on volunteer sorghum plants, both collateral and perennial hosts. Secondary spread of rust is through airborne urediniospores and *Oxalis corniculate* which acts as an alternate host for the development of aecidial stage. Under cool and humid conditions, the disease develops rapidly leading to lower yields and quality of forage (Thakur *et al*., 2007d).

Symptoms emerge on upper and lower leaf surfaces, characterized by randomly distributed purple or red flecks, with which the fleck's color depends on the pigment of the plant. In plants that are susceptible to the disease, the flecks expand, appearing like a swelling, which is brown to dark in color while in less susceptible plants, the flecks do not enlarge. Symptoms on the peduncle include stretched lesions that are reddish brown to blackish brown in color (Thakur *et al*., 2007d).

2.4.4 Downey mildew

Peronosclerospora sorghi is an Oomycete that causes downy mildew of sorghum resulting in crop damage particularly in the tropics (Tesso *et al*., 2012). The means of reproduction adopted by the pathogenic fungi involves use of conidia (asexual) and sexual means via oospores (Thakur *et al*., 2007c). The fungus infects seedlings by systemic action, colonizing the young meristematic tissues and later causing chlorosis in foliage (Thakur *et al*., 2007c). Cooler temperatures and low moisture conditions in the soil enhance infection of crops by oospores while elevated humidity and cool temperatures ranging between 15-20°C result in conidia formation, disease infection and

development in plants (Thakur *et al*., 2007c). Some of the hosts for the fungus, which act as inoculum sources include Sudan grass, *Sorghum halepense* and soil when land is left to rest (Thakur *et al*., 2007c). The disease infection is characterized as both systemic and local, where in systemic, meristematic tissues of seedlings are attacked, with symptoms emerging inform of chlorosis and stunted crop, leading to dying of seedlings.

Chlorosis, which first appears underneath the leaf surface, expands to cover bigger area of the leaf and also the plant. Under favorable conditions, the lower leaf surface infected with chlorosis usually produces sufficient white conidia (spores). New leaves that emerge when the plant is growing show green and white stripes, and with time, death of interveinal white tissues and shredding of infected leaves which comprise of many oospores takes place (Thakur *et al*., 2007c). Bock and Jager (1996) noted that in plants that are aging, pale yellow streaks from young leaves change to reddish brown with interveinal tissue appearing necrotic. Local infections are characterized by chlorotic lesions which turn to purple tan with edges elongating parallelly irrespective of the sorghum leaf (Bock and Jager, 1996). The fungus also generates white conidia and conidiophores on the lesions which are dispersed by wind to other plants resulting in secondary infection. Moreover, these conidia from local infections can also infect the young meristematic tissues leading to systemic infections (Thakur *et al*., 2007c).

2.4.5 Head smut

Head smut of sorghum is caused by a soil borne fungus *Sporisorium reilianum* (Kuhn) (synonym: *Sphacelotheca reiliana* or *Sporisorium holci-sorghi*) infects plants at early stages of plant growth, systematically develops its infection and expresses itself during booting stage. The disease has become severe because of sowing varieties that are less tolerant and the emergence of pathogenic fungi races with high virulence. The fungus lives in active meristem tissues that are growing and its spores can be introduced to new fields through seeds when attached on them (Ramasamy *et al*., 2007).

The disease is favored by dry soil temperature of 24°C up to when plants attain 3-4 leaves. However, temperatures exceeding 31°C and lower than 21°C decrease the rate of

disease infection. A smut gall with a membrane that is dense and whitish in color (Ramasamy *et al*., 2007) covers the disease seldomly, affects the leaves as compared to inflorescent which when infected. Prior to head emergence, the membrane ruptures releasing an aggregation of powdery teliospores that are dark brown to black mixed together with different vascular strands (Wagari, 2019; Ramasamy *et al*., 2007). Other symptoms of head smut include; stunted growth due to failure of the peduncle to elongate, reduced plant height, premature tillering and phyllodial phenotype of panicles (Little *et al*., 2012; Ramasamy *et al*., 2007). Although head smut has a low infection percentage in fields estimated at 10%, yield loss is significant with no treatment. Moreover, 80% infection rates have also been recorded (Wagari, 2019). Ngugi *et al*. (2002) recorded more than 25% head smut incidences in 3% of surveyed fields and an approximated percentage of yield reduction at 4% based on panicle that was 60% diseased.

2.4.6 Covered Kernel Smut

Covered kernel smut is a seed-borne disease caused by fungus *Sporisorium sorghi* (synonym: *Sphacelotheca sorghi*) which infects sorghum pants systemically from seedling stage up to inflorescence. This disease is prevalent in sorghum growing zones, and is regarded an ordinary disease where untreated seeds that are planted (Thakur, 2007b). Wagari (2019) noted that continuous growing of sorghum in warm and wet soils (15.5°- 32°C) favors disease development. Hayden (2002), noted covered kernel smut often infected sorghum plants in Uganda compared to other smuts with average incidence and grain loss significantly varying between 2.5% -22.5% and 1.3% -43.0% respectively. Ngugi *et al*. (2002) also estimated a yield reduction of 0.9% based on panicle that was 60% damaged in Kenya. In Nigeria, a study by Paul and Daniel (1999), also established covered kernel smut as most prevalent disease with highest severity ranging between 7.3-7.7 and incidence between 24.8%- 29.5% of fields surveyed. Symptoms include kernels being replaced by conical to oval smut sori enclosed by peridia, which is bigger in size than an average grain. At first, each sori is enclosed with a membrane that is pink or silver white in color, which then raptures to expose brown to black spores (Thakur, 2007b).

2.5 Management of sorghum diseases

2.5.1 Chemical control

Chemical control can be applied in two different ways; either as seed treatment prior to planting (Gwary *et al*., 2007; Gwary and Asala, 2006) and as preventive against diseases when a crop has already grown (Gwary and Asala, 2006). The use of fungicides for control of fungal diseases has been used for a long period from 1950's (Wagari, 2019). Dressing of seeds with fungicides protects growing seedlings from diseases that arise from infected seeds and inoculum in the soil, however, spraying is also administered to enhance prevention from further infection (Gwary and Asala, 2006).

Systemic fungicides which are easily taken up and redistributed in the plant system are more effective in management of diseases in sorghum than contact fungicides which only protect parts of the plant where the chemical is deposited and can also be washed off (Fromme *et al*., 2017). Treating of seeds with chemicals has proved effective in control of diseases spread through untreated or contaminated seeds for example; covered kernel smut and loose kernel smut (Wagari, 2019). Gwary *et al*. (2007), recorded significant reduction in incidences of smut ranging between 4.8% to 7.23% in plots where seed dressing and spraying fungicides were applied compared to untreated plots with higher incidence of 11.25%. Although the study listed Apron star (20% Metalaxyl –m+20% Thiamethoxam+2% Difenoconazole), Dress force (20% Metalaxyl –m+20% Imibaclopriv+ 20% Cevaconazole) and Forte Plus (32.5% Mancozeb +27.5% Thiophanate methyl+15% Diazinon) as effective in suppressing both anthracnose and smut infection, significant variations were recorded on smut severities and grain yield between the varieties and fungicides used in treatment, with Apron plus (20% Metalaxyl –m+20% Thiamethoxam+2% Difenoconazole) being ranked the best in effective management of smuts but also in attainment of highest grain yield.

Fungicides with active ingredient Metalaxyl have proved to be effective in inhibiting fungal infections. In vitro study by Idowu *et al*. (2012) showed that Apron star consisting of 20% thiamethoxam, 2% w/w metalaxyl-M and 2% w/w difenoconazole significantly reduced the growth of *Colletotrichum graminicola* by 20.9% compared to other two

fungicides namely; Benomyl (50% Methyl 1-(butylcabamoyl) benzimidazole) and Mancozeb (2.5% Zn ion + 2% Mn as in maneb) (34.3%) which inhibited by 34.3% an 58.2% respectively.

Some of the disadvantages of application fungicides are; not only being uneconomical but also leads to negative impacts on environment and buildup of resistance by pathogens if fungicides are used continuously (Basavaraju *et al*., 2009). Hayden (2002) also acknowledged that although fungicide Thiram (dimethyldithiocarbamate) can be used in treatment of sorghum seeds in return for control of the disease, it is still lowly adopted by small scale farmers with limited resources in Uganda, Tanzania and Kenya.

2.5.2 Biological control

Biological control refers to the use of either an organism or various organisms except man to control pathogens by reducing diseases or inoculum (Alabouvette *et al*., 2006). Biocontrol agents used in biocontrol methods usually control crop pathogens either by secreting lytic enzymes, generating antibiotics or through competing for space and nutrients. Although microorganisms e.g., fungi, bacteria and viruses are utilized in biocontrol of plant pathogens, best agents are rated according to ability to multiply, grow and live in different ago-ecosystem (Dukare *et al.*, 2020). Dukare *et al*. (2020) also notes that bacterial species namely: *Bacillus*, Pseudomonas, and fungal species of *Trichoderma* are microorganisms majorly used as biocontrol agents.

The use of natural products extracted from plants is advantageous since they are target specific, environmentally friendly with short shelf life and not harmful for antagonist microorganisms (Zaker, 2016). Nigusie and Ademe (2020) acknowledged that use of botanicals has gained attention due to their availability and assured safeness of products. Some of the ways in which biochemicals extracted from plants can be utilized in development of natural products include through crude extracts, introduction of a new different mode of action that can degrade resistance and acting as building blocks for manufacture of complex compounds (Zaker, 2016). Gakuubi *et al*. (2017) also notes that

phytochemicals possess the potential of being utilized in developing of Phyto-fungicides, which are environmentally safe.

Zaker (2016) reviewed that plant products for example; gums, essential oils, resins and plant extracts have proved effectively in suppressing fungal pathogens both in-vitro and in-vivo, however, plant extracts and essential oils have been majorly used in management of plant diseases. In Ethiopia, the use of crude extracts in slurry form from roots of Bocha (*Dolichos kilimandscharicus*), has been applied in seed treatment against sorghum smuts grown under field conditions (Tegegnea *et al*., 2008). Although there is great potential for use of botanicals by the resource poor farmers (Wagari, 2019), Tegegnea *et al*. (2008) opposes that it has been less effective and adopted on small size over the preceding years. Sisay *et al*. (2012) noted that though the use of leaf extract from *Maesa lanceolata* in control of smut in Ethiopia proved effective in management of the disease, however, the disease incidence was not only higher but also the yields were lower compared to fungicide thiram treatment.

In Kenya, a study by Gakuubi *et al*. (2017) established that essential oil derived from *Eucalyptus camaldulensis* leaves effectively reduced growth of mycelial in five pathogenic species of *Fusarium* namely: *Fusarium subglutinans*, *Fusarium solani*, *Fusarium oxysporum*, *Fusarium proliferatum*, and *Fusarium verticillioides*, however, antifungal activity differed among the pathogens. Similarly, Siameto *et al*. (2010) observed two isolates of *Trichoderma farzianum* namely: 051E and 015E reduced growth of five pathogenic fungi: *Fusarium graminearum*, *Rhizoctonia solani*, *Fusarium oxysporum* f. sp *Lycopersici*, *Pythium sp*. and *Fusarium oxysporum* f. sp *phaseoli* by 50%, hence were superior when compared to other isolates.

2.5.3 Cultural control

Some of the cultural practices that can be applied in management of sorghum fungal diseases include; planting materials that are free from fungal diseases, appropriate timing on sowing, rotation of crops and removal of infected plant residues and alternate hosts for example wild sorghum hosts (Abuhaye, 2018; Chala and Tronsmo, 2012). Wagari (2019)

acknowledged that cultural practices are important in management of diseases that are seedborne and occur during early stages of plant growth for example Covered kernel smut and Loose kernel smut.

Crop rotations are effective in management of head smut due to the ability of fungus spores to reside in soil for years (Mohan *et al*., 2013). Chala and Tronsmo (2012) reviewed that anthracnose could also be managed by altering of sowing dates, removal of plant debris and alternate hosts such as wild sorghum, use of certified seeds and crop rotation. However, according to Hadush (2019), in order for altering of planting dates to be effective, one should have the knowledge of disease cycle and the duration when the disease attains its peak level in the farm.

Different cultural and traditional methods have also been used for an extended period by smallholder farmers with scarce resources in control of smut diseases in sorghum (Wagari, 2019). A study by Girma *et al*. (2008) reported that the use of urine from cow not only reduced Covered kernel smut and Loose kernel smut by 81% and between 26-70% in 1999 and 2000, but also increased yield by 95% and 38% in the respective years. Similarly, goat urine diluted with water and stored for different days also reduced smut by 50%-80% in the first year and 55%- 85% in the second-year while at the same time increased yields. Although cultural methods are cheap, easy to apply, environmentally friendly, and have played a role in the management of different crop diseases worldwide (Chala and Tronsmo, 2012), they are effective if applied under large scale systems (Chala and Tronsmo, 2012; Abuhaye, 2018).

2.5.4 Host plant resistance

Host plant resistance implies the application of simple and effective methods in screening traits for genetic resistance so that they can be developed in crop varieties through breeding to enhance disease resistance (Thakur *et al*., 2007a). It is an effective method in enhancing crop disease control due to being environmentally safe, sustainable and affordable (Mengistu *et al*., 2018). Ramasamy *et al*. (2007), stated that host plant

resistance less important in cases where a disease is controlled by either agronomic management or treatment of seeds with a chemical e.g., head smut disease.

Plants use both chemicals e.g., phytoalexins and physical methods involving structural barriers to counter pathogens from infecting them. In sorghum plants, young leaves usually stock phytoalexins, which have antifungal properties against pathogenic fungi while physical barriers for example dense waxy and accumulated epidermal cells, erecting of leaves and closing of spikelet glumes at anthesis stage are, reported strategies in sorghum against pathogen *Colletotrichum sublineolum* (Abuhaye, 2018).

Understanding of the pathogen biology and disease epidemiology is key in developing screening methods, which are effective (Thakur *et al*., 2007a). Moreover, according to (Marley *et al*., 2005), in order to identify a lasting resistance, sorghum varieties have to be screened against various pathogens at several sites. The need for identifying more resistant sources is of importance since host plant resistance is usually limited by various pathogens that rapidly evolve (Abuhaye, 2018; Tesso *et al*., 2012).

In Alupe, Kenya, a study by Thakur *et al*. (2007a) identified sorghum lines: IS 875, IS 6928 and IS 12467 as highly resistant to anthracnose disease and therefore had the potential of being incorporated in breeding of tolerant varieties. Sharma *et al*. (2000) noted when restorer lines: IS 8891, ICSV 197, DJ 6514 and IS 27103 were combined with three cms lines, the hybrids that resulted were resistant to leaf diseases namely; rust, leaf blight, zonate leaf spot and anthracnose. The study also further established sorghum genotypes: ICSB 88020, IS 27103, ICSB 88019, DJ 6514, ICSV 197 and IS 8891 were resistant to leaf diseases.

Although a number of resistant lines are used in developing of resistant varieties, the development of host plant resistance in plants is faced with challenges due to not only the degradation of resistance in plants but also with emergence of new pathogenic races (Wagari, 2019). Gwary *et al*. (2007) also pointed out that the progress of developing

resistant varieties has not only been slow but also, it’s rare for cultivars to have multiple resistances to all major diseases.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Evaluation of the effect of fungal diseases on performance of sorghum germplasm

3.1.1 Description of study areas

The field experiment was conducted at two sub-centers of KALRO namely: Kiboko and Ithookwe as displayed on figure 3.1. Both sub-centers receive a bimodal rainfall pattern with the long rains falling between March and May (MAM) and short rains falling from October to December (OND) (Muui, 2014). Kiboko lies between longitudes 37.7235°E, latitudes 2.2172°S and an altitude of 975 meters above sea level (CIMMYT, 2013). Temperatures range from 22.6°C (mean annual minimum) to 28.6°C (mean annual maximum) with rainfall ranging between 545 -629 mm per year (CIMMYT, 2013). Ithookwe temperature ranges between 16-34°C with an average rainfall of between 835-1079 mm. Altitude range is 1158 meters above sea level (Muui, 2014). It lies between latitude 01° 22′34″ S and longitude 037° 58′43″ E (Mutisya *et al*., 2016). Soils at KALRO Ithookwe are sandy clay loam (Ferric, Acrisols) with pH ranging between 5.5-7.0 (Gichangi *et al*., 2017 and Omwakwe, 2023) while at KALRO Kiboko are dark reddish brown to dark red, friable sandy clay to clay (Acri-Rhodic Ferassols) (CIMMYT, 2013). The sites were selected because they are not only hotspots for fungal diseases due to favorable climate, but also lie in different agroecological zones (Koima *et al*., 2022), hence least differences in disease composition, incidence and severity were recorded (Ngugi *et al*., 2002). Kiboko lies in lower midland 4 (Warm transitional), while Ithookwe lies between upper midland 3 to 4 (Temperate to sub-humid) (Jactzold and Schmidt, 2010).

