BACTERIUM (*Bacillus amyloliquefaciens*) AS A BIOCONTROL AGENT AGAINST FUNGAL DISEASES OF SORGHUM

KIPROP KOECH PATRICK

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DECLARATION

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KIPROP KOECH PATRICK

_____ **Date** _____

SAGR/SCH/M/016/18

Approval by the supervisors

This thesis has been submitted with our approval as the University supervisors

_____ **Date** _____

Dr. Javan O. Were

School of Agriculture and Biotechnology

Department of Seed, Crop and Horticultural supervisors

University of Eldoret, Eldoret

_____ **Date** _____

Prof. Linnet S. Gohole

School of Agriculture and Biotechnology

Department of Seed, Crop and Horticultural supervisors

University of Eldoret, Eldoret

_____ **Date** _____

Dr. Billy Mukumba

School of Science and Aerospace studies

Department of Biological sciences

Moi University, Eldoret

DEDICATION

I dedicate this work to God for granting me the strength and wisdom, to my dad, mum and sister for their unwavering assistance they have always shown through fees payments and educative support. To my pastor Amos Ngeywo, who always find time to pray for my success in school and to my colleagues, friends and mentors for their inspiration and motivation they offered daily.

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ABSTRACT

Sorghum (*Sorghum bicolor* L.) is among the main primary foods in arid and semi-arid lands and it is grown for both food and also as a forage crop. Its production is constrained by both biotic and abiotic factors. To ensure continuous production this study aimed at addressing sustainable management strategies to foliar fungal diseases of sorghum in western parts of Kenya. The first section of the experiment was set under field conditions in different sites and seasons with an attempt of ascertaining the effects of *Bacillus amyloliquefaciens* bacterium on incidences of foliar diseases on selected sorghum genotypes. This study was set in split plot arrangement under Randomized Complete Block design with genotypes as the main plot and treatments as the subplots replicated thrice. The second experimental phase aimed at determining the inhibitory potentials of the strains of Bacillus amyloliquefaciens on single fungal pathogens isolated in vitro. The study design involved the use of three replicating in a randomized complete block study design. The experiment was followed by a greenhouse experiment that tested the effectiveness of different concentrations of the bacterium in treating sorghum covered -kernel disease. Randomized complete block trial was also conducted using different rates of inoculation of Bacillus amyloliquefaciens. Field, lab, and greenhouse research was performed separately to assemble data. Field trials were used to record instances and the degree of sorghum foliar diseases. The percentage of mycelial inhibition in the laboratory acted as the main indicator whereas the percentage of the greenhouse was converted to the percentage of covered-kernel disease severity. The statistical processes employed to analyze all the data collected were done through the use of GENSTAT software, version 14 and the results were presented within the form of plates, figures, tables and box-and-whisker groupings. It was found that three foliar diseases (anthracnose, leaf spot and leaf blight) occurred in both sites through two seasons. The reasons were that the outbreak and severity of anthracnose were significantly more in Kibos than Sega. Varieties did not show a significant difference in their tolerance to disease pressure ($p > 0.05$) while treatments displayed a distinct efficacy in reducing disease pressures compared to control ($p < 0.05$) where dressing seeds with bacterium proved to be more efficient in managing foliar diseases. Treatments with *Bacillus amyloliquefaciens* recorded an improved plant growth and reduced disease severity and the response was directly proportional to increase in rates of the bacterium. *Bacillus amyloliquefaciens* bacterium is a growth enhancer and also reduces disease severities and therefore should be incorporated into integrated disease management system to ensure sustainable crop production. Further studies should be conducted to characterize genetic compositions of genotypes with respect to treatments that will aid in further crop improvement.

Key words: *Severity, Fungal diseases, Efficacy, Bacillus amyloliquefaciens, Sorghum bicolor* L.

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Sorghum (*Sorghum bicolor*) belongs to the family Poacea and is ranked as one of the major cereal crops grown in Kenya and rest of the world. It is majorly grown in regions characterized by scarce rainfall because of its ability to withstand low moisture content throughout its growing cycle (Amelework *et al*., 2016). It is known to be hardy crop alongside millet that are regarded as climate resilient crops and are currently produced locally and commercially with an aim of combating food insecurity in almost all the parts of the world. Sorghum is majorly grown as source of food for humans as they are used to make ugali and porridge and is also utilized as an integral crop in silage making for feeding animals (Orr *et al*., 2016). Moreover, sorghum makes a significant contribution in human diet since its rich in proteins, vitamins and carbohydrates and thus making a dependable energy food source in most developing countries (Razvy, 2016).

Sorghum production in East Africa stands at around 925 Kg per hectare which is below the maximum that has been projected to be able to feed almost half a billion people in many developing countries. This crop's stalks can be made useful in making shelters, fences or fed to livestock (Nyoni *et al*., 2020). Commercially, sorghum is used in brewing industries to make alcoholic drinks, beverages, breakfast cereals and other malting products and raw materials for the biofuel industries (Wenndt *et al*., 2023a). Despite the above-mentioned benefits, sorghum production has been constrained by both biotic and a biotic factor.

Major limiting factors to sorghum production are diseases which are caused by different pathogens and have been reported to cause great yield loses (Wenndt *et al.*, 2023b). Weeds such striga on the other hand compete with this crop for space, nutrients and light and thus leading to fewer yields in infested regions resulting to about 15-97 % depending on the prevailing weather conditions. Insect pests such as sorghum midge, termites and fall armyworms are among the major biotic factors alongside mammalian pests such as birds that contribute to significant losses in quality and quantity of sorghum (Hailu *et al*., 2021).

Many researchers have identified and proposed a number of measures to manage fungal diseases, including the adoption of healthy and disease-free seeds, the removal of alternate host plants, and field sanitations (Fromme *et al*., 2017). Alongside these cultural practices, synthetic fungicides prove to work best despite the side effects such as cost ineffectiveness, harmful effects to humans, animals and environment and also phytotoxicity effects on plants thus not sustainable (Nyambok *et al*., 2014). Resistant varieties on the other hand may become inefficient with time due to emergence of new races of the pathogens and also breakdown of resistance in the given varieties (Tsedaley *et al*., 2016). This calls for a long lasting, less costly, environmentally friendly and sustainable management strategies to ensure continuous success in sorghum production in Kenya and beyond.

1.2 Statement of the problem

Common fungal diseases include head smut (*Sporisorium reilianum*), leaf rust *(Puccinia purpurea)*, anthracnose (*Colletotrichum* spp.), and grain molds in sorghum which reduces the quality and the quantity of grains. Head smut is the most serious disease of sorghum, which thrives best in high humidity and high temperatures (Matrood & Rhouma, 2021).

These fungi can reproduce both vegetatively, asexually and sexually, thus allowing the persistence of inoculum in seeds, soil and air reservoirs. Foliar diseases reduce the photosynthetic surface area of leaves, which eventually result in inadequate filling of the grain, reduced size of the grain kernel, and often deformed grains. Some of these pathogenic fungi, especially those of the genus *Aspergillus, Fusarium and Penicillium*, are mycotoxin producers whose corresponding toxins are very dangerous to human health (Krijgsheld *et al*., 2013). Infection by fungi is a key problem in most cereal crops worldwide. The first species of *Aspergillus* were discovered in 1729 as the pathogen of maize, rice, millet, and sorghum. We have since seen reports of yield losses due to fungal infections in all the key areas of sorghum production in the world.

A recent study showed that in Africa, grain samples from Egypt had a higher percentage of aflatoxin contamination, followed by South Africa, Nigeria and Cameroon, while in Kenya, foliar diseases are rampant in western parts of Kenya which is a major sorghum producing zone and also the zone is characterized by good conditions for pathogen to thrive (Meijer *et al*., 2021; Montoya *et al*., 2020). These reports point out that those fungal pathogens that affect sorghum production are found in the environment and can be of great concern in practically every region of the world thus the need for sustainable management strategies.

Worldwide, fungal diseases are distributed in air, soil and water causing severe losses in countries with arid and dry conditions including Africa, Asia and the Middle East since these conditions favor their growth and development. In Eastern Africa, foliar diseases continue to cause significant yield losses. For instance, above 60% incidence level of leaf blight was reported across many districts in Sudan (Beshir *et al*., 2016). In Kenya, sorghum

is mostly grown by smallholder farmers of western Kenya and other areas characterized by semi-arid conditions and are considered the second staple cereal after maize and most fungal pathogens are prevalent in these zones due to warm and humid environmental conditions (Makumba, 2016).

Fungal pathogens are important in contributing to food insecurity in Kenya and in the world. *Aspergillus* causes about 70-76 % of yield losses (Panchal & Dhale, 2011). Anthracnose in sorghum causes yield losses estimated at 67 % on susceptible varieties. The fungus attacks the vegetative regions of the crop reducing the photosynthetic area and thus resulting in losses in both quality and the quantity of the grains. About 67% of the Kenyan population has been affected by chronic diseases following *aflatoxin* exposure (Mutegi *et al*., 2018).

1.3 Justification of the study

Anthracnose disease in the central and west Africa is reported to be a very important disease that when not managed in severe conditions results to late development of the plant and may contribute up to 50% losses of grain yield especially on susceptible genotypes (Cota *et al*., 2017). They can infect plants at any development stage and thus damage photosynthetic functionalities, which could lead to decreases in dry and fresh matter by 47% and 38%, respectively (Mengistu *et al*., 2019). Foliar pathogens cause several losses to seed industries because they cause seed discoloration, shriveling, loss of seed vigor, loss of seed weight, and seed rot. The damages inflicted by the pathogens decrease the market price of the seeds and grains in the local and international markets and disrupt the nutritive value of the produce (Ashkani *et al*., 2015). Extracts of lantana camara have been used to treat the disease by seed dressing. Rhizobacteria and Pseudomonas spp. have been reported

to inhibit the growth of Aspergillus spp. As seed dressers, agrochemicals like Captan, Captafol, and Ditane M 45 are reported to be effective against the disease (Yaseen *et al*., 2015). These seed dressers prevent the growth of most pathogens that are carried by the seeds. Breeding for resistance is a more sustainable tool to manage most plant diseases though it takes a long time and the use of breeding technological tools makes the whole process to be costly and since synthetic chemical residues can be detrimental to the ecosystem, botanical extracts take a long time to respond in the system and therefore other eco- friendly methods should be exploited to manage diseases (Yaseen *et al*., 2015).