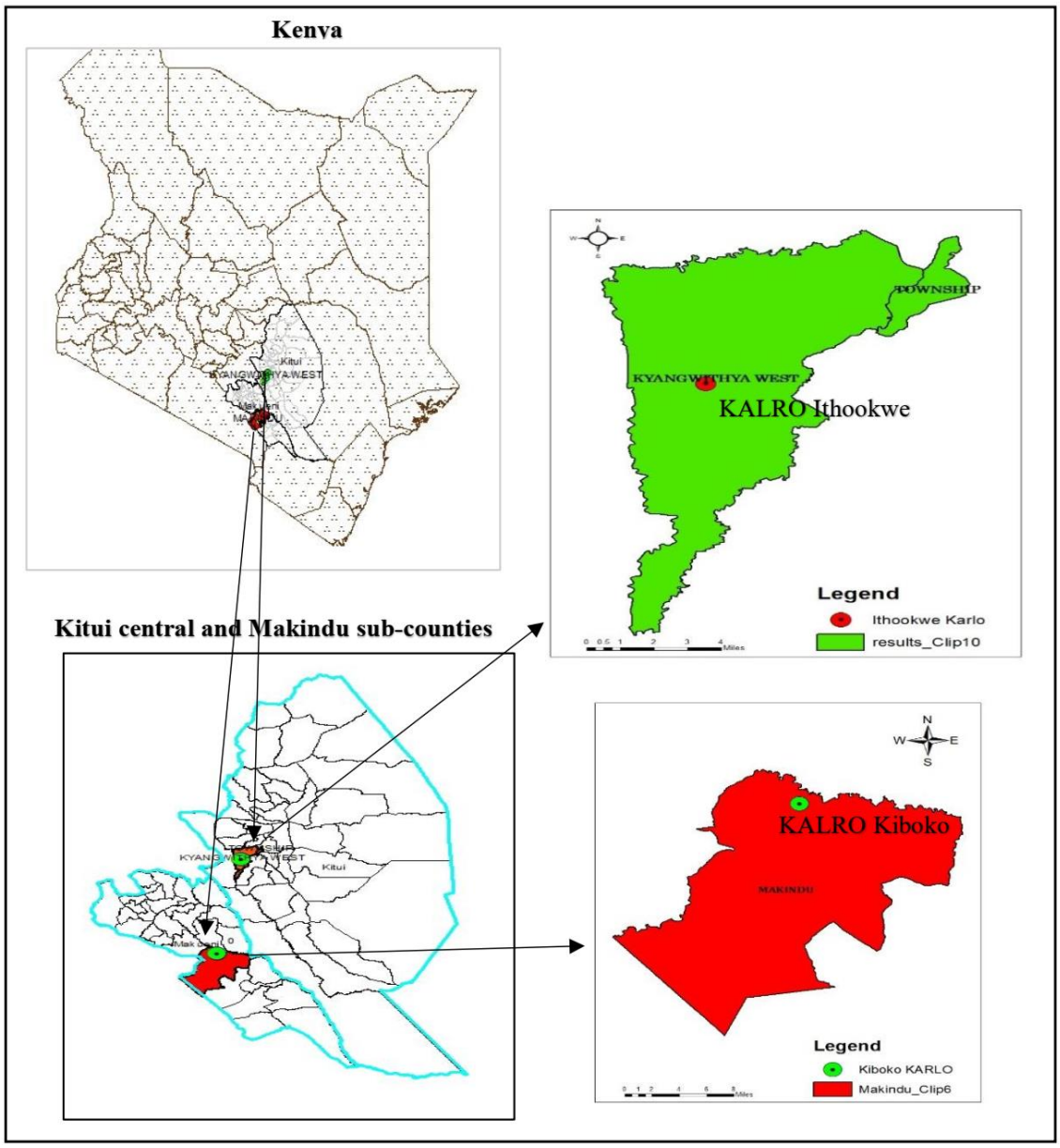


Figure 3.1: Map showing regions and locations of the study sites

3.1.2 Sorghum germplasm

Sixteen sorghum varieties, including eight improved varieties under test (Gadam, Marcia, IESV 24029 SH, KARI Mtama 1, Kiboko Local 2, Makueni Local, Serena and Seredo), one variety for guard rows (Sila), a disease spreader variety (Wagiita) from International Crops Research Institute for Semi- Arid Tropics (ICRISAT) and six local landraces (Kateng'u, Kauwi, Rasta, Mugeta, Kaguru and Dark Red) from farmers were used for the

field trial. Local varieties were first grown, then panicles with dominant traits selected at physiological maturity to enhance seed uniformity for use in field trials. The improved genotypes had been bred for drought tolerance, yield and grain quality improvement (Table 3.1). Two varieties were used as controls for the study: Kaguru as susceptible control (Ogolla *et al*., 2019) and Kateng'u as a dominant local landrace and high yielding (Njaimwe *et al*., 2017), to show the losses attained by farmers in their farms (Ngugi *et al*., 2002). Sila variety of sorghum was planted as guard rows since it is not only adapted to growing in the semi-arid areas but also its seeds have a white color that is appealing to birds (Oyier *et al*., 2016). Wagiita was used as a spreader variety due to being susceptible to fungal diseases, hence served as sources of inoculum for fungal diseases (Bock and Jager, 1996). Below is table 3.1 showing sorghum germplasm adopted for the study.

Table 3.1 : Sorghum germplasm subjected to field trials

No.	Genotype	Parents	why it was bred/ other information	Source
1	Kiboko Local 2	Landrace from Kiboko	Drought tolerance	ICRISAT
2	Makueni Local	Landrace from Makueni	Short duration, drought tolerance, and suited for dry lowland areas, moderate resistance to bird damage.	ICRISAT
3	IESV 24029SH	Gadam x IS 8193	Grain yield, moderate resistance to *Striga hermontheca.*	ICRISAT
4	Macia	F3A-115-2 (Synonym M91057)	Bred for high grain yield, stay green and dual purpose	ICRISAT
5	KARI Mtama 1	KAT 83/KAT 369, Open pollinated (pure line) variety	Bred for utilization as food (Ugali, porridge, baking, boiled grain). Adapted to short and long rain seasons (Wide adaptation) and has traits fit for industrial use i.e., brewing qualities.	ICRISAT
6	Serena	Swazi P1207 x Dobbs, Open pollinated (pure line) variety	Bred for early to medium maturity, suitable for food uses. Resistance to shoofly	ICRISAT
7	Seredo	Serena x CK60, Open pollinated (pure line) variety	Bred for utilization as food and adaptation to sub-humid and dry lowland areas	ICRISAT
8	Gadam	Selection from IS 7055. Introduced from Sudan by ICRISAT Regional Program (EARSAM).	Bred for adaptation to dry lowlands, drought tolerant, good for food (Ugali, Porridge) and brewing. Has excellent malting qualities.	ICRISAT
9	Kateng'u	*Not applicable*	Widely grown by local farmers	Local farmers
10	Kauwi	*Not applicable*	*Not applicable*	Local farmers
11	Rasta	*Not applicable*	*Not applicable*	Local farmers
12	Mugeta	*Not applicable*	*Not applicable*	Local farmers
13	Kaguru	*Not applicable*	Susceptible to fungal diseases	Local farmers
14	Dark Red	*Not applicable*	*Not applicable*	Local farmers
15	Sila	*Not applicable*	*Not applicable*	ICRISAT
16	Wagiita	*Not applicable*	Susceptible to fungal diseases	ICRISAT

Source: Sheunda (2019).

3.1.3 Experimental design

The experimental plot was laid out in Randomized Complete Block Design (RCBD) with four replications as guided by Davis *et al*. (2009) for pathological field tests. Each block consisted of fourteen plots. Each plot size measured 4.2m width by 3.0m length consisting of eight rows. This plot size increased accuracy in measuring effects and uneven distribution of fungal pathogens (Davis *et al*., 2009). The test varieties and local landraces were planted each in six rows bordered by two outer rows of Wagiita variety, one on either side. Spaces of 1meter were maintained between plots and between blocks as paths. The spacing of sorghum plants was 60cm from row-to-row and 20cm between plants as recommended by Karanja *et al*. (2006) for a sole crop. Thinning and 1st weeding was done three weeks after planting, followed by 2nd weeding after two weeks, to maintain a healthy plant stand (Karanja *et al*., 2006).

In Kiboko trial, supplementary irrigation was done from the planting date up to when plants were at grain filling phase, while Ithookwe mainly relied on rain. Infection of sorghum cultivars involved use of spreader rows, inoculation by spores from diseased plant leaves of previous season and through natural infection. The spreader rows were planted 3weeks before the test cultivars as suggested by Bock and Jager (1996) to enhance buildup of infection within the spreader rows, increasing asexual spores. Preparation of inoculum for inoculating the spreader rows was done following procedures by Shekhar and Kumar (2012) for sorghum downey mildew inoculation, with few modifications, where diseased sorghum leaves were harvested, soaked in water and washed. The suspension was sprayed in sorghum plant whorls using a hand sprayer in the evening when temperature and dew were ideal for effective fungal infection (Aliyi *et al*., 2018), at 25 and 40 days after planting (Singh *et al*., 1997). The study was carried out for two seasons to allow identification of errors and increase accuracy and estimation of data variability (Slutz and Hess, 2017).

3.1.4 Determination of sorghum growth parameters

A random sample of 10 plants was tagged from the inner two rows in each plot for data collection so as to minimize errors that arise from some of the diseases and pathogens

spread rapidly from one plot to another (Davis *et al*., 2009). Growth characters assessed are in table 3.2 below.

Table 3.2: Growth characters recorded on tagged plants

Parameter	**Description / unit of measurement**	**Source / citation**
Plant height	From the ground level to the tip of the stem (cm)	(IBPGR and ICRISAT, 1993)
Number of green leaves/ plant	Counted and average done to determine number of leaves per plant. A leaf was considered green when more than 75% its area was green.	(Shamme *et al*., 2015) (Naoura *et al*., 2019)
Leaf Area/ plant leaf area	Estimated by multiplying maximum length (cm) by maximum width (cm) of the fourth leaf from the top then by a constant 0.75.	(Bueno, 1979)
Days to 50% Flowering	Counted from the date of sowing to when the tagged plants had flowered halfway.	(Shamme *et al*., 2015)
Plant color	Recorded as either tan or pigmented following descriptors for *Sorghum bicolor* (L.)	(IBPGR and ICRISAT, 1993)

3.1.5 Visual identification of fungal diseases

Fungal diseases in the field were identified based on visual symptoms and signs, aided through magnification by hand lenses (Frederiksen and Odvody, 2000), as well as sorghum fungal disease identification keys as described by Williams *et al*. (1978) and other authors. The symptoms and signs used to identify various fungal diseases in this study are summarized in table 3.3 below.

Table 3.3: Signs and symptoms used to identify of various sorghum fungal diseases

Disease	Description of symptoms and signs	Source
Anthracnose	Small, circular, elliptical to elongated spots with straw-colored centers and margins that are dark, red or purple. Spots may enlarge to coalesce all over the leaf. When magnified with a hand lens, black hair-like structures (setae) can be seen protruding from fruiting bodies (acervuli).	(Williams *et al*., 1978; Thakur and Mathur, 2007)
Leaf blight	Long elliptical necrotic lesions consisting of centers that are straw colored. Lesions can coalesce displaying a burnt appearance. Moreover, a faint to grey bloom of conidiophores and conidia is produced on lesions.	(Williams *et al*., 1978; Mathur *et al*., 2007)
Rust	Scattered purple, tan, or red small flecks first appear on leaves. Rust pustules or uredosori then develop under the leaf surface, rupturing to release uredospores (reddish powder). Teliospores later develop either in the old uredosori, or in teleutosori, hence changing from a reddish brown to dark.	(Williams *et al*., 1978; Thakur *et al*., 2007)
Gray leaf spot	Rectangular shaped, dark red to purplish lesions in pigmented plants while lighter centers occur in tan plants develop on either on leaf blades and sheaths. These symptoms are majorly isolated but can develop into long stripes. A greyish white bloom of conidia can also appear on lesions.	(Williams *et al*., 1978)
Ladder leaf spot	Lesions characterized by pale centers and dark margins appear like a ladder on the leaf.	(Njoroge *et al*., 2018)
Oval leaf spot	Small water-soaked spots emerge first, and later develop into small circular lesions with lighter centers in which small black sclerotia are generated and dark red to brown margins. However, protruding black setae are not produced on the lesions of oval leaf spot, unlike on anthracnose.	(Williams *et al*., 1978; Njoroge *et al*., 2018)
Downy mildew	Leaves appear light green and abundant white spores (conidia and conidiophores) are produced nocturnally under the leaf surface. Subsequent leaves display parallel green and white stripes which shredding may occur when the interveinal tissue die.	(Williams *et al*., 1978; Thakur *et al*., 2007c)
Covered kernel smut	Conical to oval shaped smut sori, enclosed by a tough silver white or cream to light brown skin, replaces individual grains on the panicle. When smut sori ruptures, brownish to black smut spores are visible.	(Williams *et al*., 1978; Thakur, 2007b)

3.1.6 Assessment of sorghum fungal disease incidence

Disease incidence, expressed as a percentage of infection was determined by direct quantitative methods using the formulae suggested by Richard *et al*. (2014) where disease incidence was calculated as shown below:

$$\text{Disease incidence \%} = \frac{\text{No. of plants showing disease symptoms in the middle rows}}{\text{Total No.of plants assesed in the middle rows}} \times 100$$

3.1.7 Assessment of sorghum fungal disease severity

Ten plants within the inner two rows in each plot were tagged and assessed. Disease symptoms on top five leaves, sheathes and panicles were recorded on a monthly basis until physiological maturity as described by Ngugi *et al*. (2000). Disease assessment was based on a scale of 1 to 9 as described by Ngugi *et al*. (2002) (Table 3.4), while for panicle diseases, a scale of 1-9 by Thakur *et al*. (2007e) was adopted (Table 3.5).

Table 3.4 : Foliar fungal disease severity scale

Disease scale	Area of foliage infected
1	No disease
2	1 to 4% area of top 5 leaves
3	5 to 9%
4	10 to 19%
5	20 to 29%
6	30 to 44%
7	45 to 59%
8	60 to 75%
9	>75% of leaf area affected

Table 3.5 : Panicle fungal disease severity scale

Disease scale	Area of panicle infected
1	< 1%
2	1 - 5%
3	6 - 10%
4	11 - 20%
5	21 - 30%
6	31 - 40%
7	41 - 50%
8	51 - 75%
9	76 - 100%

3.1.8 Collection of sorghum leaf samples from the field trial

Leaf sample collection was done at soft dough stage as described by Bechem and Afanga (2017), where diseased plant leaves on tagged plants within the inner row were first taken photos, then collected. Photos enhanced availability of data for characterizing plant diseases (Mutka and Bart, 2015). Both symptomatic and asymptomatic portions of leaves were cut, stored in damp tissue paper and carried in plastic bags to the laboratory for fungal isolation.

3.1.9 Media preparation

Potato Dextrose Agar (PDA) was prepared and used as culturing medium for fungi isolation as described by Bechem and Afanga (2017). Its preparation involved adoption of manufacturer instructions where 39g of PDA powder was mixed in one litre of distilled water, autoclaved at 121°C for 20 minutes and allowed to cool to about 50°C before 0.1g/L of streptomycin was added to prevent bacterial infection. The mixture was gently stirred, transferred to sterile petri dishes measuring 9mm in diameter and allowed to solidify.

3.1.10 Isolation and purification of fungal isolates

A procedure by Tsedaley *et al*. (2016) with few modifications was adopted for isolation of fungi, where samples of infected leaves were cut into pieces measuring 1cm by 1cm using a sterile scalpel under conditions that were aseptic and surface sterilized for 2minutes in constituted 1.2% sodium hypochlorite solution. The leaf pieces were rinsed three times in sterile distilled water, with each wash lasting for 2minutes, allowed to dry in the laminar flow hood before being plated on Potato Dextrose Agar (PDA) in petri dishes and finally incubated for 7 days at 25°C. The water derived from the final wash was plated to serve as a control (Ager, 2023). Purification of fungi was done as described by Bechem and Afanga (2017), where a small section of each emerged separate colony was cut, transferred on new PDA media and incubated at 25°C for seven days

3.1.11 Cultural and morphological identification of fungal pathogens

Cultural characterization of fungal isolates was done following a protocol by Rajula *et al*. (2017), where: fungal cultures grown on PDA were studied colony color on the forward side and pigmentation on the reverse side on the petri dish on the 12th day. A slide culture technique described by Rosana *et al*. (2014) with slight modifications was used in morphological characterization of fungal pathogens. First, a glass slide was mounted on V-shaped glass rod placed inside a sterile petri dish. Square block of 1cm^2 potato dextrose agar (PDA) was cut and placed on top of the glass slide, then small portions of top mycelia was inoculated on the sides by use of a sterile inoculating needle, and finally covered with coverslips. About 2 mL of sterile distilled water was put at the base of the petri dish, and then covered. The square blocks inoculated with mycelia were then incubated for one week at 25ºC after which spores were stained with lacto-phenol cotton blue and observed under a compound microscope at low (4 x), medium (10 x) and high power (40 x) magnification.

3.1.12 Molecular characterization of fungal pathogens.

3.1.12.1 DNA Isolation

A modified protocol by Ager (2023) with few adjustments was adopted in isolating DNA of the fungal pathogens. From each ten-day old cultures of the fungal pathogens, 1 cm² sections of mycelial biomass were aseptically excised by use of sterile scalpel, placed in 1.5ml Eppendorf microcentrifuge tube containing 800 μl of lysis buffer [100 mM Tris-HCL (pH 8), 10 mM EDTA- pH 8.0) and macerated using sterile microfuge rods. Next, 537 µL of the mixture was transferred to a new sterile microfuge tube, then 3 μl of proteinase K and 60 μl of 10% SDS solution were added and homogenized with a vortex mixer for 1 minute. The microcentrifuge tube was incubated in a water bath at 65ºC for 30 minutes, after which 100 μl of 5M NaCl solution and 80 μl of 10% (w/v) CTAB was added, vortexed for 1 minute and incubated again in a water bath at 65ºC for 30 minutes. Equal volume of Chloroform: Isoamylalcohol (24:1) was added and centrifuged at 12000rpm for 15minutes. The aqueous phase was carefully transferred to a new sterile microfuge tube and the same process repeated to ensure complete removal of the interphase layer. The final aqueous phase of the suspension was transferred into sterile

microcentrifuge tube, then 0.6 of the volume of isopropanol (from -20ºC) was added and incubated at -20ºC for 60minutes. After incubation, Deoxyribonucleic Acid (DNA) was pelleted by centrifuging the mixture at 14000rpm for 15min, followed by washing the pellet twice with 100µL of 70% ethanol, then removal of the supernatant with a sterile micropipette. The DNA pellet was dried by inverting the tube over a sterile paper towel for 10 minutes. Sterile distilled water measuring 49.5 μl and 5 μl of 1mg/ml of RNAse A were added then incubated at 37ºC for 45min. The solution containing DNA was later stored at -20ºC for long term storage.