Biocontrol agents have been shown to yield credible outcomes in control of plant diseases, as well as in plant propagation. Growth and development. Bacillus amyloliquefaciens has demonstrated a broad antimicrobial spectrum with respect to a collection of barley foliar fungal. Pathogens and suggested that the metabolites that it forms in media are broad spectrum and a potential Bio control. Agent candidate to control several sorghum pathogens hence reduces the application of chemicals (Kipkogei, 2019; Makumba, 2016). In the view of the fact that past research has proposed that the use of B. amyloliquefaciens could be used as an alternative to herbicides to control different sorghum pathogens, the following study therefore sought to test its effectiveness as a growth regulator and a disease suppressor in sorghum. Contact between fungal pathogens and the biocontrol agent did lead to decreased disease occurrence in the chosen sorghum varieties. Field experimentation was pursued to determine the effectiveness of the biocontrol agent in suppressing disease regardless of its so-called potential.

Further confirmation of these so-called capabilities was obtained in the greenhouse conditions when the biocontrol agent was used as a growth enhancer. Further, dual-culture laboratory tests were conducted to determine the ability of the agent to suppress target

fungi. The combined results highlight the advantage of biocontrol agents to reduce disease and improve growth and, therefore, biocontrol agents could be effective alternatives to traditional chemical control strategies.

1.4 Study Objectives

1.4.1 Broad objective

1.4.1.1 To improve sorghum production through management of selected sorghum foliar diseases by use of *Bacillus amyloliquefaciens* as a biocontrol agent

1.4.2 Specific objectives

1.4.2.1 To determine the efficacy of *Bacillus amyloliquefaciens* as a biocontrol in the management of foliar diseases in sorghum under field conditions.

1.4.2.2 To determine the *in-vitro* inhibitory indices of *Bacillus amyloliquefaciens* on pathogenic fungi affecting sorghum

1.4.2.3 To determine the efficacy of *Bacillus amyloliquefaciens* as a biocontrol against *Sporisorium sorgi* under controlled conditions

1.5 Research Questions

Objective 1

- Which foliar diseases affect Sorghum production in Western Kenya?
- Is *Bacillus amyloliquefaciens* able to suppress the incidence and severity of identified foliar diseases of sorghum?

Objective 2

- What are the inhibitory effects of *Bacillus amyloliquefaciens* in mycelia growth of the test fungi *in vitro*?
- Which test fungi was more inhibited by *Bacillus amyloliquefaciens in vitro*?

Objective 3

- Does *Bacillus amyloliquefaciens* inhibit covered kernel disease of sorghum?

CHAPTER TWO

LITERATURE REVIEW

2.1 Sorghum production

Sorghum is an important cereal worldwide grown for both subsistence and commercial purposes. Sorghum is a major cereal crop in arid and semi-arid lands because it can withstand drought conditions. It is characterized by the C4 carbon cycle making it a photosynthesis-efficient characterized by high water utilization (Borrell *et al*., 2021). Sorghum is known to tolerate salinity and grows well in both sandy and clay soils with pH ranges 5.0 - 8.5 and also can do well in soils with less permeability. This crop has a well-developed root system extending into the soil 15-25m, and therefore, reaching deeper water supplies (Prazak, 2016). Sorghum differs from maize, wheat, and barley because it can regenerate through rhizomes after harvest and be re-harvested in the next harvest (Cox *et al*., 2018). These qualities make sorghum have a high yield potential which makes it widely adaptable to environments unlike other cereals. In most arid areas, sorghum serves as a livestock feed and/or poultry feed, since there are high carbs in the stems and protein content in the leaves. Sorghum is also a good green-manure crop that has been employed in Europe to produce hay or silage (Berenji 2004, p. 294). Sorghum is also a nutritious food because it has a high level of protein, carbohydrate, vitamin, and mineral contents (Afify *et al*., 2012).

Sorghum has allelopathic qualities that suppress the growth of weeds thus making it appropriate as an addition to the rotation of crops and as a companion crop. The product made out of sorghum (stover) finds various uses in several ways and includes being used as a source of power, fencing and is used as building material in some cultures. In the

brewing sector, there are nine companies that use sorghum in the form of molded and unmolded. The beer industry in Kenya has seen an increase in demand of the sorghum, specifically the Gadam type of sorghum being popularized by KARI and EABL (Kilambya & Witwer, 2019). Moreover, the raw sorghum is also refined into wax, dextrose agar, edible oils and starch on an industrial level (Amelework *et al*., 2016). The first type of distinguishing feature of the globally grown sorghum varieties is colour of the kernels; the most common colours are red and white, with black, brown, and purple colour regulated by the genes being reported (Khoddami *et al*., 2023). Preferential products that use white varieties for making porridge, extruded snacks, and industrial beer, and red varieties are commonly used to make beer in the majority of African environments (De Petre *et al*., 2016). The red varieties also have high levels of polyphenolic compounds and have an impact on coloration of the final products. Kenya has a wide-range of varieties nurtured such as landraces (Gesare *et al*., 2013).

Much of the world sorghum crop is being cultivated in the United States but much importance is also being laid on India, China, and Africa. From about 98 million hectares of the crop spanning the globe, Sorghum is thought to produce 6,169.5 million metric tonnes per year, and the economic loss of production limitations throughout the year is estimated USD -130 per unit (Lahouar *et al*., 2018). Among the African countries, the foremost producers of sorghum are Nigeria and Sudan with Ethiopia taking the 10th and Rwanda taking 11th place in production. There is also the cultivation of sorghum in Tanzania and Kenya (Frah, 2016). The western, eastern, and coastal parts of Kenya are mainly known to practice sorghum, which is majorly practiced by the small-holder farmers in Kenya. As a result, a disillusionment is created between what the country is able to produce and what is demanded of the crop. In case sorghum crop is planted on a bigger

scale, Kenya would record similar yields with the use of other dominant cereals like maize, rice, and wheat (Okeyo *et al*., 2020). The Kenyan country is currently importing sorghum in Uganda, Tanzania, and Sudan. Based on this, it is urgent to increase research initiatives in an attempt to improve the performance of crops by means of genetic improvement and agronomic practices that have been refined to counter biotic and abiotic limitations and sociocultural problems. In a conducive environment, a variety of pathogens originate, bringing about the disease in sorghum seedlings, roots, stalks, leaves, panicles, and grains (Kharayat, 2020).

2.2 Major fungal diseases in sorghum crops.

2.2.1 Grain molds

Grain mold is caused by several fungal pathogens that infect the panicle before physiological maturity interfering with grain development. The first symptoms of these molds are visible at the base of the grain near the pedicel, pigmentation of the spikelet and results in the formation of a premature black layer resulting in pink, black or white grains due to fungal mycelium covering grains (Vincent, 2021). It causes shriveled grains, rotting of grains and contamination with mycotoxin leading to harmful grains for consumption, affecting the market value of grains, nutritive value and planting quality of seeds. White sorghum varieties are reported to be the most susceptible varieties compared to colored grains. Inoculum for this disease is found in the field stubble, airborne and seed-borne spores (Nagaraja *et al.,* 2021). *Aspergillus* spp. is known to cause major grain contamination with mycotoxin. That result from secretions of the secondary metabolites called aflatoxin and ochratoxins. *Fusarium* spp. is known to release fumonisin which is lethal to humans and animals (Maina *et al*., 2016).

2.2.2 Post harvest diseases

Mycotoxin are secondary metabolites, toxins that are released by fungi, during aerobic respiration, as a method of security from organisms that rely on the same ecosystem and improve fitness opposed to harsh environments (Kagot *et al*., 2019). These toxins are produced by fungal pathogens such as *Aspergillus* spp., *Fusarium* spp., and *Penicilium* spp. The most widespread mycotoxin includes fumonisin, ochratoxins, T-2 toxins and aflatoxin. *Aspergillus* spp. is capable of producing aflatoxin and ochratoxins and this makes it a major threat to humans and livestock (Bhat *et al*., 2010).

It is estimated that 25% of the world's grains are contaminated with aflatoxin. Mycotoxin can damage the DNA and cause diseases and result in cancerogenic activities (Buszewska-Forajta, 2020). Aflatoxin is considered the most dangerous forms of mycotoxin that are released by fungi of the genus Aspergillus. Growth of aflatoxin is favored by temperatures between 24-38^{o} C and relative humidity > 18% (Marc, 2022); aflatoxins are reported in many seeds including; maize, peanuts, sorghum, millet, wheat and cotton in most parts of Kenya. Predisposing factors include; favorable climatic conditions, poor post-harvest handling of grains, continuous farming in the affected regions, and lack of knowledge and high levels of poverty (Mutegi *et al*., 2018).

2.3 Foliar diseases affecting sorghum production

Rust, grey leaf spot, leaf blight, Smuts and anthracnose are some of the most significant sorghum diseases which limit crop productivity in most of the sorghum-growing regions of Kenya. Smuts affect the panicle while rusts, leaf blight, leaf spots are foliar diseases (Okong'o, 2021). Anthracnose is soil-borne and is most severe during panicle formation stages under favorable conditions affecting the quality and quantity of sorghum grains and

is transmitted by conidia. These diseases are reported to cause losses of up to 90 % in susceptible varieties (Ngugi *et al*., 2002).

Sorghum diseases are seed-borne and management strategy should focus on ensuring clean disease-free seeds. Since their inoculum can be found on seeds, soil stubble, or even air, the majority of these fungal pathogens are difficult to eradicate due to their capacity to remain in the environment. (Abdulsalaam & Shenge, 2011; Makumba, 2016). In Kenya, sorghum production has stagnated without improvement in terms of yield this is attributed to biotic, socio-economic and a biotic-factors in which fungal diseases constitute makor biotic factors alongside other pests such as insects and birds, the major a biotic factor is drought as reported by (Teferi & Wubshet, 2015). Among the socio-economic factors; low-income levels, high poverty levels, low input application and lack of markets were reported (Kebeney *et al*., 2014).