3.1.12.2 Polymerase chain reaction (PCR)

Polymerase Chain Reaction (PCR) amplification was done by use of a thermal cycler (MJ MiniTM Personal Thermal Cycler–BIO-RAD Company) following a procedure described by Kutawa *et al*. (2021) with mini modifications. The PCR reaction was carried out in 40 µl reaction volume consisting of 20 µl PCR master mix (OneTaq® Quick-Load 2X Master Mix with Standard Buffer (M0486)), 17 µl of free nuclease water, universal internal transcribed spacer region (ITS) primers including: 1 µl forward primer, NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and 1 µl reverse primer, NL4 (5′ GGTCCGTGTTTCAAGACGG-3′) and 1 µl of DNA template. The reaction was subjected to initial denaturation for 7min at 94ºC, then followed by 34cycles of second denaturation at 94ºC for 40sec, annealing at 54ºC for 40sec, and extension at 72ºC for 40sec with final extension at 72ºC for 7minutes.

3.1.12.3 Agarose Gel Electrophoresis

Agarose Gel Electrophoresis was done based on combination of protocols adopted by Ager (2023) and Lee *et al*. (2012) with modifications, where 1.2% agarose gel was prepared by adding 1.2 grams of agarose in 100 mL of 1× TBE-buffer and boiled in a microwave until agarose had dissolved. The mixture was allowed to cool to about 55 ºC then 1 µl of Ethidium bromide (EtBr) solution as a stain added and swirled. The solution was poured into a casting tray having a comb to solidify and later submerged into an electrophoresis tank filled with 1× TBE-buffer. GeneRuler 1 kb Plus DNA Ladder (1.5μl) used to ascertain the size of the amplified DNA bands, was loaded on the first well

followed by 5 μl of the amplified sample and finalized with a control at the end. The gel was then connected to an electric voltage of 100 volts for 45 minutes to grant migration of PCR amplicons. The resulting DNA bands were visualized under UV transilluminator and photos taken by using by UVP Photodoc-It™.

3.1.12.4 DNA sequencing and phylogenetic analysis

Eight amplicons were taken for sequencing to Macrogen Europe Company, in Netherlands. The sequences derived from forward and reverse primer sequencing were edited with BIOEDIT software and combined to generate a single contiguous sequence which was compared with known sequences in the GenBank by use of BLAST (Basic alignment search tool) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to locate similar regions of the sequences and establish the identity of the fungal isolates (Kimaru *et al*., 2018; Bechem and Afanga, 2017). Phylogenetic analysis was performed using Molecular Evolutionary Genetics Analysis version 11.0 (MEGA 11). The evolutionary history was inferred by using neighbor-joining analysis based on the Kimura 2-parameter model (Tamura *et al*., 2021).

3.1.13 Assessment of sorghum yield characters

Yield characters presented in table 3.6 were assessed following the procedure described in subsection 3.1.3.

Table 3.6 : Yield characters recorded on tagged plants

Parameter	Description / unit of measurement	Source / citation
Panicle height	Measured by a 30cm ruler from the base to the tip of panicle on tagged plants within the inner row (cm) at maturity	(Sheunda, 2019)
Panicle width	Tagged plants panicles were measured from the widest central position at maturity	(Sheunda, 2019)
Grain weight (g)	Grain harvested from ten targeted plants was weighed and recorded in grams	(Sheunda, 2019)
Grain yield (tha^{-1})	Four central rows within the net plot were harvested, threshed and grain weighed at 12.5% moisture content. The weight was converted to tha^{-1} by using the following formulae; $GY = \frac{GW}{100A}$ Where: GY- grain weight in grams per pot Area of the net plot harvested in square meters determined by: ***A=(R×I×L)*** Where: *R*- No. of rows within the net plot *I*-Space between rows(m) *L*-Length of the rows(m)	(Sheunda, 2019)
Dry matter Yield (panicles, stalk and leaves) (tha^{-1})	weight of dried stalks within the four central rows of the net plot was recorded and converted to tha^{-1} by the following formulae; $DYLD = \frac{10DW}{A}$ Where: DW-Dry biomass per plot (kg) Area of the net plot harvested (m^2)	(Sheunda, 2019)

3.1.14 Data analysis

Data on disease incidence, disease severity rating, growth characters (Days to 50% flowering, plant height, leaf area, and number of leaves) and yield characters (panicle height and width, grain weight (g) grain yield (tha^{-1}) and dry matter yield (t/ha)) was subjected to analysis of variance (ANOVA) and means separated by fisher's protected least significant difference test at 5% ($P< 0.05$) significant level using Genstat version 15

(Baird *et al*., 2022). Disease severity rating refers to the portion of plant tissue or organ infected by a symptom or sign of a disease and is assessed visually (Madden *et al*., 2007). Correlation analysis was done to determine the effect of severity and incidence on growth and yield parameters.

CHAPTER FOUR: RESULTS

4.1 Major fungal diseases and pathogens identified

4.1.1 Symptoms of identified fungal diseases

The symptoms of major fungal diseases identified on sorghum varieties in lower Eastern Kenya included: Anthracnose, Leaf blight, Rust, Gray leaf spot, Ladder leaf spot, oval leaf spot, Downy mildew and Covered Kernel Smut (Plate 4.1).

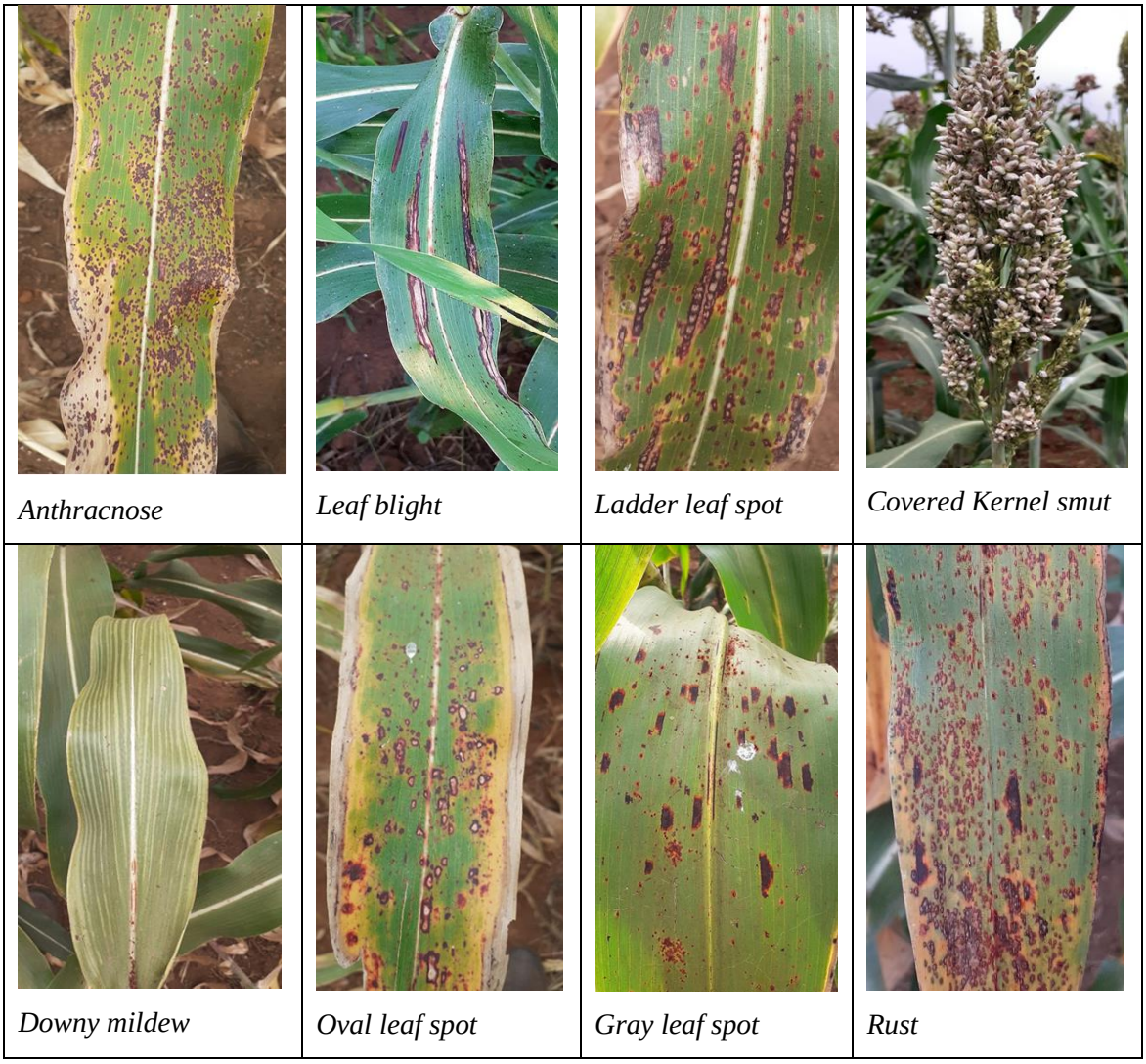


Plate 4.1 : Symptoms of anthracnose, leaf blight, ladder leaf spot, covered kernel smut, downy mildew, oval leaf spot, gray leaf spot and rust.

Foliar anthracnose: Small, circular to elliptical lesions with straw centers surrounded with purple margins. Leaf blight: long elliptical necrotic lesions of leaf blight with straw-colored centers engulfed with darker margins.Ladder leaf spot: lesions with pattern

resembling a ladder. Cone to oval shaped sori of Covered Kernel smut. Progressive chlorotic streaks of Downy mildew. Oval leaf spot: Circular lesions with dark margin. Rectangular dark red lesions of gray leaf spot. Red or purple distributed flecks of rust on the lower leaf surface.

4.1.2 Incidence and severity of identified fungal diseases

Three fungal diseases namely: Anthracnose, leaf blight and rust registered higher disease severity incidence of more than 90% while the downy mildew and covered kernel smut recorded least incidences in both seasons and sites as shown in table 4.1. All fungal diseases showed significant ($P \leq 0.05$) variation among G (Genotypes), S (Seasons), L (Locations) and interactions between Genotypes and Seasons (G*S) as displayed in appendix 3.0 KALRO, Kiboko field station exhibited higher fungal disease severity than KALRO-Ithookwe field station. In both sites, the first season recorded a higher disease severity for fungal diseases than the second season except for downy mildew. Moreover, three fungal diseases namely: leaf blight, anthracnose and rust recorded higher disease severities than other diseases. The mean fungal disease severity score ratings at KALRO-Kiboko filed station for the first season were in the order: Leaf blight, anthracnose, rust, gray leaf spot, ladder leaf spot, oval leaf spot, downy mildew and covered kernel smut while for the second season, were in the order: leaf blight, anthracnose, rust, gray leaf spot, downy mildew, oval leaf spot, ladder leaf spot and covered kernel smut. At KALRO, Ithookwe, first season disease severity scores were recorded in the order: leaf blight, anthracnose, rust, gray leaf spot, oval leaf spot, downy mildew, ladder leaf spot, and covered kernel smut while for second season were decreasing in the order: leaf blight, anthracnose, rust, downy mildew, gray leaf spot, oval leaf spot, ladder leaf spot and covered kernel smut as displayed in table 4.2

Table 4.1: Incidences of identified fungal diseases at KALRO Kiboko and Ithookwe under different seasons

Seasons	Season one								Season two							
Site	KALRO Kiboko				KALRO Ithookwe				KALRO Kiboko				KALRO Ithookwe			
	Days after sowing				Days after sowing				Days after sowing				Days after sowing			
Disease	30	60	90	120	30	60	90	120	30	60	90	120	30	60	90	120
Anth	28.3	58.3	85.9	91.8	24.4	48.8	74.7	91.4	24.2	50.4	76.0	91.8	27.2	48.9	73.0	91.6
Leaf B	16.4	37.3	75.9	100.0	6.0	32.0	65.3	100.0	8.7	36.0	75.3	100.0	6.1	33.9	65.1	100.0
Rust	0.0	0.0	59.6	100.0	0.0	0.0	45.5	100.0	0.0	0.0	53.7	100.0	0.0	0.0	47.6	100.0
Gray L.S	0.0	13.4	49.4	71.3	0.0	12.0	17.6	48.6	0.0	11.7	50.5	64.6	0.0	12.0	17.6	51.6
Ladder L.S	0.0	0.0	23.7	76.4	0.0	0.0	10.9	17.1	0.0	0.0	18.7	69.4	0.0	0.0	10.1	16.4
Oval L.S	0.0	0.0	32.3	53.5	0.0	0.0	18.7	46.9	0.0	0.0	24.3	52.2	0.0	0.0	15.7	46.8
Downy M	7.4	14.7	16.2	0.0	6.1	8.2	14.1	0.0	6.1	12.2	13.9	0.0	6.5	10.2	18.5	0.0
CKS	0.0	0.0	0.0	9.5	0.0	0.0	0.0	13.3	0.0	0.0	0.0	13.3	0.0	0.0	0.0	15.6

Anth = anthracnose; Leaf B. = leaf blight; Gray L.S =gray leaf spot; Ladder L.S=Ladder leaf spot; Oval L.S = oval leaf spot; Downy M. = downy mildew; CKS = covered Kernel smut.

Table 4.2: Severities of identified fungal diseases at KALRO Kiboko and Ithookwe under different seasons

Seasons	Season one								Season two							
Site	KALRO Kiboko				KALRO Ithookwe				KALRO Kiboko				KALRO Ithookwe			
	Days after sowing				Days after sowing				Days after sowing				Days after sowing			
Disease	30	60	90	120	30	60	90	120	30	60	90	120	30	60	90	120
Anth	1.7	3.4	4.5	5.8	1.4	2.6	3.7	5.3	1.5	2.3	3.7	5.1	1.3	2.0	3.0	4.3
Leaf B	1.1	2.9	4.3	6.5	1.1	2.0	3.3	6.0	1.1	1.9	3.5	5.7	1.0	1.7	3.1	5.2
Rust	1.0	1.2	3.4	5.7	1.0	1.0	2.5	5.1	1.0	1.2	3.1	5.0	1.0	1.0	2.3	4.2
Gray L.S	1.0	1.4	2.5	3.6	1.0	1.0	1.2	2.1	1.0	1.0	1.6	2.6	1.0	1.0	1.1	1.5
Ladder L.S	1.0	1.0	1.2	2.4	1.0	1.0	1.1	1.5	1.0	1.0	1.1	1.7	1.0	1.0	1.0	1.2
Oval L.S	1.0	1.2	1.5	3.0	1.0	1.0	1.1	1.9	1.0	1.0	1.1	2.3	1.0	1.0	1.1	1.4
Downy M	1.4	1.8	2.1	1.0	1.2	1.5	1.9	1.0	1.4	1.9	2.5	1.0	1.3	1.8	2.3	1.0
CKS	1.0	1.0	1.0	1.2	1.0	1.0	1.0	1.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Anth = anthracnose; Leaf B. = leaf blight; Gray L.S =gray leaf spot; Ladder L.S=Ladder leaf spot; Oval L.S = oval leaf spot; Downy M. = downy mildew; CKS = covered Kernel smut.

4.1.3 Fungal pathogens isolated from sorghum

A total of 69 fungi isolates were isolated from sorghum leaf samples. KALRO Kiboko registered higher number of fungal isolates than KALRO Ithookwe as shown in figure 4.1.

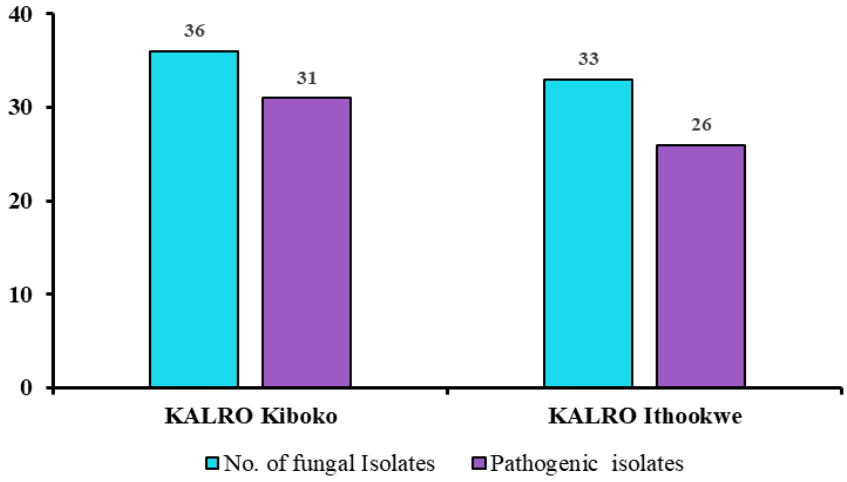


Figure 4.1 : Number of fungal isolates and pathogenic isolates at KALRO, kiboko and Ithookwe.

4.1.4 Morpho-cultural characteristics of major fungal pathogens

The fungal pathogens isolated displayed both variation and similarities in morphological and cultural characteristics. For example: *Curvularia*, *Fusarium* and *Epicoccum* isolates recorded a variation in both morphological and cultural attributes. However, *Alternaria*, *Bipolaris*, *Exserohilum* and *Colletotrichum* isolates registered similarities in cultural attributes but differed in conidial properties as illustrated in the tables and plates below.