2.4 Management of sorghum fungal diseases

2.4.1 Chemical management approaches

Most sorghum farming is currently dependent on synthetic pesticides. These fungicides have proven to work best in the management of fungal pathogens and improve sorghum yield (Ali *et al*., 2021). Many classes of antifungal agents have been utilized in the management of plant diseases as systemic, foliar sprays, seed dressers and fumigants e.g., Azoles (Dos Reis *et al*., 2021). To decrease mycotoxin contamination, most agricultural companies and other large-scale production farms depend on fumigating agricultural foods and feed products with either methyl bromide or phosphides (Dwivedy *et al*., 2016). Even though chemicals are excellent in the management of plant diseases, factors such as resistance by pests, depletion of the ozone layer, effects on animal and human health due

to their active toxicity, negative impacts on biodiversity and also synthetics are expensive limits these strategies (Mohana *et al*., 2016). Due to these limitations, it is necessity for development of alternatives that are eco-friendly, cheap and safe management strategies (Mohana *et al*., 2016).

2.4.2 Bio-control management strategies

In a broader sense, biological management of plant diseases refers to the use of organism(s) to influence the activities of other organisms by either degrading or detoxifying mycotoxin without interfering with the chemical composition of the grains or crops (Poveda *et al*., 2020). Biological agents influence other pathogens through competition, antibiosis, parasitism and lytic enzymes production. *Trichoderma harzianum* suppresses the growth of most mycotoxin fungi *invitro* (Loi *et al*., 2020), This fungus is a free-living organism that is found in the ecosystem soil and plant roots. Moreover, the antagonistic associations with other fungi make it a good bio control agent. It can secrete high concentrations of enzymes that degrade the cell wall and pores germination hyphae elongation (Kumar *et al*., 2017).

Bacillus amyloliquefaciens

Bacillus amyloliquefaciens is a rhizobacterium found on the rhizosphere of sorghum roots with antagonistic properties against plant pathogens. *Bacillus amyloliquefaciens* is described as a peritrichous flagella-bearing, rod-shaped, endospore-forming, gram-positive, non-pathogenic bacterium that is extensively dispersed. It is commonly utilized against plant, human, and animal infections and dwells in the rhizosphere and inside plants, infrequently in marine sediments or fermented foods. Because endospores can withstand harsh environments including high temperatures, biocides, pressure, and UV light, they can

endure pasteurization and continue to exist in industrial machinery. This bacterium is known to generate a range of antibacterial and antifungal substances.

Notably, genome analysis depicts most strains of *Bacillus amyloliquefaciens* carry genes that encode the enzymes glucose-1-dehydrogenase and gluconate dehydrogenase, which help produce gluconate-dissolved phosphorus and are involved in gluconic acid formation. Additionally, these bacteria promote plant growth by changing the insoluble phosphorus in the soil into soluble phosphorus, which plants may absorb and which stimulates plant growth, bacteria that have the ability of solubilizing phosphorus also exudes organic acids along with certain metal ions that is key in plant growth (Arshad Ali *et al*., 2021; X. Li *et al*., 2023).

Other bio-control agents including plant or botanical extracts such as garlic extracts, green chili and ginger were tested on *Aspergillus niger* and reported to have inhibitory effects (Ozbay & Newman, 2004). Essential oils on the other hand were tested against the mycelial growth of *Aspergillus* species and showed antifungal activities. These developments point out biocontrol as a promising management strategy with minimum to no side effects (Chemitei, 2019; Kim *et al*., 2016).

2.4.3 Pre-harvest and post-harvest management approaches

Aspergillus species causes infection both in the field and in storage. Management strategies such as crop rotation due to fluctuations in the environmental conditions enable one to have diet diversifications reducing mycotoxin contamination, proper spacing, use of certified seeds, sorting, dehulling, cleaning and milling are the best practices before storage (Shricharan *et al*., 2020). Also, harvesting time contributes to mycotoxin contamination since when crops are unharvested for a very long time, the chances of Inoculum landing on the crops becomes high and this in addition to environmental conditions and since temperature, humidity and moisture content plays a bigger part

in growth and development of fungal pathogens, controlling these conditions in storage helps in the management of mycotoxin contamination (Gemeda *et al*., 2014), e.g., use of metal silos. Grains and seeds with moisture contents lower than 14 ⁝% are usually immune to attack by pathogenic fungi (Kimatu *et al.*,2012)

2.4.4 Host plant resistance

Genetic modification of plants through breeding to cause resistance to pests and disease is currently seen as the best approach toward treatment of mycotoxigenic disorders. Most are in the world grow resistant cultivars. Such cultivars contain antifungal proteins or physical barriers (toughness of tissues, tightness of husks, hard kernels, and waxy pericarp) that hinder inoculum spores' access to the site of infection (Loi *et al*., 2020). The genes providing resistance to cultivars are transmitted to the next generation, which makes this technique cheap and sustainable compared to other strategies (Assefa and Geremew, 2018). The high breeding cost, the long breeding cycle, the disaggregation of resistance caused by geographic variations, and the interference between breeding and other desirable qualities during the process of crop improvement are the main limitations to breeding against biotic stresses in sorghum (Were *et al*., 2016); the more sustainable approaches to sorghum productivity improvement should therefore be embraced.

They also stimulate growth of plants, and they can become an alternative to synthetic fertilizers because they are environmentally friendly, cheap and not toxic to humans and animals.

2.4.5 Useful organisms.

Plant growth-promoting microorganisms (PGPMs) enhance the survival of plants and can be utilized instead of synthetic fertilizers as it is not only environmentally friendly but also it is cheap and not harmful to humans and animals. These growth regulators help in the acquisition of nutrients, control phytohormonal interactions, and alleviate biotic and abiotic stresses. They can either arise naturally in the environment or be applied onto the planting surface, either through seed inoculation or root system colonization, naturally or artificially (Lopes *et al*., 2021; Maksimov *et al*., 2015). This has been reported to help in promoting plant growth with Pseudomonas spp. being known to play antagonistic roles with other microorganisms thereby helping to control disorders in plants. Among other benefits, arbuscular mycorrhizal fungi do not harm but rather enhance the vigor of plants, as well as stimulate the use of contaminated soils to bioremediate them (Preston, 2004; Xun *et al*., 2015). Bacillus amyloliquefaciens was reported to penetrate root knot nematodes and promote plant growth; it also prevents bacterial wilt and net blatch barley growth, and, as a result, leads to further plant growth (Abdallah *et al*., 2019; Khan *et al*., 2008).

2.5 Gaps and way forward

Continuous use of synthetic chemicals and fertilizers improves plant yield and also controls pests, however, it poses negative impacts to the ecosystem as they may kill non-target pests, health risks to animals and humans, increases the costs of production, can persist in the environment for a long time, pests can develop resistance over time, can have high residue levels in plants and also may pose killing effects to our crops if they are not measured accurately as well as not having a potential of other additional importance such as improving plant growth and development.

Cultural and physical management strategies to plant diseases have limitations such as non-consistencies in their efficiency, time consuming, most cultural practices are preventative and not effective in a case of pest surge, and these measures as well might not be effective under an intensive cropping system and requires proper understanding of pest and plant biology. This calls for exploitation of more sustainable and environmentally friendly methods of disease management. The present study therefore, aimed at investigating the potential use of *Bacillus amyloliquefaciens* bacterium in management of sorghum foliar diseases, promoting sorghum plant growth and development and also determine the most suitable rates that can be used by farmers to realize these remarkable results.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Efficacy of *Bacillus amyloliquefaciens* in management of major fungal diseases of sorghum under field conditions

3.1.1 Sorghum genotypes selection

A total of five sorghum genotypes namely: N57, N68, Nyadundo 1, Serena and Nyadundo 2, sourced from McKnight foundation crop improvement project we used. These varieties are reported to have incidences of foliar diseases and therefore were used in this study so as to allow maximum disease expression. Using these genotypes also helped to minimize variation within the treatments and any that arises was due to the various treatments applied.

3.1.2 Experimental sites and site characteristics

Field experiment was set at KALRO Kibos research farm located in Kisumu County within the coordinate's latitude 0º 05` S and longitude 34º 48` E. This area receives rainfall of 1180 mm annually and is situated at an altitude of 1130 meters above the sea level (Anton, 2016). Long rains start from March-June whereas the short rains start from Mid-August to November. Temperatures in this area ranges from 21 ºC to 35 ºC. Sega farm was the second site for this experiment which is located along the Kisumu-Busia road. It lies between latitude 0º 3̓ North and longitude 34º 25` East. Long rains fall from March-June whereas short rains run from Early September to November. The soils are red loam and acidic with average pH levels of 4.7. This area receives up to 2000 mm rainfall per year. Selected sites are the major sorghum growing areas of Kenya, hot spot areas for major foliar diseases

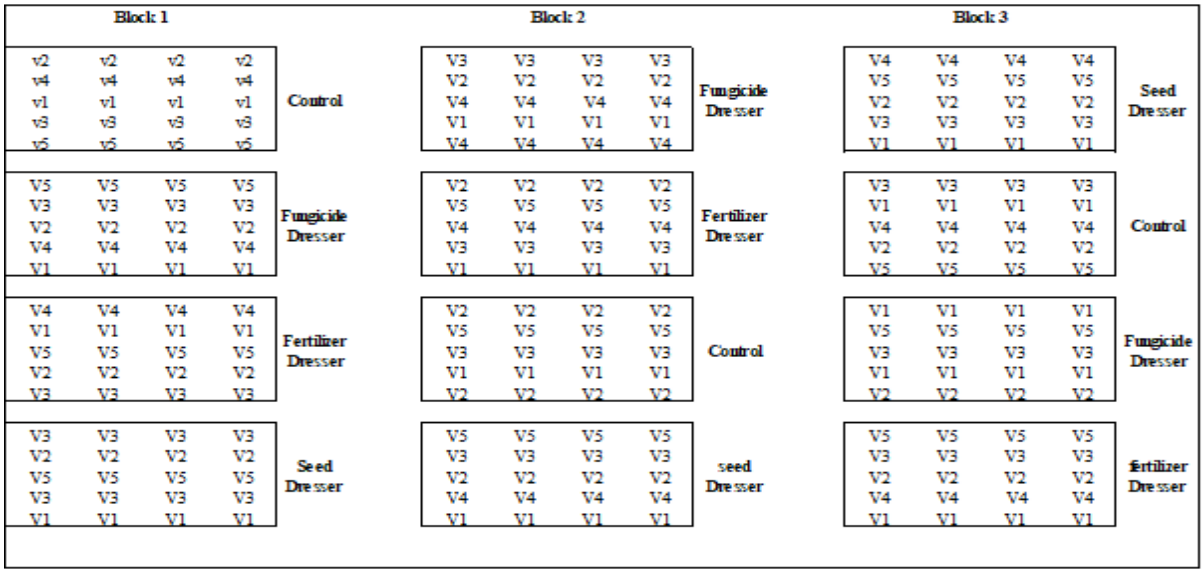
of sorghum representing the major sorghum growing zones of Kenya (Ogello *et al*., 2021). Most research work for the crop improvement project are done in these sites and thus was found suitable for the study.