Table 4.3: Morpho-cultural characteristics of *Curvularia* isolates

Groups	Isolates code	Symptom description under field trial	Mycelial characteristics	Forward side	Reverse side	Conidia characteristics	No. of Septa
Group-1	K9b, I6b, I11c	long elliptical necrotic lesions	Sparse mycelium, fimbriated margin	dark to brown	Concentric ringed, dark at the centre and light brown at the margin	ovoid to slightly curved at the second cell	3
Group-2	K3a, K7d, I7b	long elliptical to sub-circular necrotic lesions	abundant aerial mycelium, fimbriated margin	white to grey	3 concentric rings, dark brown at the centre and light brown towards the end	oviform and round at both of the ends	2-3
Group-3	K12c, K11a	long elliptical to sub-circular necrotic lesions	abundant aerial mycelium, cottony, fimbriated margin	Olivaceous grey	olivaceous black	ellipsoidal to ovoid, smooth-walled, rarely curved and dark brown	2-3
Group-4	K5c, I1d	long elliptical to sub-circular necrotic lesions	Sparse mycelium, fimbriated margin	grey olivaceous, pale mouse grey zones	Dark-brown, concentric, hyaline margin	smooth-walled, slightly curved with both apical and basal cells being paler	3-4
Group-5	I4b, K14e	long elliptical necrotic lesions	Aerial velvety mycelium, fimbriated margin	dark to brown	dark brown and 3 concentric rings	smooth-walled, ellipsoidal to ovoid, curved, with paler apical and basal cells	3

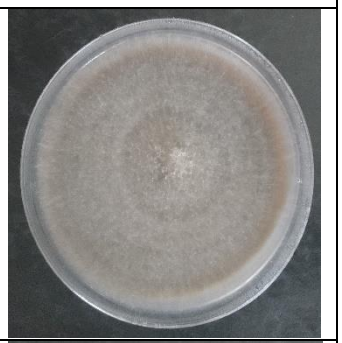
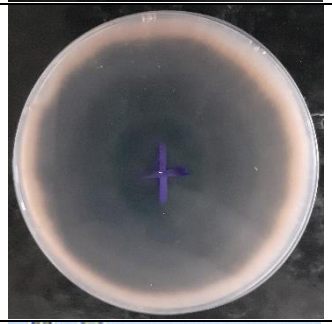
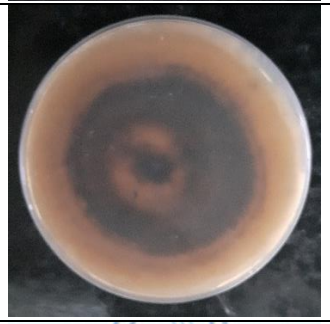
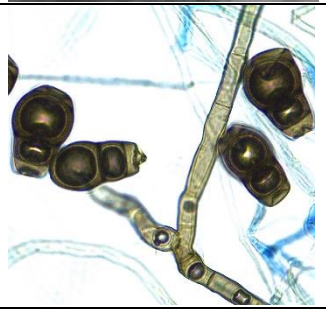
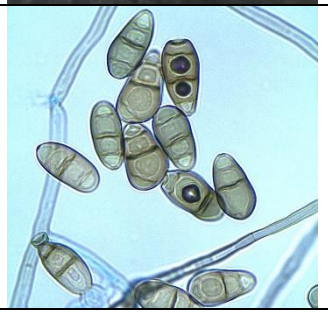
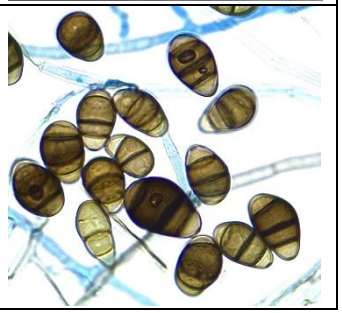
	Group-1	Group-2	Group-3	Group-4	Group-5
Forward side					
Reverse side					
Conidia					

Plate 4.2: Variation in morpho-cultural characteristics of *Curvularia* isolates

Table 4.4: Morpho-cultural characteristics of *Fusarium* isolates

Groups	Isolates code	Symptom description under field trial	Mycelial characteristics	Forward side	Reverse side	Conidia characteristics	No. of Septa
Group-1	K8a	Circular to irregular greyish to dak brown lesions	Flat mycelia	whitish	white to cream	Sickle shaped macro conidia	0-2
Group-2	I2a, I4a	Circular to Irregular grey, dak brown lesions	Sparse mycelia	whitish	Whitish	Falciform macro conidia	3-7
Group-3	K12b, K2f, K1c,K2a	Circular greyish to dak brown spots	Flat mycelial growth	white and pinkish (at the center)	white to pinkish	oval to kidney shaped micro conidia	0
Group-4	I14f, K13c, K9b	Circular to irregular greyish to dak brown spots	Sparce mycelia	whitish	Plum	Hyaline micro conidia varied in sizes from obovoid with truncate base to fusiform. Hyaline macroconidia with slightly curved apical cells.	0-1 3-5
Group-5	K3b, K1a, K10b, I5c, I8e, I13a	Circular greyish to dak brown spots	Sparce mycelia	whitish	whitish to cream	oval shaped micro conidia	0

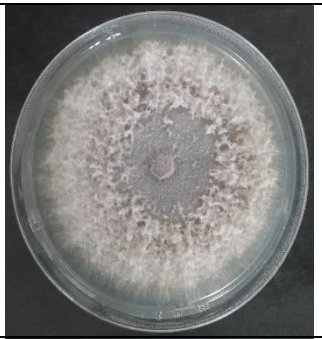
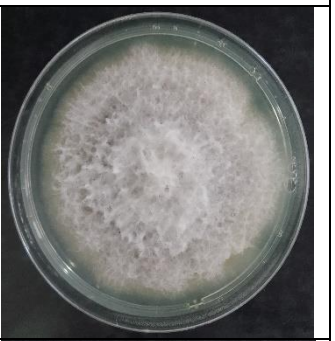
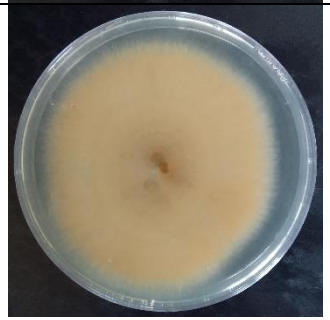
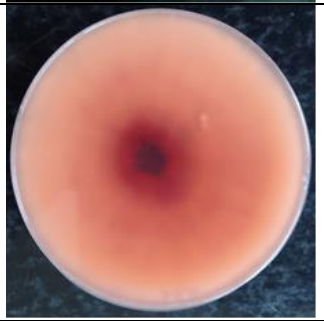
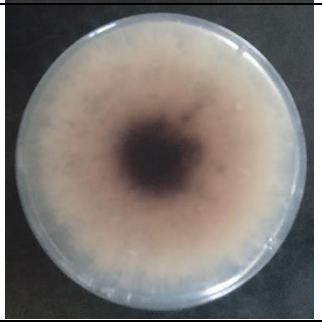
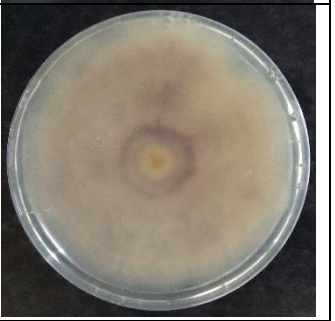
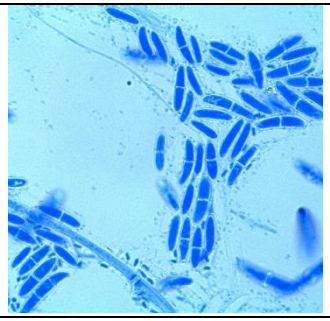
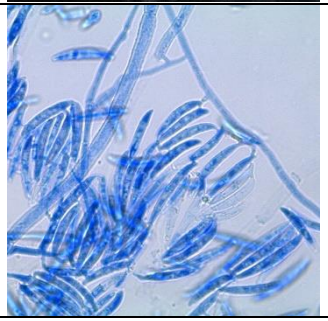
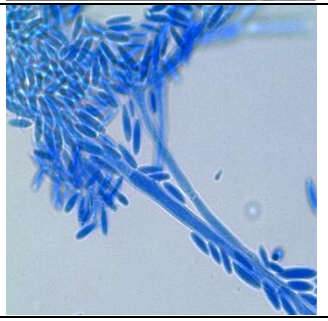
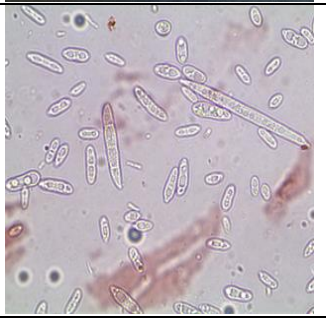
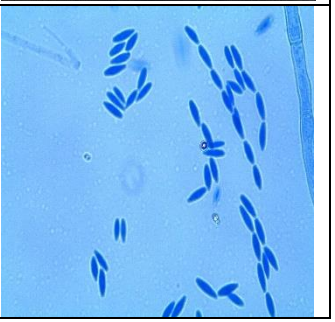
	Group-1	Group-2	Group-3	Group-4	Group-5
Forward side					
Reverse side					
Conidia					

Plate 4.3: Variation in morpho-cultural characteristics of *Fusarium* isolates

Table 4.5: Morpho-cultural characteristics of *Alternaria* isolates

Groups	Isolates code	Symptom description under field trial	Mycelial characteristics	Forward side	Reverse side	Conidia characteristics	No. of Septa
Group-1	K11a, I5e, K12g, I10f, K1f	Nearly circular lesions with lighter centers bordered by darker margins	Sparse mycelia	Grey	Olivaceous grey	Oval brown and inverted clavate conidia	0-4 longitudinal septa
Group-2	I8d, I13b, I7e,	Small roundish to irregular spots	Sparse mycelia	whitish grey	Olive grey	Oval brown and inverted clavate conidia	0-4 longitudinal septa
Group-3	K2c, I14a,	Small roundish to irregular spots	Sparse mycelial growth	Grey	Olive grey	Oval brown and inverted clavate conidia	0-4 longitudinal septa
Group-4	K9a, I8c,	Small roundish to irregular spots	sparse mycelia	Olivaceous grey	whitish dark	Oval brown and inverted clavate conidia	0-4 longitudinal septa

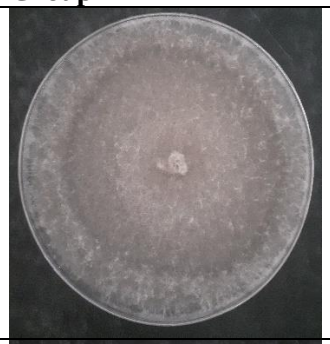
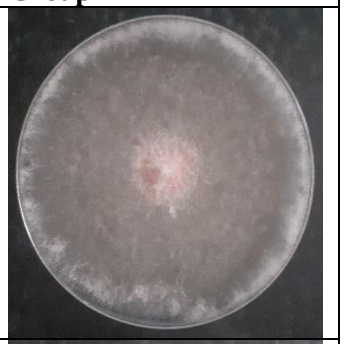
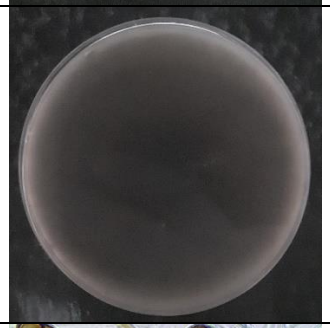
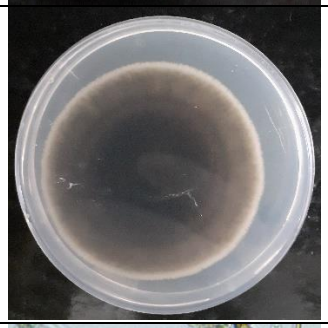
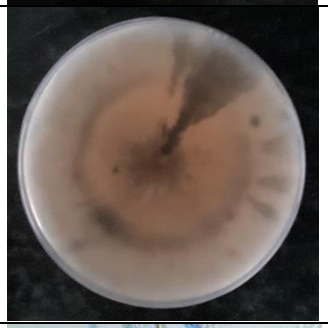
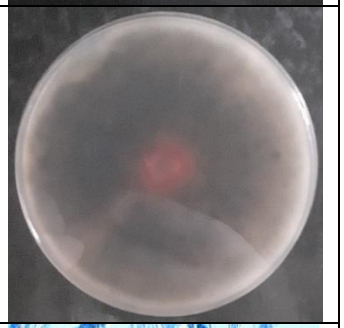
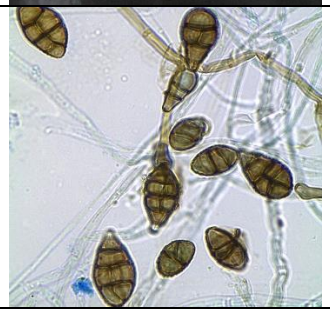
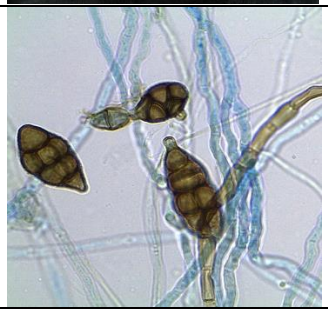
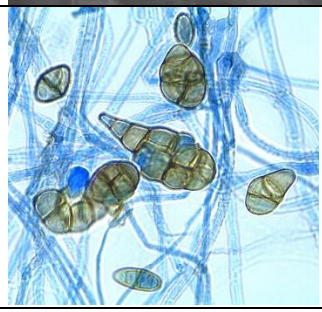
	Group-1	Group-2	Group-3	Group-4
Forward side				
Reverse Side				
Conidia				

Plate 4.4: Variation in morpho-cultural characteristics of *Alternaria* isolates

Table 4.6: Morpho-cultural characteristics of *Bipolaris* isolates

Groups	Isolates code	Symptom description under field trial	Mycelial characteristics	Forward side	Reverse side	Conidia Characteristics	No. of Septa
Group-1	K13h, K2d	Fusiform to long strip shaped lesions	Fluffy cottony appearance and irregular margin	whitish (at the center) to slightly gray mycelia	Slightly gray at the center and dark towards the margin	Rod-shaped, geniculate, light brown	0-7
Group-2	I4j	Fusiform or elliptical shaped lesions	Abundant aerial mycelia	Slightly gray to dark gray	Dark grey	Rod-shaped, geniculate, light brown	0-7
Group-3	I10a, 15g	Elongated strip shaped lesions	Fluffy cottony appearance and regular margin	Slightly gray	Slightly gray	Rod-shaped, geniculate, light brown	0-7
Group-4	K7a	Fusiform to long strip shaped lesions	Fluffy cottony appearance and irregular margin	Slightly gray (at the center) to dark gray	Dark	Rod-shaped, geniculate, light brown	0-7

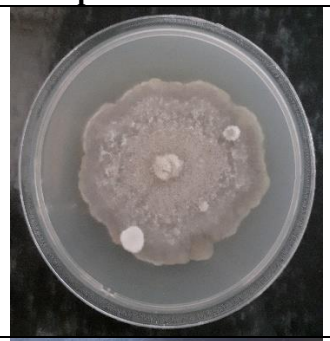
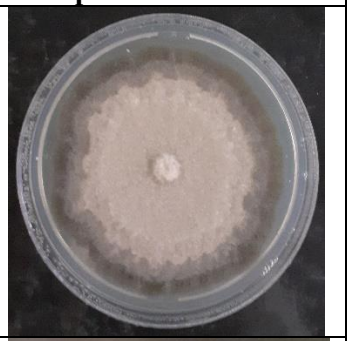
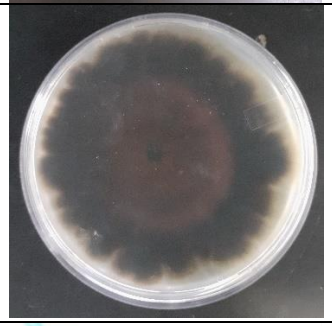
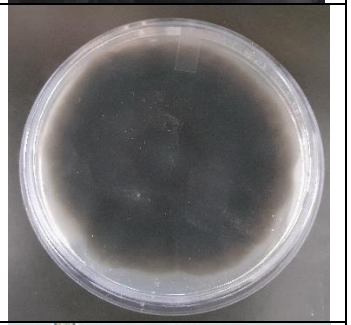
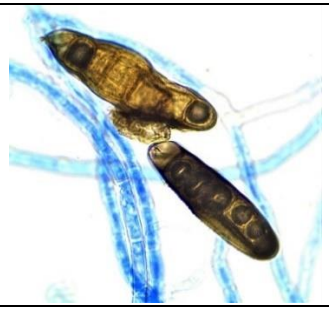
	Group-1	Group-2	Group-3	Group-4
Forward side				
Reverse side				
Conidia				

Plate 4.5: Morpho-cultural characteristics of *Bipolaris* isolates

Table 4.7: Morpho-cultural characteristics of *Exserohilum* isolates

Groups	Isolates code	Symptom description under field trial	Mycelial characteristics	Forward side	Reverse side	Conidia characteristics	No. of Septa
Group-1	K3f, K11c	Elongated gray streaks	Flat mycelia with irregular margin	Dark grey	Dark	Cylindrical, pyriform to oval, deep brown, with protruding hilum	4-8
Group-2	I6h	Elongated cigar shaped blighted lesions	Cottony appearance and irregular margin	Slightly grey on the upper side with irregular margin	Dark	Cylindrical, pyriform to oval, deep brown, with protruding hilum	4-8
Group-3	K14e	Elongated cigar shaped blighted lesions	Abundant aerial mycelia, Fluffy cottony appearance and irregular margin	Deep brown	Grey to dark grey	Cylindrical, pyriform to oval, deep brown, with protruding hilum	4-8

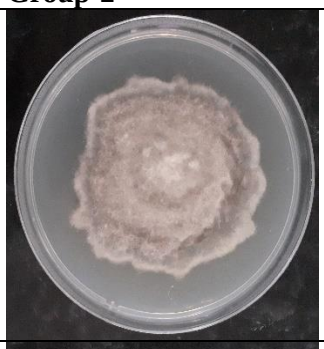
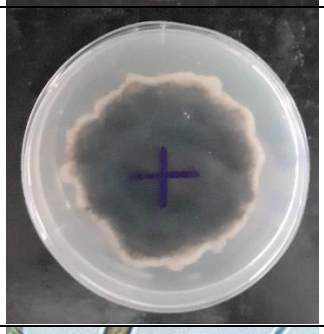
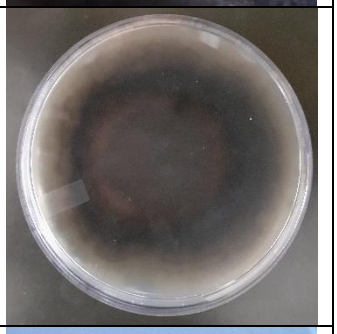
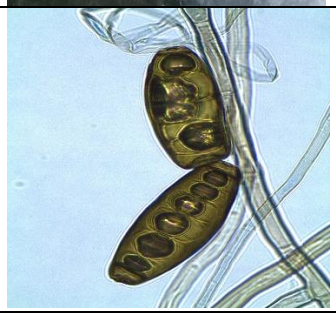
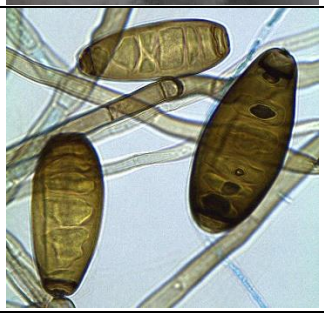
	Group-1	Group-2	Group-3
Forward side			
Reverse side			
Conidia			

Plate 4.6: Morpho-cultural characteristics of *Exserohilum* isolates

Table 4.8: Morpho-cultural characteristics of *Colletotrichum* isolates

Groups	Isolates code	Symptom description under field trial	Mycelial characteristics	Forward side	Reverse side	Conidia characteristics	No. of Septa
Group-1	K1h, I8f, I8e	Small, circular to elliptical dark or red or purple lesions with straw colored centers. Redish discoloration on mid rib	Dense aerial mycelia with regular margin	white	Greyish orange with concentric rings	Sickle shaped, hyaline	None
Group-2	K2b	Small, circular red lesions with straw colored centers	Dense aerial mycelia mycelia with regular margin	white	Yellowish	Sickle shaped, hyaline	None

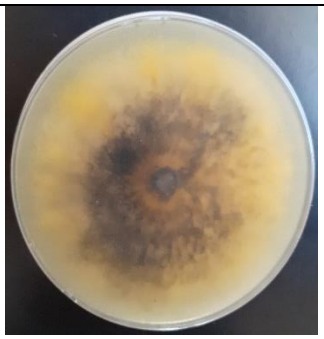
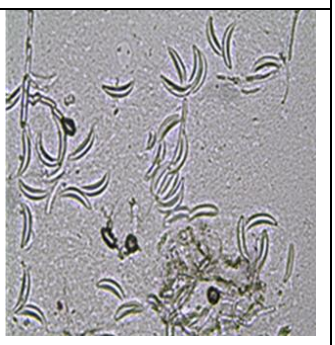
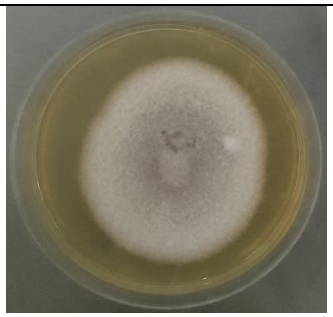
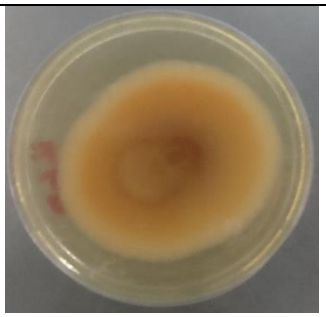
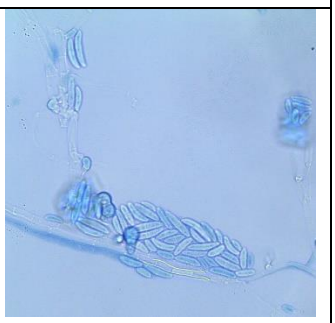
	Forward side	Reverse side	Conidia
Group- 1			
Group- 2			

Plate 4.7: Morpho-cultural characteristics of *Colletotrichum* isolates

Table 4.9: Morpho-cultural characteristics of *Epicoccum* isolates

Groups	Isolates code	Symptom description under field trial	Mycelial characteristics	Forward side	Reverse side	Conidia characteristics	No. of Septa
Group-1	K2f, I8a	Small, circular dark to red lesions coalese and later replaced with straw colored centers	Cottony aerial mycelia with regular margin	light purple in color	Dark to Orange with concentric rings	Unicellular (nearly spherical in shape) to multicellular (elongated in shape) chlamydospores	None
Group-2	K13b	Small, circular dark to red lesions coalese and later replaced with straw colored centers	Velvety aerial mycelia with regular margin	White with a light pink, flocculent center	Light orange with Light purple concentric rings	Unicellular (nearly spherical in shape) to multicellular (elongated in shape) chlamydospores	None

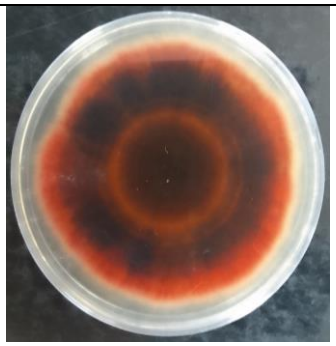
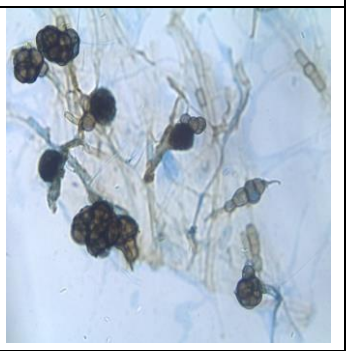
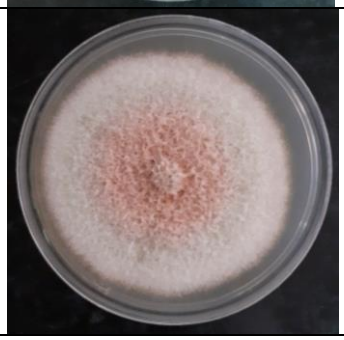
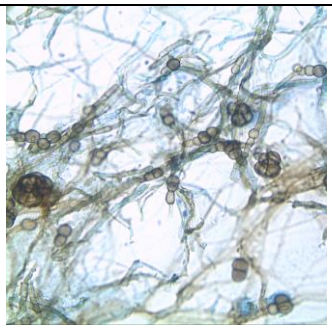
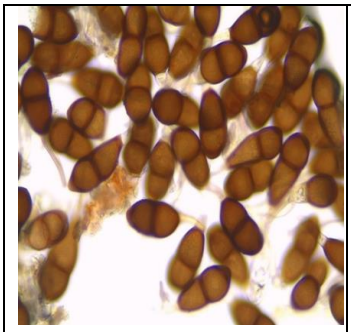
Group	Forward side	Reverse side	Conidia
Group-1			
Group-2			

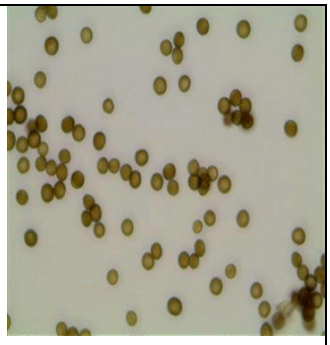
Plate 4.8: Morpho-cultural characteristics of *Epicoccum* isolates.

4.1.4.1 Other fungal pathogens identified

Puccinia purpurea conidia was orange, obovate shaped urediniospores consisting of small spikes on their surface while *Sporisorium sorghi* conidia was characterized by dark brown oval shaped teliospores as shown in Plate 4.8, *ii*.



Puccinia purpurea



Sporisorium sorghi

Plate 4.9: Identified conidia of *Puccinia purpurea* (rust) and *Sporisorium sorghi* (covered kernel smut)

4.1.5 Molecular characteristics of major fungal pathogens

4.1.5.1 Gel electrophoresis

The amplified DNA fragments from the 8 isolates ranged between 500 to 700bp as displayed by the gel electrophoresis photo (plate 4.9).

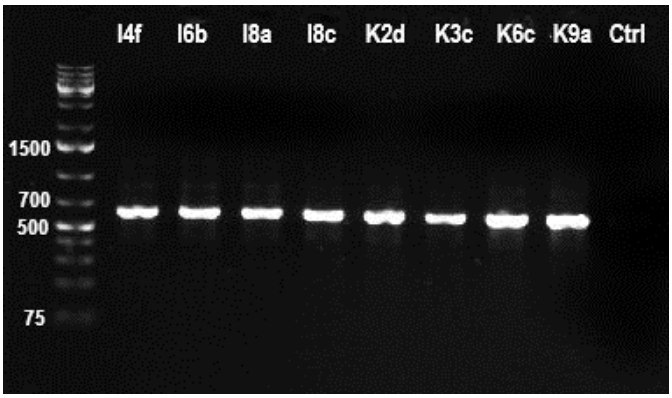


Plate 4.10: Agarose gel showing an approximately between 500-700bp product amplified from the fungal isolates

4.1.5.2 BLASTn results

The BLASTn searches revealed the sequences of the 8 isolates had base pairs ranging between 570 to 610. The ITS sequences of the eight isolates were more than 99% identical with closest species available in the gene bank. The top BLASTn results for each query sequence are presented in Table 4.10.

Table 4.10: BLASTn results of the major fungal isolates

No.	Sample	Size (bp)	Closest Match in Blast	ITS Accerssion	% similarity
1	K6c	604	*Curvularia akaiiensis*	MW644950	100
2	I6b	572	*Curvularia lunata*	MT516307	99.83
3	K3c	578	*Exserohilum rostratum*	MT516299	99.83
4	K2d	602	*Bipolaris secalis*	MH876123	99.83
5	I4f	575	*Fusarium napiforme*	MH862670	100
6	I8c	607	*Alternaria alstroemeriae*	NG_069882	99.68
7	K9a	583	*Alternaria alstroemeriae*	NG_069882	100
8	I8a	584	*Epicoccum sorghinum*	MK516207	100

4.1.5.3 Phylogenetic relationship of fungal isolates

Phylogenetic relationship clustered isolate K6C with *Curvularia akaiiensis* species supported by a bootstrap value of 91.9%, isolate I6b with *Curvularia lunata* species supported by a bootstrap value of 85.5%, isolate K2d with *Exserohilum rostratum* species supported by a bootstrap value of 92.4% and K3c with *Bipolaris secalis* species supported by a bootstrap value of 95.6 as displayed in figure 4.2. Isolates K9a and I8c clustered with *Alternaria alstroemeriae* species supported by a bootstrap value of 95.7% while isolates I8a and I4f clustered with species of *Epicoccum sorghinum* and *Fusarium napiforme* supported by bootstrap values of 95.8% and 78.2% respectively as shown in figure 4.3.

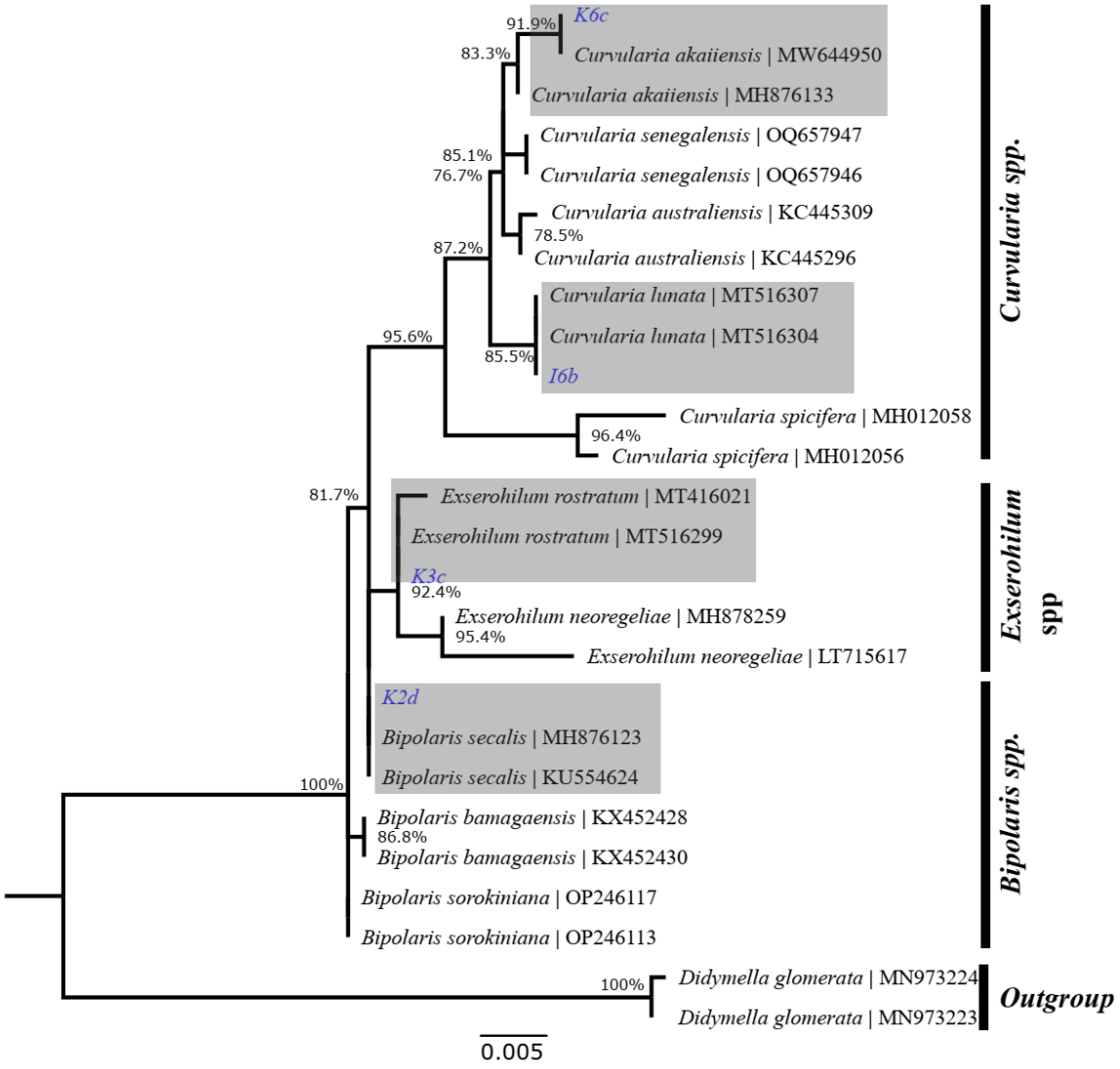


Figure 4.2 : Phylogenetic relationship for *Curvularia akaiiensis*, *Curvularia lunata*, *Exserohilum rostratum*, and *Bipolaris secalis* isolates constructed with ITS sequences.

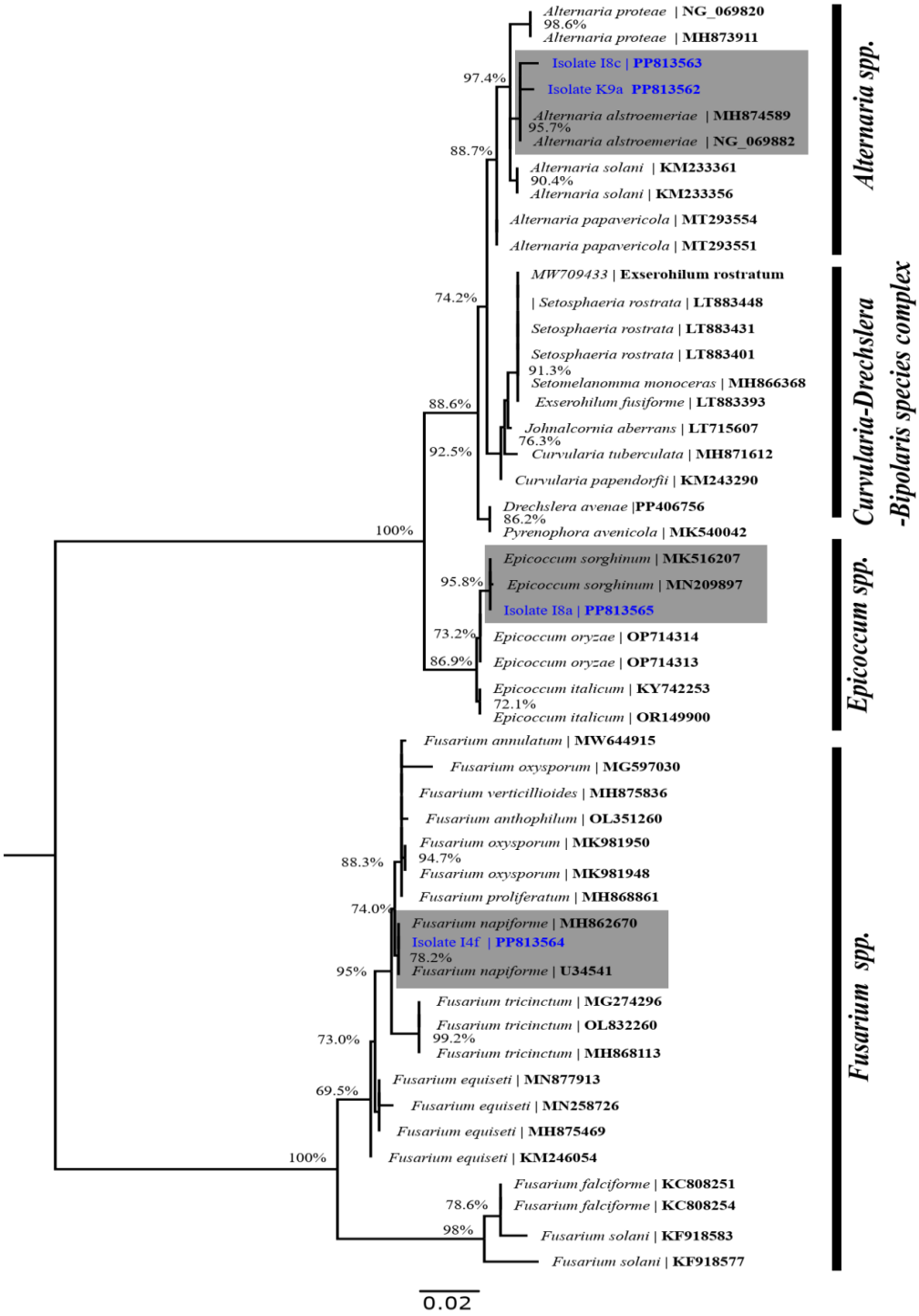


Figure 4.3: Phylogenetic relationship for *Alternaria alstroemeriae*, *Epicoccum sorghinum* and *Fusarium napiforme* isolates constructed with ITS sequences.