3.1.3 Experimental layout and statistical linear model

Field experiment was set in a split plot arrangement in a randomized complete block design (RCBD) with five genotypes as the main plots and four treatments as the sub plots replicated three times in each site under two seasons the long rains and the short rains seasons (Table 1). In each site, sorghum genotypes were planted in plot sizes measuring 3 m by 1.5 m separated by 0.5 m path between blocks and plots to facilitate accessibility resulting to 4 lines in each block with 9 plants in each line. Four treatments were administered in this experiment namely: seeds dressed (where sorghum genotypes seeds were inoculated with bacterium before planting), fertilizer dressed (where fertilizers to be used during planting were mixed with bacterium), fungicide dressed (where seeds to be used were dressed with the fungicide before planting) and control (without any treatment on seeds were used). The bacterium was applied at the rate of 300ml/1kg unit.

These application strategies aimed at managing seed borne and soil borne foliar fungi in sorghum. Treatments were randomly allocated to each main plot and subplots. On each subplot, six plants in every variety were selected for data scoring and collection. Randomization using a die was done and below is the layout for the experiment and statistical linear model that aided analysis of collected data.

Table 1: Randomization plan for the response of different genotypes to *Bacillus amyloliquefaciens* treatments in different sites and seasons



Where: **V1** is Nyadundo 1, **V2** is N57, **V3** Nyadundo 2, **V4** N68 and **V5** is Serena genotypes. Genotypes are main plots while treatments are subplots replicated thrice The model for the above design is as outlined below:

Yijk = μ + Bi + Tj + ε (a) ij Vk+ TVjk + εijkl where:

Xijk = Total observation

μ = Overall mean

Bi = i^{th}effect due to block

Tj = j^{th} Effect due to main plot (Dressing with bacteria)

ε (a) ij = Residual error (a) due to i^{th} and j^{th} interaction effect

Vk = kth Effect due to subplot (varietal treatment)

TVjk=j^{th} and k^{th} effect due to the Interaction (dressing treatment and varietal treatment)

εijkl = Residual (b) effect due to block, dressing treatments, varietal treatments

3.1.4 Inoculum preparation and application

Bacillus amyloliquefaciens was cultured on conical flasks in Potato Dextrose Broth (PDB) and kept on a shaker rating 180 rpm for 24 hours at 28^{o}C (Kipkogei, 2019). Sorghum seeds were sterilized using sodium hypochlorite at 1 % for 4 - 5 mins and rinsed with sterile distilled water four times. This was done to ensure that the traces of sterilizing agent were completely removed. For each kilogram of seed, 300 ml inoculum was applied and thoroughly mixed ensuring all seeds were immersed and left to settle for five minutes (Gleń- Karolczyk *et al*., 2021). These seeds were put on trays and under a shade for a day and then put on khaki bags to be used for planting.

First, ploughing and harrowing was done early before planting to obtain a fine tilth. Planting was done in mid- march 2019 in both sites. Both sites were planted in mid-march 2019. Second season planted in August 2019 at recommended rate of 75 kg/ha between rows. 60 cm distance and 15 cm distance between plants. The depth of the furrow containing the seeds was 5 cm. The rate of application of NPK Fertilizer was 75 kg/acre this was done to supplement soils. it is described as slightly acidic (Wamalwa *et al*, 2018).

3.1.5 Data collection

Data on incidence and severity of the diseases were measured in all the plots after the. application of treatments. Plants in the inner rows have been selected to reduce variations. These data were rated on all the foliar diseases of sorghum when early symptoms appeared and during. The plant cycles. Disease incidence was presented as a percentage of disease infected plants with respect to all plants in the plot. Assessed.

Whereas disease severity scoring was done on a scale of 1 -5 where score 1 represents plants with no symptoms of any foliar diseases while score 5 represents plants with about

51 – 100% foliar diseases incidence. These values gave the host status ratings in a scale of 1-5 describing their abilities to resist the foliar fungal disease attack (Table 2).

Table 2 : Host status ratings following their responses to treatments

Host status rate	% Disease incidence	Description
1	0%	Highly resistant
2	1-10%	resistant
3	11-25%	Moderately susceptible
4	26-50%	Susceptible
5	51-100%	Very susceptible

Disease severity data were scored using different scales depending on the disease identified. For instance; anthracnose and leaf blight diseases were scored using a scale of 1-5 as described by (Deep & Thakur, 2007), where; 1- no visible symptoms, 2- (1-10%) leaf area covered by the disease, (11-25%) leaf area covered by the disease, 4 - (26-50%) of the leaf area covered by the disease and 5- (51-100%) leaf area covered by the disease. However, for the leaf spot disease, a scale of 1-5 was used as described by (Odvody G., & Madden D., 1984), where (1) - No visible symptoms, (2)- 1 or more diseased sheath per plant at 25 -50 %, (3)- 1 or 2 diseased sheaths per plant at 51 -100 %, (4)- 3 or more diseased sheaths per plant at 25 – 50 % and (5)- All diseased sheath at 100 %

3.1.6 Data analysis

Collected data was subjected to analysis of Variance (ANOVA) at 5 % level of significance using GenStat Release 16.1, VSN International Ltd. Mean separation was done using Duncan Multiple Range Test (DMRT). These results were presented in form of table of

means, line graphs (standard error of means), figures and plates demonstrating the effects of treatments and additive effects of genotypes as well as their combined effects on foliar disease severities and plant performance.

3.2 Assessment of the *in-vitro* inhibitory effect of *Bacillus amyloliquefaciens* on isolated foliar diseases of sorghum

3.2.1 Isolation and identification of foliar fungal pathogens of sorghum

Media preparations and culturing

Fungal isolation was done through the agar plate method with the use of potato dextrose agar media. Thirty-nine (39) grams of the PDA were weighed and added to one litre of sterile distilled water. After thorough mixing using a magnetic stirrer on a hot plate maintained at 60 ^{o}C, this media was sterilized in an autoclave at 15 PSI and 121 ^{o}C for 15 minutes, after which sterile media was dispensed onto sterile Petri dishes in laminar flow hood and left to solidify (Mwatabu *et al*., 2023).

Plant parts sampled showing symptoms of foliar diseases identified in the field were analyzed at the crop protection laboratory at University of Eldoret department of seed crop and horticultural sciences. Small infected sections were carefully excised within the boundary of the lesions to about 2mm pieces and surface sterilized with 10 % sodium hypochlorite for 20 seconds to remove any external organisms that may have accompanied the samples. These samples were thoroughly rinsed three times with sterilized distilled water to remove any remains of the sterilizing agent and put in sterile filter papers to dry. Clean and dry plant parts were put on Petri dishes and incubated for seven days (Sarlia Dorley *et al.*, 2023).

Identification of isolated fungi

After seven days, growing fungi was identified by describing the cultural and microscopic characteristics. Plant pathological manuals, reference journals and microscope were used to differentiate and identify the isolates (Mwatabu *et al*., 2023; Sarlia Dorley *et al.*, 2023).

3.2.2 Evaluation of inhibitory effects of *Bacillus amyloliquefaciens* on identified fungal pathogens of sorghum

Experimental layout and linear model

Evaluation of *Bacillus amyloliquefaciens* was done using dual culture technique where an experiment comprising of two treatments was set in a completely randomized design (CRD) with identified fungal isolates such as *Colletotrichum sublineola* causing anthracnose disease of sorghum, *Sporisorium sorghi* isolated from sorghum samples showing symptoms of covered kernel disease, *Exserohilum turcicum* isolated from sorghum leaf blight samples and *Gloeocerspora sorgi* causing zonate leaf spot of sorghum. Bio control treatments such as *Bacillus amyloliquefaciens* and a control comprising of fungal isolates without any control agent were applied to these isolates each replicated thrice. Assessment was done on the inhibitory potential of treatments applied on mycelial growth characteristics of the test fungi.

Freshly grown fungal culture, seven days old was cultured at the center of the plate containing PDA along the diameter line. At the periphery of the plate, along the diameter line a spot of the bacterium was smeared and incubated at room temperatures. A control was set in which a fungal isolate was cultured with sterile distilled water in place of *Bacillus amyloliquefaciens*. Inhibitory potential of *Bacillus amyloliquefaciens*, was done by measuring the area covered by the fungus treated with the bacterium in comparison to the area covered by the control culture (Kipkogei, 2019). While percentage inhibition was

done by subtracting area covered by the test fungi under treatment (r) from area covered under control as a subject to control (R) expressed as a percentage as shown in the formula below;

$$\frac{(R - r)\ X\ 100}{R}$$

Where r is the radius of the fungal colony opposite the bacteria and R is the radius of the fungal colony away from the bacteria.