4.2 Evaluation of the performance of selected sorghum varieties under field trials.

4.2.1 Agronomic performance of various sorghum genotypes at KALRO Kiboko

Highly significant ($P \leq 0.001$) differences were recorded for: Days to 50% flowering, plant height (cm), leaf area, number of leaves, panicle length(cm), panicle width (cm), Dry biomass yield (tha^{-1}), grain yield (tha^{-1}) and grain yield for 10 sampled plants (g) among the sorghum varieties. Local varieties: Kateng'u, Rasta, Kaguru, Kauwi, Mugeta, and one improved variety: Gadam took lesser mean number of days to attain 50% flowering. Highest plant height above the total mean average of 176.8cm was recorded on improved varieties: Makueni Local and Kiboko Local 2, while largest leaf area above the total mean of 294.7cm^2 in KARI Mtama 1, Makueni Local, Kiboko Local 2, IESV 24029 SH, Marcia and Serena. Varieties with the higher dry biomass yield were: Kiboko Local 2 and Makueni Local while the best yielding varieties were in the order: Makueni Local, Kiboko Local 2, IESV 24029 SH, Seredo and KARI Mtama 1 as displayed in table 4.11.

4.2.2 Agronomic performance of various sorghum genotypes at KALRO Ithookwe

Highly significant ($P \leq 0.001$) differences were noted for agro-morphological parameters namely: Days to 50% flowering, plant height (cm), leaf area, number of leaves, panicle length(cm), panicle width (cm), Dry biomass yield (tha^{-1}), grain yield (tha^{-1}) and grain yield for 10 sampled plants (g) among the sorghum varieties. The earliest flowering varieties included four locals namely: Kateng'u, Rasta (59days), Kaguru, Kauwi, Mugeta and one improved variety, Gadam. Varieties Makueni Local and Kiboko Local 2 recorded the highest plant height compared to other varieties. Improved varieties: Makueni Local and Kiboko Local 2, KARI Mtama1, IESV 24029 SH, Marcia and Serena recorded highest mean leaf area. Higher mean dry biomass yield was recorded in Kiboko Local 2 and Makueni Local. Improved varieties: Makueni Local, Kiboko Local 2, IESV 24029 SH, Seredo and KARI Mtama 1 registered higher grain yield than controls Kateng'u and Kaguru as shown in table 4.12.

Table 4.11: Agronomic performance of sorghum varieties at KALRO Kiboko in 2020-2021 seasons

Variety	DF	PH	LA	NL	PL	PW	DMY	GY	GW
Gadam	63.6b	137ab	249abc	2.9bcd	20.1bc	6.6ab	5.3abc	1.5bcd	355.8ab
Kateng'u	56.1a	207ef	237a	2.3a	23.2de	8.9def	4.9a	1.5abc	352.4ab
Marcia	72.9f	127a	332bcde	4.0f	26fg	8.8def	5.8abcd	2.0def	568.7d
IESV 24029 SH	69.8de	143ab	337cde	3.6ef	23.1dde	7.6bc	6.1abcd	2.2f	525.9d
Kauwi	62.9b	198e	267abcd	2.7abc	21.5bcd	6.4a	7.3d	1.6bcde	364abc
KARI Mtama 1	70.1e	157abc	373e	3.3de	23.5def	8.1cd	6.7cd	2.1ef	560.8d
Kiboko Local 2	70.6e	229f	359e	3.3de	26.1g	9.5f	9.3e	2.3f	533.4d
Rasta	56.0a	206ef	243ab	2.3a	22.9de	8.8def	5.1ab	1.4abc	356.9ab
Makueni Local	70.7e	233f	370e	3.4def	23.8defg	11g	9.1e	2.4f	587d
Serena	67.8cd	144ab	315abcde	2.4 ab	24efg	8.3cde	5.7abc	1.9cdef	468.9bcd
Mugeta	64.8b	182cde	253abc	2.7abc	20b	6.3a	6.4bcd	1.0a	260.6a
Seredo	67.5c	154abc	283abcde	2.4 ab	23.6def	7.6bc	6.0abcd	2.1f	492.6cd
Kaguru	56.2a	192de	245ab	2.3a	22.6cde	9.4ef	4.9ab	1.4abc	368.6cd
Dark Red	69.7de	166bcd	264abc	3.2cde	13.3a	6.6ab	6.3abcd	1.3ab	341.6ab
Means	**65.6**	**177**	**295**	**2.9**	**22.4**	**8.1**	**6.3**	**1.8**	**438.4**
FPr	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
l.s.d.	2	30.5	92.1	0.6	2.5	1.1	1.5	0.5	130.3
CV%	3.1	17.4	31.5	20.2	11.1	14	24	29.5	30

Means within each column (agromorphological and yield characters) that are not followed by the same letter are significantly different (P <.05), while those followed by the same letter are insignificantly different at (P <.05). Where: DF= Days to 50% flowering, PH = plant height (cm), LA = Leaf area, NL = number of leaves, PL= Panicle length, PW= Panicle width, DMY= Dry Matter yield (t/ha), GY=Grain Yield (t/ha) GW= Grain weight per 10 sampled plants.

Table 4.12: Agronomic performance of sorghum varieties at KALRO Ithookwe in 2020-2021 seasons

Variety	DF	PH	LAI	NL	PL	PW	DMY	GY	GW
Gadam	64.9c	115.1a	318.3cd	3.6de	19.2c	8.1ab	5.8abc	1.6bc	374.4bc
Kateng'u	58.7a	203.7g	247.3a	2.4ab	19.8cd	13.1d	5.0a	1.7bc	376.4bc
Marcia	74.1g	121.1ab	340.2de	5.3f	23.8fg	9.5bc	6.0abc	2.3de	483.2cd
IESV 24029 SH	72.3fg	127.4bc	410.6fg	3.8e	22.4ef	8.0ab	6.3bcd	2.6ef	518.2d
Kauwi	64.1bc	194.9g	347.7de	3.5de	20.4cd	7.0a	8.1e	1.9c	377.5bc
KARI Mtama 1	72.6fg	155.4e	385.7ef	3.8e	21.4de	8.8b	7.1d	2.3de	531.9d
Kiboko Local 2	71.2ef	234.5h	444g	3.5cde	25.4cd	11.0c	10.2f	2.7f	580.9d
Rasta	59a	200.6g	234.4a	2.3a	20.1cd	13.4d	5.0a	1.6bc	392.8bc
Makueni Local	72.5fg	240.6h	385.9ef	3.8e	15.1b	16.6e	10.0f	2.8f	553.2d
Serena	69.8de	137cd	329.3d	3.0f	23.1f	8.8b	5.6ab	2.2d	474.3cd
Mugeta	62.4b	180.8f	239.7a	3.5de	19.5c	6.5a	5.3ab	1.1a	227.9a
Seredo	69.1d	141.9d	299.3bcd	2.8abc	22.3ef	7.8ab	5.9abc	2.4def	517.5d
Kaguru	58.9a	201g	252ab	2.4ab	20.4cd	14.9de	5.1a	1.6bc	392.9bc
Dark Red	71.1ef	158.2e	278.5abc	3.8e	12.4a	6.8a	6.7cd	1.4b	304ab
Means	**67.2**	**172**	**322**	**3.4**	**20.4**	**10**	**6.6**	**2**	**436.1**
FPr	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
l.s.d.	2	10.2	49.1	0.7	1.7	1.8	1	0.3	117.6
CV%	3	6	15.3	19.9	8.2	18.1	16	16.9	27.2

Means within each column (agromorphological and yield characters) that are not followed by the same letter are significantly different (P <.05), while those followed by the same are insignificantly different at (P <.05). Where: DF= Days to 50% flowering, PH = plant height (cm), LA = Leaf area, NL = number of leaves, PL= Panicle length, PW= Panicle width, DMY= Dry Matter yield (t/ha), GY=Grain Yield (t/ha) GW= Grain weight per 10 sampled plants.

4.3 Effect of fungal diseases on sorghum growth and yield

4.3.1 Correlation of sorghum agromorphological characters to fungal disease incidence and severity of fungal diseases under field trials.

There was a negative correlation between fungal disease severity and days to 50% flowering (r= -0.741, P≤0.002), number of green leaves (r= -0.813, P≤0.001), leaf area (r= -0.543, P≤0.045). However, insignificant negative association was noted between fungal disease severity and dry matter yield (r= -0.338, P≤0.237), grain yield (r= -0.268, P≤0.355), grain weight for 10 tagged plants (r= -0.293, P≤0.309), and panicle length (r= -0.163, P≤0.577). Similarly, fungal disease incidence was negatively associated with days to 50% flowering (r= -0.647, P≤0.012) and number of green leaves (r= -0.754, P≤0.002). Insignificant negative correlation was noted between fungal disease incidence and leaf area (r= -0.449, P≤0.107), dry matter yield (r= -0.224, P≤0.441), grain yield (r= -0.175, P≤0.550), grain weight for 10 tagged plants (r= -0.236, P≤0.417) and panicle length (r= -0.185, P≤0.528) as displayed in table 4.13.

Table 4.13: Correlation coefficients (r) of sorghum agronomic characters to fungal disease incidence and severity.

	FDS	FDI	D.F	P.H	N.L	L.A	D.M	G.Y	G.W	P.L	P.W
FDS	1	0.977**	-0.741**	0.434	-0.813**	-0.543*	-0.338	-0.268	-0.293	-0.163	0.507
FDI	0.977***	1	-0.647*	0.439	-0.754**	-0.449	-0.224	-0.175	-0.236	-0.185	0.490
D.F	-0.741**	-0.647*	1	-0.330	0.821**	0.809**	0.557*	0.671**	0.672**	0.117	-0.220
P.H	0.434	0.439	-0.330	1	-0.339	0.008	0.523	0.040	-0.016	-0.039	0.627*
N.L	-0.813***	-0.754**	0.821**	-0.339	1	0.647*	0.429	0.415*	0.449	0.068	-0.211
L.A	-0.543*	-0.449	0.809**	0.008	0.647*	1	0.748**	0.879**	0.863**	0.424	0.100
D.M	-0.338	-0.224	0.557*	0.523	0.429	0.748**	1	0.609*	0.532*	0.083	0.203
G.Y	-0.268	-0.175	0.671**	0.040	0.415*	0.879**	0.609*	1	0.977**	0.556*	0.331
G.W	-0.293	-0.236	0.672**	-0.016	0.449	0.863**	0.532*	0.977**	1	0.585*	0.369
P.L	-0.163	-0.185	0.117	-0.039	0.068	0.424	0.083	0.556*	0.585*	1	0.225
P.W	0.507	0.490	-0.220	0.627*	-0.211	0.100	0.203	0.331	0.369	0.225	1

***Correlation is significant at P ≤ 0.001 level; **Correlation is significant at P ≤ 0.01 level; *Correlation is significant at P ≤ 0.05 level; FDS= Fungal disease severity, FDI= Fungal disease incidence, D.F= Days to 50% flowering, P.H= plant height (cm), N.L= number of leaves, L.A= Leaf area, D.M= Dry Matter yield (t/ha), G.Y=Grain Yield (t/ha) G.W= Grain weight per 10 sampled plants(g), P.L= Panicle length, P.W= Panicle width.

4.3.2 Disease tolerance among sorghum germplasm

Local pigmented varieties namely: Kateng'u, Rasta and Kaguru showed higher severity for: anthracnose, leaf blight, rust, gray leaf spot, ladder leaf spot and oval leaf spot than other varieties at KALRO, Kiboko and Ithookwe. Improved tan varieties: Marcia and KARI Mtama 1 recorded lower disease severity for anthracnose, ladder leaf spot, oval leaf spot and rust at both sites. Most varieties were recorded lower disease severity for covered kernel smut (Table 4.14) and (Table 4.15). Improved varieties namely: Makueni local, Kiboko local 2, IESV 4029 SH, KARI Mtama 1, Seredo, Marcia and Serena recorded higher grain yield than local varieties at both sites. Improved tan varieties registered lower disease incidence for anthracnose, gray leaf spot and ladder leaf spot at KALRO, Kiboko and Ithookwe. Local pigmented varieties recorded highest disease incidence for: anthracnose, leaf blight, rust, gray leaf spot, ladder leaf spot and oval leaf spot at both sites. Most varieties recorded least disease incidence for covered kernel smut except Kauwi variety at both sites the experiment was done (Table 4.9) and Table 4.10).

Table 4.14 : Categorization of sorghum germplasm based on disease reaction, disease severity and yield at KALRO, Kiboko

Variety	Plant color	Anth	Leaf B.	Rust	Gray L.S	Ladd. L.S	Oval L.S	Downy M.	CKS	Yield (t/ha)	DR
Makueni local	Pigmented	6.3c	6.1abcde	5.2bc	3.1c	1.9c	2.8f	1.0a	1.0a	2.4f	Tolerant
Kiboko local 2	Pigmented	6.3c	6.1abcd	5.1bc	3.1c	2.0c	2.8f	1.0a	1.0a	2.3f	Tolerant
IESV 4029 SH	Pigmented	5.9c	6.0ab	4.7b	3.0c	1.7bc	2.2d	2.4b	1.0a	2.2f	Tolerant
KARI Mtama 1	Tan	1.0a	6.6e	4.0a	2.2a	1.0a	1.5b	2.9bc	1.0a	2.1ef	Tolerant
Seredo	Pigmented	6.2c	6.1abcd	5.0b	2.9ab	1.9c	2.3de	3.0cd	1.0a	2.1f	Tolerant
Marcia	Tan	1.0a	6.5bcde	3.9a	2.0a	1.0a	1.0a	1.0a	1.0a	2.0def	Tolerant
Serena	Pigmented	6.2c	6.1abc	5.0b	3.1c	1.8bc	2.1d	3.0c	1.0a	1.9cdef	Tolerant
Kateng'u	Pigmented	8.1d	5.7a	7.4d	4.4d	3.5d	4.5g	3.7e	1.0a	1.5abc	Susceptible
Rasta	Pigmented	8.1d	5.7a	7.4d	4.3d	3.5d	4.5g	3.3cde	1.0a	1.4abc	Susceptible
Kaguru	Pigmented	8.1d	5.7a	7.4d	4.3d	3.6d	4.5g	3.5de	1.0a	1.4abc	Susceptible
Dark Red	Pigmented	6.1c	5.8a	5.0b	3.0ab	1.9c	2.6ef	3.8e	1.0a	1.3ab	Susceptible
Mugeta	Mixed	3.3b	6.6de	4.2a	2.4ab	1.4ab	1.7bc	1.0a	1.0a	1.0a	Susceptible
Gadam	Pigmented	6.2c	5.9a	5.5c	2.9ab	1.8bc	2.3de	1.0a	1.0a	1.5bcd	Susceptible
Kauwi	Mixed	3.7b	6.6cde	4.7b	2.8ab	1.8bc	2.1cd	1.0a	1.1b	1.6bcde	Susceptible

Means within each column (disease severity scores and yield) that are not followed by the same letter are significantly different (P <.05), while those followed by the same are insignificantly different at (P <.05). Where: Anth = anthracnose; Leaf B. = leaf blight; Gray L.S =gray leaf spot; Ladd. L.S=Ladder leaf spot; Oval L.S = oval leaf spot; Downy M. = downy mildew; CKS = covered Kernel smut; DR = disease reaction.

Table 4.15 : Categorization of sorghum germplasm based on disease reaction, disease severity and yield at KALRO, Ithookwe

Variety	Plant color	Anth	Leaf B.	Rust	Gray L.S	Ladd. L.S	Oval L.S	Downy M.	CKS	Yield (t/ha)	DR
Makueni local	Pigmented	5.7de	5.7bcde	4.3bc	1.9c	1.3ab	1.6c	1.0a	1.0a	2.8f	Tolerant
Kiboko local 2	Pigmented	5.8e	5.9bcde	4.3cd	1.8c	1.4b	1.6c	1.0a	1.0a	2.7f	Tolerant
IESV 4029 SH	Pigmented	5.1d	5.6bcde	4.0abc	1.5abc	1.2ab	1.3abc	2.0b	1.0a	2.6ef	Tolerant
KARI Mtama 1	Tan	1.0a	6.0cde	3.7ab	1.4a	1.0a	1.1a	2.2b	1.0a	2.3de	Tolerant
Seredo	Pigmented	5.4de	5.5b	4.2abc	1.7abc	1.3ab	1.3abc	3.1cd	1.0a	2.4def	Tolerant
Marcia	Tan	1.0a	6.0bcde	3.7a	1.4a	1.0a	1.0a	1.0a	1.0a	2.3de	Tolerant
Serena	Pigmented	5.4de	5.6bcd	4.2abc	1.7abc	1.3ab	1.3abc	2.9c	1.0a	2.2d	Tolerant
Kateng'u	Pigmented	7.1f	4.8a	6.4e	2.4d	1.8c	2.7d	3.2cd	1.0a	1.7bc	Susceptible
Rasta	Pigmented	7.1f	4.9a	6.4e	2.4d	1.9c	2.8d	3.3cd	1.0a	1.6bc	Susceptible
Kaguru	Pigmented	7.1f	4.9a	6.4e	2.5d	1.9c	2.8d	3.1cd	1.0a	1.6bc	Susceptible
Dark Red	Pigmented	5.5de	5.5bc	4.3bc	1.8bc	1.3ab	1.6bc	3.5d	1.0a	1.4b	Susceptible
Mugeta	Mixed	2.3ab	6.0de	3.8abc	1.4ab	1.2ab	1.2a	1.0a	1.0a	1.1a	Susceptible
Gadam	Pigmented	5.4de	5.6bcde	4.9d	1.7abc	1.3b	1.3abc	1.0a	1.0a	1.6bc	Susceptible
Kauwi	Mixed	3.0c	6.1de	4.2abc	1.6abc	1.3b	1.3ab	1.0a	1.1b	1.9c	Susceptible

Means within each column (disease severity scores and yield) that are not followed by the same letter are significantly different (P <.05), while those followed by the same are insignificantly different at (P <.05). Anth = anthracnose; Leaf B. = leaf blight; Gray L.S =gray leaf spot; Ladd. L.S=Ladder leaf spot; Oval L.S = oval leaf spot; Downy M. = downy mildew; CKS = covered Kernel smut; DR = disease reaction.

Table 4.16 : Disease incidence of sorghum genotypes at KALRO Kiboko

Variety	Plant color	Anth	Leaf B.	Rust	Gray L.S	Ladder L.S	Oval L.S	Downy M.	CKS
Makueni local	Pigmented	100.0	100.0	100.0	68.3	70.8	53.8	0.0	0.0
Kiboko local 2	Pigmented	100.0	100.0	100.0	69.8	66.8	56.3	0.0	0.0
IESV 4029 SH	Pigmented	100.0	100.0	100.0	67.8	66.6	43.0	12.5	0.0
KARI Mtama 1	Tan	0.0	100.0	100.0	30.4	0.0	8.3	10.6	0.0
Seredo	Pigmented	100.0	100.0	100.0	70.2	66.7	44.6	14.2	0.0
Marcia	Tan	0.0	100.0	100.0	29.1	0.0	0.0	0.0	0.0
Serena	Pigmented	100.0	100.0	100.0	69.2	66.7	43.8	12.5	0.0
Kateng'u	Pigmented	100.0	100.0	100.0	97.3	100.0	100.0	21.4	0.0
Rasta	Pigmented	100.0	100.0	100.0	96.5	100.0	100.0	19.6	0.0
Kaguru	Pigmented	100.0	100.0	100.0	95.6	100.0	100.0	16.6	0.0
Dark Red	Pigmented	100.0	100.0	100.0	71.5	73.3	52.2	21.6	0.0
Mugeta	Mixed	44.9	100.0	100.0	39.1	15.1	13.2	0.0	0.0
Gadam	Pigmented	100.0	100.0	100.0	67.4	65.8	45.0	0.0	0.0
Kauwi	Mixed	56.7	100.0	100.0	68.7	68.8	21.1	0.0	11.4

Anth = anthracnose; Leaf B. = leaf blight; Gray L.S =gray leaf spot; Ladder L. S=Ladder leaf spot; Oval L.S = oval leaf spot; Downy M. = downy mildew and CKS = covered Kernel smut

Table 4.17 : Disease incidence of sorghum genotypes at KALRO Ithookwe

Variety	Plant color	Anth	Leaf B.	Rust	Gray L.S	Ladder L.S	Oval L.S	Downy M.	CKS
Makueni local	Pigmented	100.0	100.0	100.0	47.1	16.2	38.9	0.0	0.0
Kiboko local 2	Pigmented	100.0	100.0	100.0	51.1	15.0	35.7	0.0	0.0
IESV 4029 SH	Pigmented	100.0	100.0	100.0	36.7	7.1	28.8	12.5	0.0
KARI Mtama 1	Tan	0.0	100.0	100.0	33.3	0.0	21.9	10.6	0.0
Seredo	Pigmented	100.0	100.0	100.0	43.2	12.4	31.5	14.2	0.0
Marcia	Tan	0.0	100.0	100.0	34.5	0.0	23.3	0.0	0.0
Serena	Pigmented	100.0	100.0	100.0	41.2	10.8	26.7	12.5	0.0
Kateng'u	Pigmented	100.0	100.0	100.0	80.5	29.1	100.0	21.4	0.0
Rasta	Pigmented	100.0	100.0	100.0	79.1	27.4	100.0	19.6	0.0
Kaguru	Pigmented	100.0	100.0	100.0	77.9	30.3	100.0	16.2	0.0
Dark Red	Pigmented	100.0	100.0	100.0	47.5	11.5	29.3	21.6	0.0
Mugeta	Mixed	37.3	100.0	100.0	40.1	10.9	22.7	0.0	0.0
Gadam	Pigmented	100.0	100.0	100.0	41.0	10.9	36.3	0.0	0.0
Kauwi	Mixed	46.7	100.0	100.0	48.5	16.5	34.6	0.0	14.6

Anth = anthracnose; Leaf B. = leaf blight; Gray L.S =gray leaf spot; Laddder L. S=Ladder leaf spot; Oval L.S = oval leaf spot; Downy M. = downy mildew and CKS = covered Kernel smut

CHAPTER FIVE: DISCUSSION

5.1 Major fungal diseases affecting sorghum under field conditions

The major sorghum fungal diseases recorded at both KALRO, Kiboko and KALRO Ithookwe included Leaf blight, anthracnose, rust, gray leaf spot, ladder leaf spot, oval leaf spot, downy mildew and covered kernel smut. Among the foliar fungal diseases, the severity of leaf blight, anthracnose and rust was higher compared to other diseases. This concurs with a survey done by Koima *et al*. (2022) that listed the three diseases as most prevalent in agroecological zones of lower eastern Kenya. The severity of these major fungal diseases was higher during the first season compared to the second season in both sites with KALRO Kiboko attaining higher severity than KALRO Ithookwe. This maybe attributed to higher initial adequate pathogen inoculum coupled with favorable climatic conditions characterized by varying temperatures that were high and adequate rainfall (warm and wet) during the first season than the second season that had lower temperatures and moisture conditions. The susceptibility of genotypes used for the study trials to fungal diseases might have also played a role for the registered severities. These findings agree with a study by Thakur *et al*. (2007) that listed prevailing environmental conditions and inoculum density among factors that influence disease severities. Koima *et al*. (2022) reported environmental conditions characterized by high levels of moisture and temperature favored development of anthracnose and leaf blight, while warm, humid and moderate temperature accelerated rust infection in agro-ecologies of lower eastern Kenya.

Leaf blight severity was higher among the varieties compared to other fungal diseases. This may be attributed to the high percentage frequency in major fungal pathogens isolated that cause leaf blight namely: *Alternaria spp, Bipolaris spp, Colletotrichum spp, Exerohilum spp* and *Fusarium spp,* in both sites that the trials were done. These findings agree with studies by Khanal (2021), Ogolla *et al*. (2018), Tralamazza *et al*. (2018), Sun *et al*. (2020), Dai *et al*. (2019) and Waller and Brayford (1990) that identified *Colletotrichum sublineola, Exerohilum spp, Alternaria spp, Bipolaris spp, Curvularia spp and Fusarium spp,* respectively are important pathogens causing leaf blight related symptoms in cereals.

Weather conditions recorded at the start of each season within the sites involving: adequate rainfall, humidity and temperature propelled anthracnose development at the first month and progress to reach its peak at the fourth month. These findings contradict a study by Ngugi *et al*. (2000) who reported that anthracnose development started after anthesis stage and progressed steadily to reach its peak at maturity. Lower temperatures accompanied with dry weather conditions between the third and final month during the second season may have constrained anthracnose progress to higher severity (Ngugi *et al*., 2000). Teferi and Wubshet (2015) reported that anthracnose epidemics developed rapidly when unexpected rainfall occurred during anthesis and dough stages, coincided with moderate to high temperatures of between 10ºC to 27ºC.

Rust infection started after the second month and reached final peak at the maturity in both seasons and sites. This study concurs with a study by Karunakar *et al*. (1996) that noted rust severity was high in sorghum plants that were 2 to 3 months old while young plants were free from infection due to elevated concentrations of hydrocyanic acid in the leaves. Reduced temperature and moisture conditions during third and fourth month of the second season in both sites might have limited severe rust score. These weather conditions do not fall in line with descriptions by Koima *et al*. (2022) for high rust severity development.

Gray leaf spot, oval leaf spot and ladder leaf spot symptoms pattern of starting to develop after the second month especially on susceptible local varieties are similar to a study by Thomas (1991) who reported gray leaf spot infection started few days to anthesis stage, slowly progressed and attained its highest score at physiological maturity stage in sorghum. This trend can be associated with polycyclic nature of the pathogens that cause gray leaf spot (Ringer and Grybauskas, 1995). Benson (2013), listed: present amount of inoculum to initiate disease development, pathogen reproduction rate and proportion of uninfected plant tissue which the pathogen can infect as key factors that influence gray leaf spot development. The amount of inoculum required to initiate the disease is determined by practices that involve conservation tillage and previous season infected

plant debris which primary inoculum can develop when favorable conditions arise (Denazareno *et al*. 1992) while the rate at which the pathogens can reproduce is dependable on weather parameters namely: humidity, rainfall, and temperature. (Benson, 2013). Warmer temperatures ranging between 24 to 29°C and elevated humidity and rainfall favor gray leaf spot development (Ringer and Grybauskas, 1995). Therefore, low disease severity recorded in this study can be associated with initial inoculum deficiency at early stages of sorghum growth, which with time, buildup up later in the season rising late at maturity stage (Bhardwaj *et al*., 2021) coupled with unsteady rainfall throughout the season (Ringer and Grybauskas, 1995).

The severity of foliar diseases: leaf blight, anthracnose, rust, gray leaf spot, ladder leaf spot and oval leaf spot reached its peak during the 4th month (physiological maturity). This can be associated with senescence of the crops. Tsedaley *et al*. (2016) noted that a plant's rigidity is lost when it approaches physiological maturity hence fungal pathogens have an easy way to rapidly penetrate and develop.

5.1.1 Isolation and identification of sorghum fungal pathogens

Morpho-cultural characteristics of the major fungal pathogens grown on potato dextrose agar displayed variation not only among individual species but also classified groups. Major variations in cultural and morphology involving: reverse view, forward view and conidia may be because of differences in agro-ecologies involving altitude and climatic factors where the field trials were done. These findings agree with Ogolla *et al*. (2018) who noted variation in *Exerohilum turcicum* isolates cultural and morphological characteristics was due to differences in altitude, rainfall and temperature between highlands and the semi-arid lands of Tharaka Nithi county in Kenya. The variation in both cultural and morphological characteristics of *Curvularia* isolates recorded are similar findings by Yuvarani *et al*. (2021) and Kusai *et al*. (2016).

The colony color characteristics of *Fusarium* species varied from white, white to pinkish, plum and white to creamish as recorded by Okungbowa and Shittu (2012). However, they were categorized into four morphotypes based on microscopic characterization.

Morphotype one which consisted of group one isolates, had 0 to 2 septa micro-conidia as noted by Teixeira *et al*. (2017). Morphotype two included group two isolates which had falciform macro-conidia with septa ranging between 3 to 7 as noted by Hafizi *et al*. (2013). Morphotype three included group three and five isolates which consisted of non-septate oval to kidney shaped micro-conidia agree with previous description by Fourie *et al*. (2011). Morphotype four comprised of group four isolates which has I to 2 septate microconidia with shapes ranging from straight to curved were identical to the findings of Okungbowa and Shittu (2012).

Alternaria isolates displayed less variation in cultural characteristics, with oval and inverted clavate conidia that agree with previous descriptions by Marin Felix *et al*. (2019). *Bipolaris* isolates exhibited variation in colony characteristics ranging from whitish (at the center) to slightly gray, slightly gray to dark gray, slightly gray (at the center) to dark gray with identical conidial morphology agrees with descriptions by Sun *et al*. (2020) and Koima *et al*. (2022). *Exserohilum* isolates displayed less variation in colonies which ranged from dark grey to deep brown as described by Marin Felix *et al*. (2019), while conidia were similar in shape, characterized by a protruding hilum as noted by Lin *et al*. (2011). Although the conidial morphology of *Colletotrichum* isolates shared similar characteristics of being non-septate, sickle shaped and hyaline as described by Tsedaley *et al*. (2016), cultural characteristics differed. The whitish top view with greyish orange pigmentation on the reverse of group one isolates were consistent with the descriptions by Kimaru *et al*. (2018), while group two isolates forward and reverse descriptions of being whitish and yellowish, respectively are in line with findings by Koima *et al*. (2022).

The recorded variation in cultural characteristic of the groups of *Epicoccum* isolates, ranging from light purple to white with a light pink, flocculent center at the top view while the reverse side characterized by dark to Orange and light orange with light purple concentric rings are similar to findings of a study by Li *et al*. (2020). The similarity in conidial characteristics including unicellular to multicellular chlamydospores concurs with studies by Chen *et al*. (2021) and Li *et al*. (2020). The orange, obovate shaped

urediniospores of *Puccinia purpurea* descriptions recorded in this study are identical to findings by Dinanty *et al*. (2022). The morphology of *Sporisorium sorghi* conidia described in this study corroborates with findings by Wagari *et al*. (2019).

5.2 Effect of major fungal pathogens on performance of selected sorghum varieties

The significant negative association between fungal diseases severities and incidences and growth parameters (days to 50% flowering, number of green leaves and leaf area) indicates that these diseases inhibit sorghum growth. Moreover, local varieties that were the genotypes took less days to attain 50% flowering and had the highest fungal diseases severities and incidences. The negative correlation between the number of green leaves, leaf area, and fungal disease severity and incidence indicates that the number of green leaves and their leaf area reduce with increase in fungal diseases severity and incidence due to destruction of the green leaf area resulting in premature wilting and defoliation (Kutama *et al*., 2010; Little and Perumal, 2019).

The insignificant negative correlation between fungal diseases severities and incidences and yield characters (dry matter yield, grain yield, grain weight for 10 tagged plants and panicle length) suggests that there were less yield losses caused by fungal diseases. This could be attributed to lower fungal diseases severities from the vegetative to grain filling phases, which occurred between 1st months to 3rd month. Fungal diseases can cause significant crop losses including yield, depending on the stage in which the disease sets in, susceptibility of genotype and environmental conditions at the time of infection (Anitha *et al*., 2020). Little and Perumal (2019) reported that sorghum yields can be significantly reduced when fungal diseases infect top leaves during grain filling. Several studies have classified stages in which individual fungal diseases can affect yields negatively. For example: TeBeest *et al*. (2004), reported that leaf anthracnose causes substantial reduction in yield when high severity occurs prior to or during grain filling period while for leaf blight, Mathur *et al*. (2007) and Spurlock *et al*. (2004) noted that, when it sets in prior to flowering stages losses may be high unlike when it occurs from flowering to physiological maturity where minimal losses are recorded.

5.2.1 Disease tolerance among sorghum germplasm

Low yield attainment by local pigmented varieties (Kateng'u, Rasta and Kaguru) compared to improved varieties which included: Serena, KARI Mtama 1, Marcia, Seredo, IESV 24029 SH, Kiboko Local 2 and Makueni Local, maybe related to high fungal disease severities and incidences for fungal diseases namely: anthracnose, rust, gray leaf spot, ladder leaf spot and oval leaf spot. These results are in line with a study by Njoroge *et al*. (2018) who also noted that some local landraces were more susceptible to fungal diseases than improved varieties. Improved varieties that exhibited a tan color (KARI Mtama 1 and Marcia) recorded not only lowest severity of 1.0 to both anthracnose and ladder leaf spot, but also percentage disease incidence for anthracnose, rust, gray leaf spot, ladder leaf spot, oval leaf spot and downy mildew. Plant color (Tan, Red or Purple) is the pigmentation that is viewed when a plant is injured (Erpelding and Prom, 2006; Williams- Alanis *et al*., 1999). This plant color is a result of various composition of pigments (Siame *et al*., 1993). Tan sorghum varieties posses' high concentration of flavones while pigmented or purple varieties accumulate 3-deoxyanthocyanidins (Dykes, 2008). The two main 3-deoxyanthocyanidins in sorghum are apigeninidin and luteolinidin (Siame *et al*., 1993) while flavones are apigenin and luteolin (Stutts and Vermerris, 2020). These main phytoalexin compounds have been reported to be key in disease tolerance in sorghum (Du *et al*., 2009) and are secreted in sub-cellular inclusions in the epidermal cell that is on the verge of being attacked by a pathogenic fungus (Snyder and Nicholson, 1990). Poloni and Schirawski (2014), reviewed that intensive accumulation of 3-deoxyanthocyanidins is faster in cells of resistant varieties compared to susceptible varieties, hence, highlights the significance of accumulating phytoalexins at early stages of fungal infection which hinders the growth and colonization of fungi.

Although sorghum seedlings secrete phytoalexins for defense against *Colletotrichum graminicola*, susceptible varieties tend to lose the capacity to counter infection by fungal pathogens as they mature (Snyder and Nicholson,1990). Therefore, the tolerance nature of tan varieties to anthracnose maybe associated with accumulation of flavones: apigenin and luteolin which have been associated with fungal inhibition, although the current did not quantify the pigments in the two tan varieties or any other variety. In vitro bioassays

by Du *et al*. (2009) showed 20 lM of Luteolin inhibited approximately 60% of spore germination while an increased concentration of 50 lM luteolin further limited spore growth over 80%. Apigenin performed less better than Luteolin by limiting less than 50% germination of spores at a concentration of 50 lM. Therefore, by comparison, Luteolin was effective in reduction of *Colletotrichum sublineola* growth against apigenin. This study findings contrasts with Siame *et al*. (1993) who reported that pigmented sorghum plants were superior in inhibiting both fungal and bacterial invasion than tan plants and that plant color was not related to sorghum resistance or susceptibility to diseases.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The major sorghum fungal diseases identified in lower eastern Kenya were: leaf blight, anthracnose, rust, gray leaf spot, ladder leaf spot, oval leaf spot, downy mildew and covered kernel smut. The first season in both sites registered high fungal disease severity than the second season due to adequate pathogen inoculum and favorable weather conditions. High Leaf blight severity recorded compared to other fungal diseases can be related with: favorable weather conditions, frequency and variation among fungal pathogens isolated and susceptibility of the germplasm used for the study. The lack of initial inoculum during early growth stages of sorghum plants may have caused symptoms of gray leaf spot, oval leaf spot and ladder leaf spot be clearly visible at maturity. Most foliar fungal diseases reached their peak severity score at physiological maturity (4^{th} month) due to reduction in plant's rigidity, hence paving way for rapid fungal infiltration and infection. Representative fungal pathogens identified by phylogenetic relationship included: *Curvularia akaiiensis, Curvularia lunata, Bipolaris secalis, Exserohilum rostratum, Fusarium napiforme, Alternaria alstroemeriae and Epicoccum sorghinum.*

Fungal disease severity was significantly negatively correlated with growth parameters namely: days to 50% flowering, number of green leaves and leaf area while minimally negatively associated with yield characters which included: dry matter yield, grain yield, grain weight of 10 tagged plants, and panicle length. This reveals that fungal diseases reduce growth of sorghum plants by not only reducing the green leaf area but also their number. The minimal reduction in grain yields highlights that when fungal disease infection occurs at late stages of sorghum growth then insignificant losses in yield maybe recorded. Local pigmented varieties (Kateng'u, Rasta and Kaguru) were susceptible to fungal diseases than improved tan varieties (Marcia and KARI Mtama 1) and improved pigmented varieties namely (Makueni Local, IESV 24029 SH, Kiboko Local 2, Seredo and Serena) due to not only attaining low yields but also high fungal disease severity and incidence. This clearly showed stability of improved genotypes released by ICRISAT.