3.2.3 Data collection and analysis

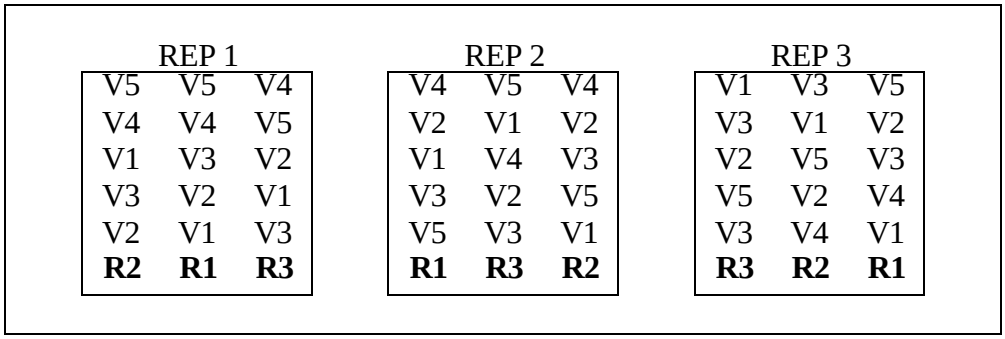
Growth patterns of the fungus following the treatment with the bacterium were assessed at the interval of 24 hours for seven days after which growth inhibition was calculated. These data were subjected to analysis of Variance (ANOVA) at 5 % level of significance using GenStat Release 16.1, VSN International Ltd. Mean separation using Duncan Multiple Range Test (DMRT). These results were presented in form of table of means, line graphs, figures and plates.

3.3 Assessment of *Bacillus amyloliquefaciens* as a bio control of covered kernel disease of sorghum under greenhouse conditions

3.3.1 Experimental design and layout

One sorghum genotype that was reported to be susceptible to major fungal diseases of sorghum was used for this study. Seeds were dressed with the Inoculum of *Bacillus amyloliquefaciens* before planting in pots and this experiment was set in a completely randomized design in three replicates. Regular agronomic management practices such as watering, weeding and close monitoring were employed to ensure optimal growth throughout the study. The following is an experimental layout and a linear model that

guided analysis thereafter.



Yij = μ + αi + βj + εijk where:

Yijk = Total observation

μ = Overall mean

αi = ith effect of bacterium

βj = jth effect of genotype

εijk = Error term/residual effect

3.3.2 Data collection and analysis

After bacterium inoculation, regular scouting for the covered kernel disease under different rates of bacterium was done and scored on a severity scale of 1-5.

These data on plant performance were subjected to analysis of Variance (ANOVA) at 5 % level of significance using Gen Stat Release 16.1, VSN International Ltd. Mean separation was perfomed using Duncan Multiple Range Test (DMRT). These results were presented in form of table of means, line graphs, figures and plates.

CHAPTER FOUR

RESULTS

4.1 *Bacillus amyloliquefaciens* as a biocontrol agent of foliar diseases of sorghum under field conditions

Foliar fungal diseases of sorghum where described and identified as follows: Anthracnose disease was characterized by small red colored spots that appears on both sides of the leaf. The center of the spot is normally white colored and surrounded by red, purple or brown margin. When diagnosed closely, numerous small black dots like acervuli are seen on the white surface of these lesions (Koima *et al*., 2023). Leaf spot disease was characterized by small reddish- brown water-soaked leaf spots sometimes with a narrow pale green halo. t became larger, blackened to a crimson color and began to stretch in a direction parallel to the veins (Heo *et al*., 1999). Conversely, the disease is defined by reddish-purple or tan macules that merge into large holes (Beshir *et al*., 2015).

4.1.1 The effects of Bacillus amyloliquefaciens on incidences and severities of sorghum foliar diseases

Three different fungal diseases were observed on the foliage: anthracnose, leaf blight, and leaf spot. It was also noted that the prevalence of anthracnose at both locations was the highest, and the minimum at the leaf spot, regardless of the site. The site-specific determination indicated that anthracnose was highest in Kibos as it was in leaf spot; however, the intensity of leaf blight was higher in Sega (Appendix xii). In all sites, control plots showed increased disease incidence of each foliar disease identified.

In overall observations, site factors did not affect treatment performance during the long rainy seasons (Appendix (iii)). There was a significant difference $p< 0.05$ in the

performance of treatments in influencing disease incidences. Seed dressing with bacterium was most effective in preventing disease infections followed closely by seed treated with fungicide which was a positive check and then fertilizer mixed with the bacterium before planting. Control treatment recorded highest disease incidence and severities across all the diseases identified.

4.1.2 Incidence and severity of foliar disease under different treatments

Generally, anthracnose disease recorded the highest incidence and severity followed by leaf blight disease and finally leaf spot disease was the least in incidence and severity across all the treatments. In terms of treatment performance, seeds treatment with bacterium recorded the lowest incidence and severities of the identified sorghum foliar diseases followed by mixing fertilizer with the fungicide and dressing seeds with the fungicide. Control treatment recorded high incidences in the three foliar diseases thus proving bacterium to be effective in managing sorghum foliar diseases (Figure 1).

Dressing seeds with bacterium, significantly reduced anthracnose disease severity while on the other hand, planting sorghum without any control agent increases the chances of disease infection and disease progress. This is seen as the control treatment record the highest disease incidence and severity over the assessment period. Mixing fertilizers with the bacterium had similar potentials as treatment of seeds with a fungicide in inhibiting anthracnose disease incidences and severities (Figure 1a).

Significant variation was noted between treatments with bacterium and those with no treatments as more leaf blight disease incidences and severities were recorded under control. Treatments of sorghum seeds with bacterium, mixing the fertilizer before planting with the bacterium and treating seeds with the fungicide did not have a significant

difference in limiting disease occurrences. Among the treatments, applying the bacterium to the sorghum seeds appeared to be relatively efficient in inhibiting leaf blight incidence (Figure 1b).

Sorghum leaf spot disease was significantly reduced by the treatment, these treatments did not show a significant difference in their performance. Sorghum seeds planted with no treatment agents had a higher sorghum leafspot incidence. Fungicide treatment recorded relatively low incidence of sorghum leaf spot disease while fertilizer mixed with the bacterium and seeds dressed with the bacterium had a similar response in reducing sorghum leaf spot disease (Figure 1c).

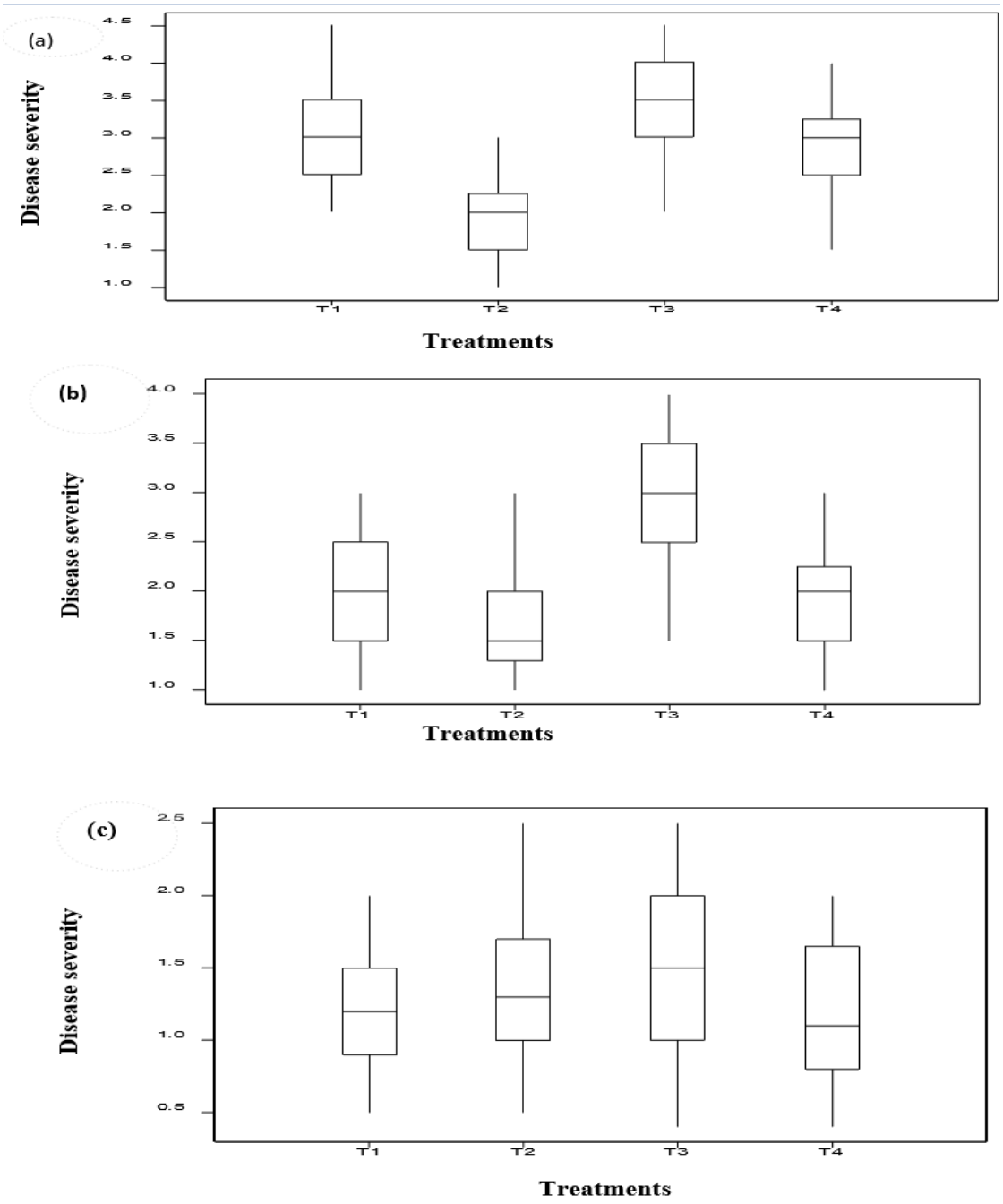


Figure 1: Incidences and severities of sorghum foliar disease; Anthracnose (a), Leaf blight (b) and Leaf spot (c) diseases following application of treatments such as mixing fertilizer with bacterium (T1), dressing seeds with the bacterium (T2), control without any treatment (T3) and dressing seeds with fungicide (T4) under field conditions

4.1.3 Response of different sorghum varieties to sorghum foliar diseases

Among the five tested genotypes, Serena variety recorded the least severity to Anthracnose and was not significantly different from Nyadundo 1, Nyadundo 2, N57 and N68 (Figure 2a). For leaf blight disease all varieties responded similarly in their tolerance abilities except for Nyadundo 2 variety which appeared to be more susceptible to leaf blight disease since it recorded slightly higher disease severity (Figure 2b). N68 and Nyadundo 2 recorded the least severity levels to leaf spot disease while Serena, Nyadundo 1 and N57 had similar responses which as slightly higher (Figure 2c). Mean disease severities were not significantly different from each other across all the varieties and this implies that varieties did not influence the disease incidences and severities.

The mean disease severities were; anthracnose 3, Leaf blight 2.5 and leaf spot 1.5 and when these means were rated on a scale of 1-5 (Table 2), tested sorghum varieties were generally grouped as moderately susceptible to anthracnose disease and resistant to both leaf blight and leaf spot diseases (Figure 2).

When displayed phenotypically, severity was highest under control treatments across all the foliar diseases identified. Seed dressing with the bacterium significantly reduced disease severity across all the three diseases bacterium compared to other treatments (Plate 1)

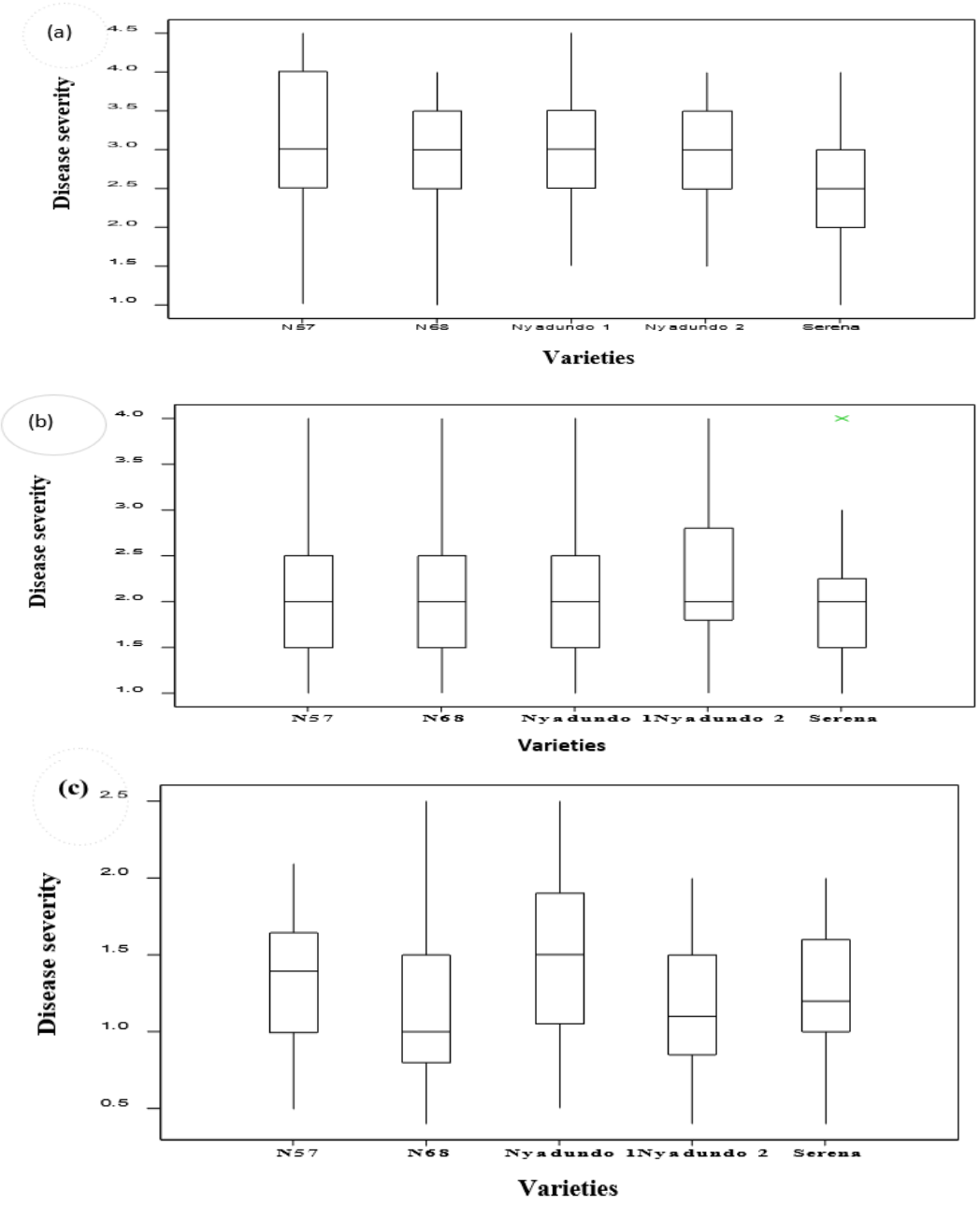


Figure 2: Incidences and severities of sorghum foliar disease; Anthracnose (a), Leaf blight (b) and Leaf spot (c) diseases on different sorghum varieties such as; N57, N68, Serena, Nyadundo 1 and Nyadundo 2 under field conditions

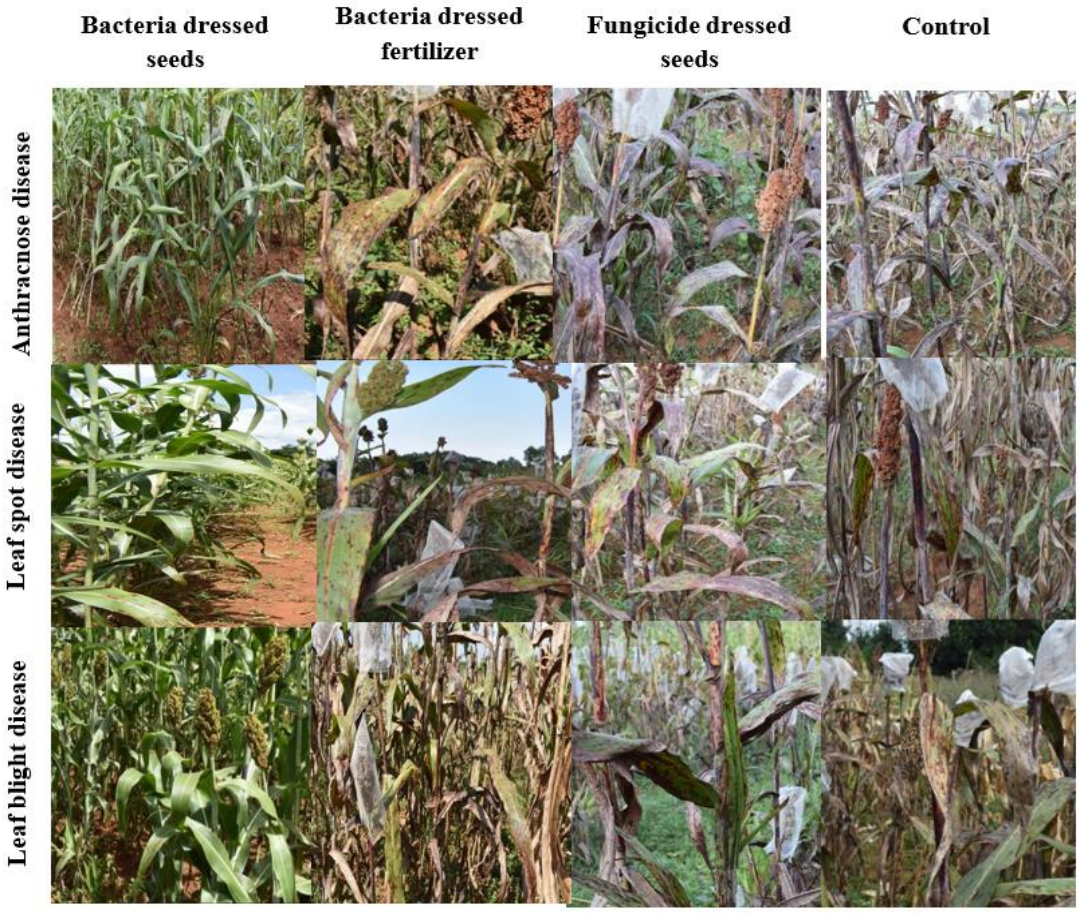
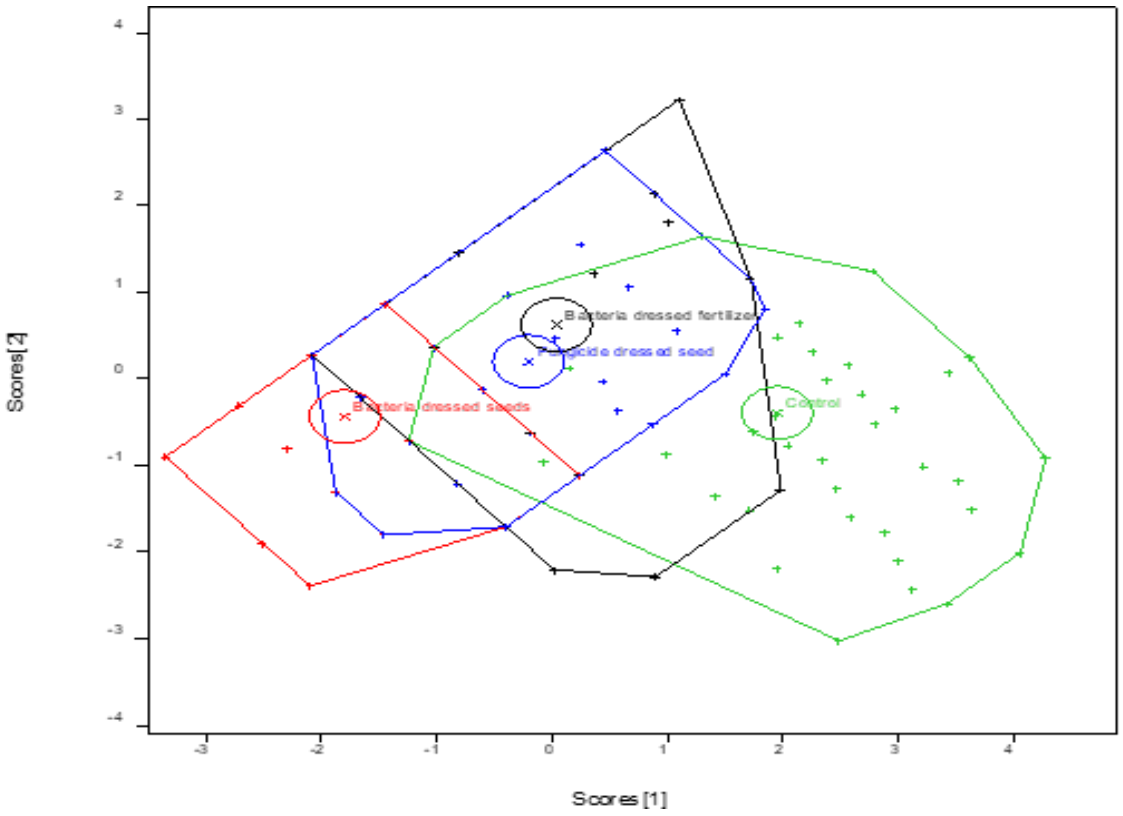


Plate 1: Severity of anthracnose, leaf spot and leaf blight following *Bacillus amyloliquefaciens* bacterium. (Source Author 2019)

4.1.4 Similarities and differences among different treatments in terms of foliar disease severities

Disease severity under control treatment depicted a distinctive display according to intergroup distances compared to treatments such as dressing with bacterium and the fungicide. However, based on the 95% confidence circles around the group means, trends in disease severity under fertilizer mix with the bacterium and when a fungicide was used to dress the seeds, had no significant difference hence an overlap in group polygons (Figure

3).