6.2 Recommendations

i. Future studies need to focus on evaluating and developing biocontrol methods to manage fungal pathogens infecting sorghum crops in lower Eastern Kenya.

ii. Most improved varieties from ICRISAT showed they are superior than local landraces derived from farmers. They could be tested in other agro-ecological zones of Arid and semi-arid lands to confirm tolerance stability.

iii. Tan improved varieties showed tolerance to anthracnose and ladder leaf spot. Therefore, they could be as categorized as potential fungal disease tolerant sources to be utilized in crop improvement programs.

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APPENDICES

Appendix i: Kiboko weather conditions during the two seasons.

Year	Month	Min. Temp	Max. Temp	Av. Temp	Rain
2020	October	16.6	32.7	24.7	0.2
2020	November	18.1	31.3	24.7	10.1
2020	December	17	30.8	23.9	1.6
2021	January	15.7	30.9	23.3	0.8
2021	February	15.8	32.9	24.4	4.5
	Mean	**16.6**	**31.7**	**24.2**	**3.4**

Year	Month	Min. Temp	Max. Temp	Av. Temp	Rain
2021	April	18.5	34.4	26.5	1.5
2021	May	16.2	30.4	23.3	4.2
2021	June	13.9	28.5	21.2	0
2021	July	13	28.2	20.6	0
2021	August	14.1	29.4	21.8	0
	Mean	**15.1**	**30.2**	**22.7**	**1.1**

Appendix ii: Ithookwe weather conditions during the two seasons.

Year	Month	Min. Temp	Max. Temp	Av. Temp	Rain
2020	October	17.6	29.4	23.5	28.5
2020	November	18.4	27.5	23	506.6
2020	December	17.5	27.7	22.6	57.1
2021	January	16.7	27.2	22	11.8
2021	February	17.7	29.4	23.6	5.4
	Mean	**17.6**	**28.2**	**22.9**	**121.9**

Year	Month	Min. Temp	Max. Temp	Av. Temp	Rain
2021	April	19	29.3	24.2	270.5
2021	May	16.9	27.3	22.1	57.3
2021	June	15.7	25.6	20.7	0
2021	July	14.8	25.6	20.2	0
2021	August	14.6	27.2	20.9	0
	Mean	**16.2**	**27**	**21.6**	**65.6**

Appendix iii: ANOVA for fungal diseases under different seasons and locations.

Source of var.	d.f.	s.s.	m.s.	v.r.	F pr.
Anthracnose					
B	3	1.11156	0.37052	6.63	
G	13	1024.353	78.79642	1409.22	<.001
S	1	40.37504	40.37504	722.08	<.001
L	1	25.98969	25.98969	464.81	<.001
G*S	13	17.10558	1.31581	23.53	<.001
G*L	13	5.60094	0.43084	7.71	<.001
S*L	1	0.69754	0.69754	12.48	<.001
G*S*L	13	0.93558	0.07197	1.29	0.225
Residual	165	9.22594	0.05591		
Total	223	1125.395			
Leaf_blight					
B	3	0.64942	0.21647	2.24	
G	13	29.87469	2.29805	23.82	<.001
S	1	32.48254	32.48254	336.7	<.001
L	1	15.69862	15.69862	162.73	<.001
G*S	13	3.18683	0.24514	2.54	0.003
G*L	13	2.14076	0.16467	1.71	0.064
S*L	1	0.08254	0.08254	0.86	0.356
G*S*L	13	0.59183	0.04553	0.47	0.938
Residual	165	15.91808	0.09647		
Total	223	100.6253			
Rust					
B	3	2.6616	0.8872	8.83	
G	13	257.4218	19.8017	197.14	<.001
S	1	35.0445	35.0445	348.89	<.001
L	1	26.1945	26.1945	260.78	<.001
G*S	13	3.0193	0.2323	2.31	0.008
G*L	13	3.5768	0.2751	2.74	0.002
S*L	1	0.3945	0.3945	3.93	0.049
G*S*L	13	1.7193	0.1323	1.32	0.208
Residual	165	16.5734	0.1004		
Total	223	346.6055			
Gray_leaf_spot					
B	3	0.36835	0.12278	3.92	
G	13	65.08558	5.00658	159.97	<.001
S	1	36.88754	36.88754	1178.6	<.001
L	1	96.8629	96.8629	3094.87	<.001
G*S	13	2.70558	0.20812	6.65	<.001
G*L	13	8.10272	0.62329	19.91	<.001
S*L	1	3.88504	3.88504	124.13	<.001
G*S*L	13	1.51058	0.1162	3.71	<.001
Residual	165	5.16415	0.0313		
Total	223	220.5725			

Ladder_leaf_spot					
B	3	1.08906	0.36302	16.44	
G	13	69.93969	5.37998	243.64	<.001
S	1	18.57254	18.57254	841.09	<.001
L	1	27.09112	27.09112	1226.87	<.001
G*S	13	5.39183	0.41476	18.78	<.001
G*L	13	17.05326	1.31179	59.41	<.001
S*L	1	2.0254	2.0254	91.72	<.001
G*S*L	13	1.10147	0.08473	3.84	<.001
Residual	165	3.64344	0.02208		
Total	223	145.9078			
Oval_leaf_spot					
B	3	0.54393	0.18131	5.21	
G	13	160.7721	12.36709	355.12	<.001
S	1	17.16071	17.16071	492.77	<.001
L	1	54.21446	54.21446	1556.78	<.001
G*S	13	5.45679	0.41975	12.05	<.001
G*L	13	14.01804	1.07831	30.96	<.001
S*L	1	0.75446	0.75446	21.66	<.001
G*S*L	13	1.47054	0.11312	3.25	<.001
Residual	165	5.74607	0.03482		
Total	223	260.1371			
Downy_mildew					
B	3	1.3134	0.4378	2.64	
G	13	257.5198	19.8092	119.35	<.001
S	1	9.6114	9.6114	57.91	<.001
L	1	1.5445	1.5445	9.31	0.003
G*S	13	9.6761	0.7443	4.48	<.001
G*L	13	3.2205	0.2477	1.49	0.125
S*L	1	0.6007	0.6007	3.62	0.059
G*S*L	13	1.8968	0.1459	0.88	0.576
Residual	165	27.3866	0.166		
Total	223	312.7698			
Covered_kernel_smut					
B	3	0.03192	0.01064	0.83	
G	13	45.82558	3.52504	273.63	<.001
S	1	3.52504	3.52504	273.63	<.001
L	1	0.0054	0.0054	0.42	0.518
G*S	13	45.82558	3.52504	273.63	<.001
G*L	13	0.07022	0.0054	0.42	0.961
S*L	1	0.0054	0.0054	0.42	0.518
G*S*L	13	0.07022	0.0054	0.42	0.961
Residual	165	2.12558	0.01288		
Total	223	97.48496			

Legend for above ANOVA tables: B= Block; G= Genotype; S= Season; L= Location; G*S= Genotype*Season interaction*;* G*L= Genotype*Location interaction*; S*L*= Season*Location interaction*; G*S*L*= Genotype*Season*Location interaction, *d.f* = degree of freedom; *s.s* = sum of squares; *m.s* = mean squares; *v.r* = variance ratios; *F pr.*= probabilities for variance ratios ANOVA with P≥0.05 are insignificantly different; ANOVA with P≤*0.*05 are significantly different.

Appendix iv: Sequences of fungal isolates, accession number and their closest match in Blast

Isolate ID	GeneBank Accession number	Sequences	Species
I6b	PP813558	GGAGGAAAAGAAACCAACAGGGATTGCCCTA GTAACGGCGAGTGAAGCGGCAACAGCTCAAAT TTGAAACTCTGGCGTCTTTGGCGTCCGAGTTGT AATTTGCAGAGGGCGCTTTGGCATTGGCAGCG GTCCAAGTTCCTTGGAACAGGACGTCACAGAG GGTGAGAATCCCGTACGTGGTCGCTAGCCTTT ACCGTGTAAAGCCCCTTCGACGAGTCGAGTTG TTTGGGAATGCAGCTCTAAATGGGAGGTAAAT TTCTTCTAAAGCTAAATACTGGCCAGAGACCG ATAGCGCACAAGTAGAGTGATCGAAAGATGA AAAGCACTTTGGAAAGAGAGTTAAAAAGCAC GTGAAATTGTTGAAAGGGAAGCGCTTGCAGCC AGACTTGCCTGTAGTTGCTCATCCGGGTTTTTA CCCGGTGCACTCTTCTACGGGCAGGCCAGCAT CAGTTTGGGCGGTTGGATAAAGGTCTCTGTCA TGTACCTCCTCTCGGGGAGAACTTATAGGGGA GACGACATGCAACCAGCCCGGACTGAGGTCCG CGCATCTGCTAGGATGCTGGCGTAATGGCTGT AAGCGGCCCG	*Curvularia lunata*
K6c	PP813559	GCATATCAAATAAGCGGAGGAAAAGAAACCA ACAGGGATTGCCCTAGTAACGGCGAGTGAAGC GGCAACAGCTCAAATTTGAAATCTGGCTCTTTT AGGGTCCGAGTTGTAATTTGCAGAGGGCGCTT TGGCTTTGGCAGCGGTCCAAGTTCCTTGGAAC AGGACGTCACAGAGGGTGAGAATCCCGTACGT GGTCGCTAGCTATTGCCGTGTAAAGCCCCTTCG ACGAGTCGAGTTGTTTGGGAATGCAGCTCTAA ATGGGAGGTAAATTTCTTCTAAAGCTAAATAT TGGCCAGAGACCGATAGCGCACAAGTAGAGTG ATCGAAAGATGAAAAGCACTTTGGAAAGAGA GTCAAACAGCACGTGAAATTGTTGAAAGGGAA GCGCTTGCAGCCAGACTTGCTTGCAGTTGCTCA TCCGGGCTTTTGCCCGGTGCACTCTTCTGCAGG CAGGCCAGCATCAGTTTGGGCGGTGGGATAAA GGTCTCTGACACGTTCCTTCCTTCGGGTTGGCC ATATAGGGGAGACGTCATACCACCAGCCTGGA CTGAGGTCCGCGCATCTGCTAGGATGCTGGCG TAATGGCTGTAAGCGGCCCGTCTTG	*Curvularia akaiiensis*
I4f	PP813564	AGGAAAAGAAACCAACAGGGATTGCCCTAGT AACGGCGAGTGAAGCGGCAACAGCTCAAATTT GAAATCTGGCTCTCGGGCCCGAGTTGTAATTT GTAGAGGATACTTTTGATGCGGTGCCTTCCGA GTTCCCTGGAACGGGACGCCATAGAGGGTGAG AGCCCCGTCTGGTTGGATGCCAAATCTCTGTA AAGTTCCTTCGACGAGTCGAGTAGTTTGGGAA TGCTGCTCTAAATGGGAGGTATATGTCTTCTAA AGCTAAATACCGGCCAGAGACCGATAGCGCAC AAGTAGAGTGATCGAAAGATGAAAAGCACTTT GAAAAGAGAGTTAAAAAGTACGTGAAATTGTT	*Fusarium napiforme*

		GAAAGGGAAGCGTTTATGACCAGACTTGGGCT TGGTTAATCATCTGGGGTTCTCCCCAGTGCACT TTTCCAGTCCAGGCCAGCATCAGTTTTTGCCGG GGGATAAAGACTTCGGGA	
I8a	PP813565	GGAGGAAAAGAAACCAACAGGGATTGCCCTA GTAACGGCGAGTGAAGCGGCAACAGCTCAAAT TTGAAATCTGGCGTCTTTGGCGTCCGAGTTGTA ATTTGCAGAGGGCGCTTTGGCATTGGCAGCGG TCCAAGTTCCTTGGAACAGGACGTCACAGAGG GTGAGAATCCCGTACGTGGTCGCTAGCCTTTA CCGTGTAAAGCCCCTTCGACGAGTCGAGTTGT TTGGGAATGCAGCTCTAAATGGGAGGTAAATT TCTTCTAAAGCTAAATACTGGCCAGAGACCGA TAGCGCACAAGTAGAGTGATCGAAAGATGAA AAGCACTTTGGAAAGAGAGTTAAAAAGCACGT GAAATTGTTGAAAGGGAAGCGCTTGCAGCCAG ACTTGCCTGTAGTTGCTCATCCGGGTTTTTACC CGGTGCACTCTTCTACGGGCAGGCCAGCATCA GTTTGGGCGGTTGGATAAAGGTCTCTGTCATGT ACCTCCTCTCGGGGAGAACTTATAGGGGAGAC GACATGCAACCAGCCCGGACTGAGGTCCGCGC ATCTGCTAGGATGCTGGCGTAATGGCTGTAAG CGGCCCG	*Epicoccum sorghinum*
K9a	PP813562	GAGGAAAAGAAACCAACAGGGATTGCCCTAG TAACGGCGAGTGAAGCGGCAACAGCTCAAATT TGAAATCTGGCTCTTTTAGAGTCCGAGTTGTAA TTTGCAGAGGGCGCTTTGGCTTTGGCAGCGGT CCAAGTTCCTTGGAACAGGACGTCACAGAGGG TGAGAATCCCGTACGTGGTCGCTGGCTATTGC CGTGTAAAGCCCCTTCGACGAGTCGAGTTGTTT GGGAATGCAGCTCTAAATGGGAGGTACATTTC TTCTAAAGCTAAATATTGGCCAGAGACCGATA GCGCACAAGTAGAGTGATCGAAAGATGAAAA GCACTTTGGAAAGAGAGTCAAACAGCACGTGA AATTGTTGAAAGGGAAGCGCTTGCAGCCAGAC TTGCTTACAGTTGCTCATCCGGGTTTTTACCCG GTGCACTCTTCTGTAGGCAGGCCAGCATCAGT TTGGGCGGTAGGATAAAGGTCTCTGTCACGTA CCTCCTTTCGGGGAGGCCTTATAGGGGAGACG ACATACTACCAGCCTGGACTGAGGTCCGCGCA TCTGCTAGGATGCTGGCGTAATGGCTGTAAGC GGCCCG	*Alternaria alstroemeriae*
I8c	PP813563	CATATCAATAAGCGGAGGAAAAGAAACCAAC AGGGATTGCCCTAGTAACCGAGTGMAGCGGC AACAGCTCAAATTTGAAATCTGGCTCTTTTAGA GTCCGAGTTGTAATTTGCAGAGGGCGCTTTGG CTTTGGCAGCGGTCCAAGTTCCTTGGAACAGG ACGTCACAGAGGGTGAGAATCCCGTACGTGGT CGCTGGCTATTGCCGTGTAAAGCCCCTTCGAC GAGTCGAGTTGTTTGGGAATGCAGCTCTAAAT GGGAGGTACATTTCTTCTAAAGCTAAATATGG CCAGAGACCGATAGCGCACAAGTAGAGTGATC GAAAGATGAAAAGCCTTTGGAAAGAGAGTCA AACAGCACGTGAAATTGTTGAAAGGGAAGCGC TTGCAGCCAGACTTGTTACAGTTGCTCATCCGG	*Alternaria alstroemeriae*

		GTTTTTACCCGGTGCACTCTTCTGTAGGCAGGC CAGCATCAGTTTGGGCGGTAGGATAAAGGTCT CTGTCACGTACCTCCTTTCGGGGAGGCCTTATA GGGGAGAGACATACTACCAGCCTGGATGAGGT CCGCGCATCTGCTAGGATGCTGGCGTAATGGC TGTAAGCGGCCCGTCTTGAAACA	
K3c	PP813560	AAAGAAACCAACAGGGATTGSCCTAGTAACGG CGAGTGAAGCGGCAACAGCTCAAATTTGAAAT CTGGCTCTTTCAGAGTCCGAGTTGTAATTTGCA GAGGGCGCTTTGGCTTTGGCAGCGGTCCAAGT TCCTTGGAACAGGACGTCACAGAGGGTGAGAA TCCCGTACGTGGTCGCTAGCTATTGCCGTGTAA AGCCCCTTCGACGAGTCGAGTTGTTTGGGAAT GCAGCTCTAAATGGGAGGTAAATTTCTTCTAA AGCTAAATATTGGCCAGAGACCGATAGCGCAC AAGTAGAGTGATCGAAAGATGAAAAGCACTTT GGAAAGAGAGTCAAACAGCACGTGAAATTGTT GAAAGGGAAGCGCTTGCAGCCAGACTTGCTTG CAGTTGCTCATCCGGGCTTTTGCCCGGTGCACT CTTCTGCAGGCAGGCCAGCATCAGTTTGGGCG GTGGGATAAAGGTCTCTGTCATGTACCTCTCTT CGGGGAGGCCTTATAGGGGAGGCGACATACCA CCAGCCTAGACTGAGGTCCGCGCATCTGCTAG GATGCTGGCGTAATGGCTGTAAGCGGCCCG	*Exserohilum rostratum*
K2d	PP813561	GCATATCAATAAGCGGAGGAAAAGAAACCAA CAGGGATTGSCCTAGTAACGGCGAGTGAAGCG GCAACAGCTCAAATTTGAAATCTGGCTCTTTCA GAGTCCGAGTTGTAATTTGCAGAGGGCGCTTT GGCTTTGGCAGCGGTCCAAGTTCCTTGGAACA GGACGTCACAGAGGGTGAGAATCCCGTACGTG GTCGCTAGCTATTGCCGTGTAAAGCCCCTTCGA CGAGTCGAGTTGTTTGGGAATGCAGCTCTAAA TGGGAGGTAAATTTCTTCTAAAGCTAAATATT GGCCAGAGACCGATAGCGCACAAGTAGAGTG ATCGAAAGATGAAAAGCACTTTGGAAAGAGA GTCAAACAGCACGTGAAATTGTTGAAAGGGAA GCGCTTGCAGCCAGACTTGCTTGCAGTTGCTCA TCCGGGCTTTTGCCCGGTGCACTCTTCTGCAGG CAGGCCAGCATCAGTTTGGGCGGTGGGATAAA GGTCTCTGTCACGTACCTCTCTTCGGGGAGGCC TTATAGGGGAGACGACATACCACCAGCCTAGA CTGAGGTCCGCGCATCTGCTAGGATGCTGGCG TAATGGCTGTAAGCGGCCCGTCTT	*Bipolaris secalis*