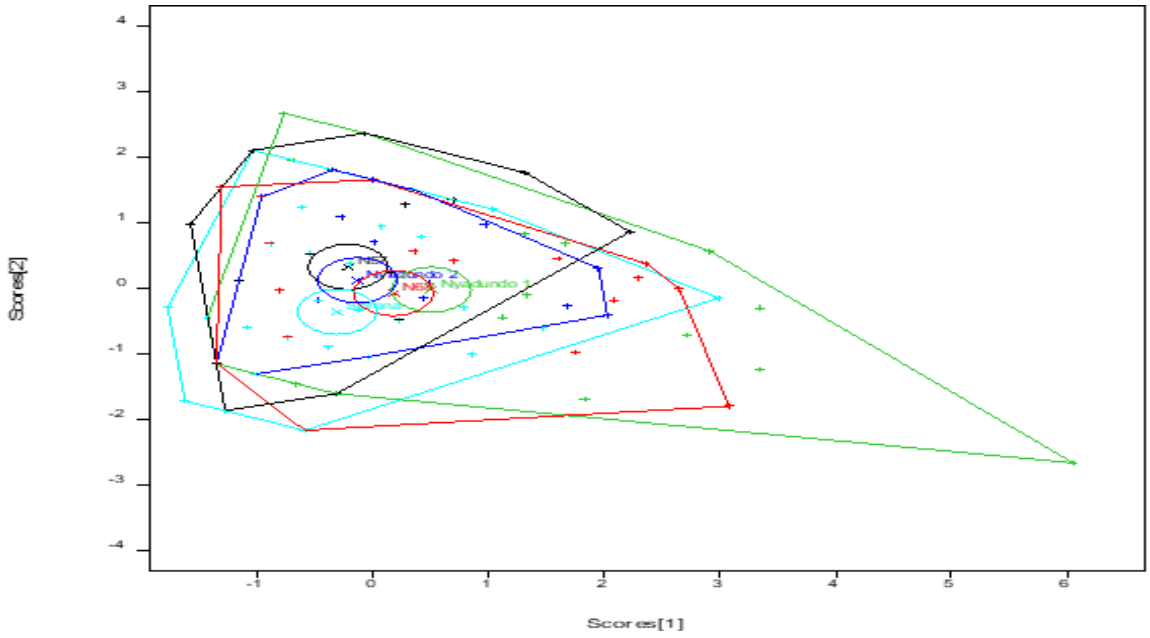
Intergroup distances-Mahalanobis (D-squared)

Bacteria dressed fertilizer (BDF)	0.0			
Bacteria dressed seeds (BDS)	4.5	0.0		
Control (CTR)	4.7	14.1	0.0	
Fungicide dressed seed (FDS)	0.4	3.1	5.1	0.0
	BDF	**BDS**	**CTR**	**FDS**

Figure 3*:* Intergroup distances among the *Bacillus amyloliquefaciens* bacterium as displayed by the polygons generated through Mahalanobis discriminant analysis approach. The analysis and displayed results were based on sorghum foliar disease severities

4.1.5 Similarities and differences among different sorghum varieties in terms of foliar disease severities

Variety responses to disease pressures did not differ significantly as depicted by an overlap of the 95% confidence circles around the group means. Nyadundo 1 appeared to be more susceptible to sorghum foliar diseases while Nyadundo 2 appeared to be the most tolerant this is due to larger and smaller polygons respectively (Figure 4).



Intergroup distances-Mahalanobis (D-squared)

N57	0.0				
N68	0.3	0.0			
Nyadundo 1	0.6	0.1	0.0		
Nyadundo 2	0.1	0.1	0.4	0.0	
Serena	0.5	0.3	0.8	0.3	0
	N57	**N68**	**Nyadundo 1**	**Nyadundo 2**	**Serena**

Figure 4 : Intergroup distances among the sorghum varieties as displayed by the polygons generated through Mahalanobis discriminant analysis approach. The analysis and displayed results were based on sorghum foliar disease severities

4.2 *In vitro* effects of *Bacillus amyloliquefaciens* on mycelial growth of identified fungus

Samples of fungal disease infection that were found in the field in both sites and seasons were cultured in the laboratory for isolation of causal fungal pathogens and from these samples, it was found that Anthracnose disease was caused by *Colletotrichum sublineola, Exserohilum turcicum* and *Gloeocercospora sorgi* were found to cause sorghum leaf blight and sorghum leaf spot respectively. From the covered kernel diseased samples *Sporisorium sorgi* was isolated as the causal agent.

Colletotrichum sublineola was identified based on cultural characteristics such as raised cottony mycelium, whitish to yellowish pigmentation, hyaline and smooth conidia with no septations (Koima *et al*., 2023). *Exserohilum turcicum* were found to form whitish grey colonies on potato dextrose agar medium and the cultures turned darkish grey during sporulation. Conidiophores were in small groups with septations, there margins were smooth and straight and dark brownish in color (Vinay & Sataraddi, 2019).

Gloeocercospora sorgi formed yellowish to brownish mycelial growth and formed long conidia, curved and hyaline with numerous septations. These cultures produced numerous mass of spores with sclerotia being hyaline, curved and septate (Watanabe & Hashimoto, 1978). *Sporisorium sorgi* was characterized by brownish mycelium. The spore were found to be thick walled, globose to cylindrical in shape (El-Dawy *et al*., 2023; McTaggart *et al.*, 2012). These four fungi displayed the following results in their response to *Paenibacillus polymyxa* treatments: Mycelial growth in *Colletotrichum sublineola* was the most inhibited by *Paenibacillus polymyxa* by 58 % while *Exserohilum turcicum* was least inhibited by the bacterium ((35%) which had a lower significance difference in terms of mycelial growth

inhibition from *Sporisorium sorgi* (42%) and *Gloeocercospora sorgi* (45%) (Figure 5).

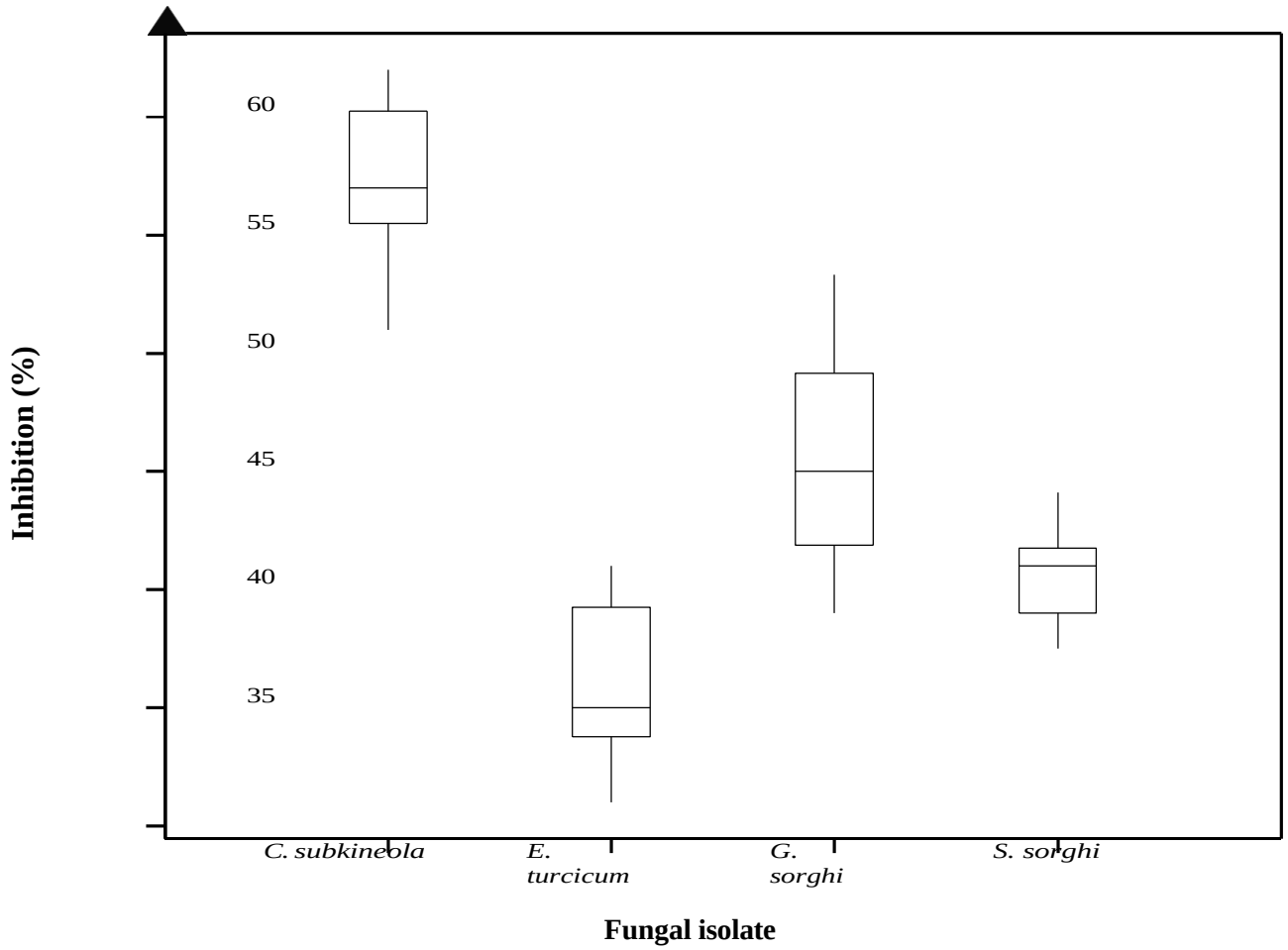


Figure 5: Percentage inhibition of selected fungal pathogens of sorghum by *Bacillus amyloliquefaciens*

Mycelial inhibition was least in *Sporisorium sorgi* while *Colletotrichum sublineola* had the highest inhibition. *Gloeocercospora sorgi* and *Exserohilum turcicum* had moderate inhibition. This was following a dual culture technique where isolated foliar fungal diseases of sorghum were cultured together with *Bacillus amyloliquefaciens* bacterium at equidistant positions and the rate of inhibition examined at intervals for seven days by measuring the distance away and to the fungus (Plate 2).

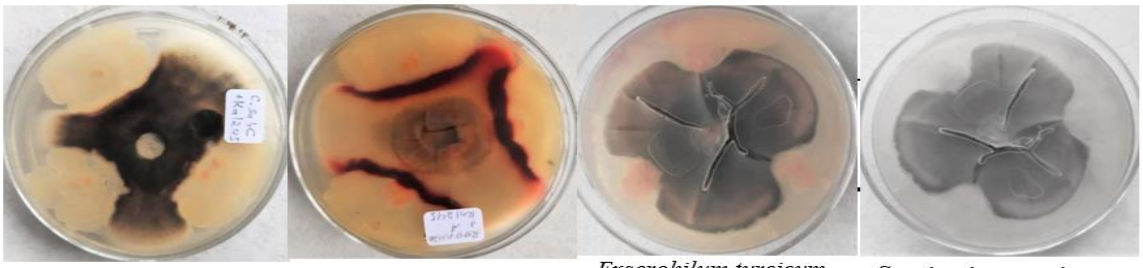


Plate 2: Effects of Bacillus amyloliquefaciens bacterium on mycelial growth of isolated fungal pathogens. (Source: Author 2019)

4.3 Effects of *Bacillus amyloliquefaciens* on covered kernel disease severity of selected sorghum genotypes

A significant difference was noted on covered kernel disease severity under different rates of bacterium $p < 0.05$ (Appendix iv). On a severity scale of 1-5, sorghum genotype seeds treated with 400 ml of bacterium per a kilogram of seed had the lowest disease severity. Increasing bacterium treatment rates consequently reduced disease severity compared to control where no bacterium was applied. When compared with a positive check, fungicide; bacterium at a higher rate responded almost similarly in suppressing disease severity (Figure 6).

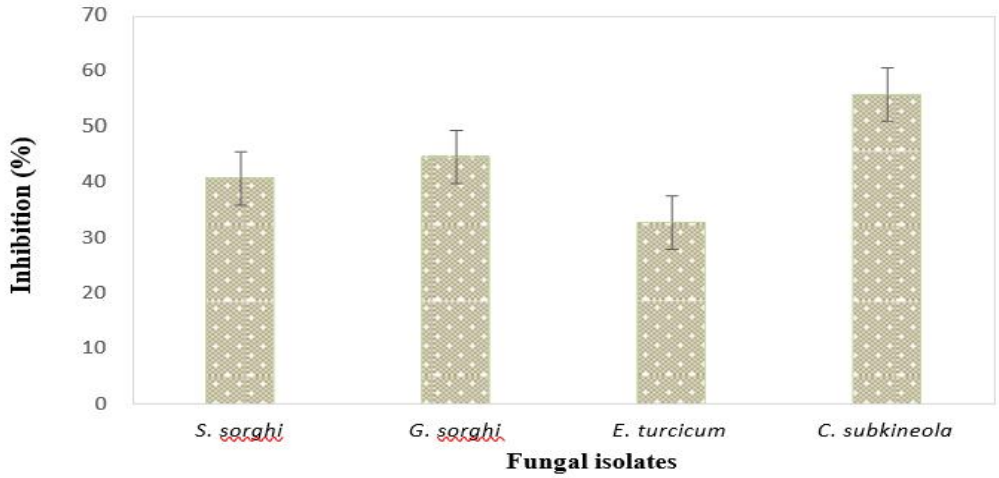


Figure 6: In vitro inhibition of sorghum foliar disease pathogens by *Bacillus amyloliquefaciens* bacterium. Error bars represents standard errors

Disease severity was inversely proportional to the rates of *Bacillus Amyloliquefaciens* bacterium this is evident in plate 6 where the highest severity was recorded in control treatment and the lowest rate (200 ml/kg of seeds)

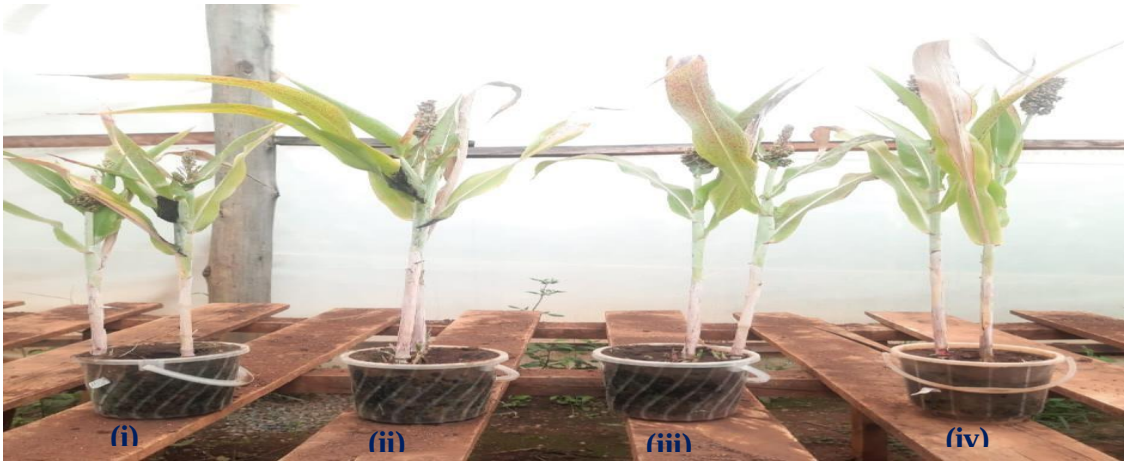


Plate 3: Severity of covered kernel disease at different rates of bacterium: (i) - 0ml/kg (ii) - 200ml/kg, (iii)-300ml/kg & (iv) -400 ml/kg of seeds. (Source: Author 2019)

CHAPTER FIVE

DISCUSSIONS

5.1 Effects of *Bacillus amyloliquefaciens* as a Biocontrol on incidences of selected Foliar diseases in sorghum and crop growth performance under field conditions.

Study conducted in Kibos recorded a higher incidence of sorghum foliar diseases. This is due to site factors such as high inoculum reservoirs and site factors in which the fungal pathogens may have adapted to over the years. Therefore, the development of diseases is preconditioned by soil properties, especially, phosphorus status, and can be the reason behind the observed difference (Nyambok *et al*., 2014). Nyambok *et al*. (2014) tested the causes of these differences. The severity of the diseases was maximum in brief rainy seasons compared to other seasons, this tendency can be explained by the fact that moisture is required to trigger infection, and the existence of good temperature regime that promoted reproduction of pathogens and colonization of tissues (Gadoury *et al*., 2012; Redman *et al*., 2011). *Bacillus amyloliquefaciens* was found to reduce the occurrence of the foliar disease through the generation of secondary metabolites, which are found to have antagonism towards foliar fungal pathogens (Abdallah *et al*., 2019). The inhibition of certain pathogens and especially the fungi is an interaction process that is cyclical in nature and requires the viable bacteria to be present. Raza *et al*. (2008) have found that magnesium ions counteract antifungal compounds released by the bacterium. The foliar diseases of sorghum are by nature seed borne; and thus, the treatment of the seed with a control agent helps to reduce the inoculum levels and could explain the observed results mentioned after seed dressing with *Bacillus amyloliquefaciens*.

It has been known that fungicides inhibit disease infection by suppressing the growth and development of fungal pathogens by inhibiting respiratory activity of the fungi (Odvody and Hepperly, 1992). At each location, different sorghum species had inconsistent performance, and had unequal reactions to disease infections. The differences observed can be attributed to the different genetic abilities (Ahmad *et al*., 2010). Bacillus amyloliquefaciens promotes plant growth; this is because the bacterium is at the forefront of colonizing the roots, taking up nutrients and adjusting soil pH to facilitate plant growth this is made possible through development of hyphae network for absorption of nutrients. To attain these effects, the bacterium alters soil physical, chemical, and biological properties (Bashan and De-Bashan, 2010; Olanrewaju *et al*., 2017).

5.2 *In vitro* effects of *Bacillus amyloliquefaciens* on mycelial growth of identified fungus

Bacillus amyloliquefaciens was found to inhibit mycelial growth of sorghum foliar diseases *in vitro* and this may be attributed to production of enzymes, antibiotics and sideophores that may have triggered antagonistic reactions between the fungus and the bacterium resulting to observed changes (Sessitsch *et al*., 2004). In some instances, the bacterium may act through hyper parasitism or direct competitions. The degrees of inhibition were less in other fungal pathogens; for instance, *Exserohilum turcicum* was less inhibited by the bacterium since it may have caused a zone of inhibition making the fungus resist the actions of the bacterium (Köhl *et al*., 2019). These results corresponds to earlier scholars who concluded that bacterial organisms produce enzymes that degrades fungal structures inhibiting their growth and development (Alharbi, 2022), some strains attach, penetrate and lyse the fungal hyphae. Furthermore, some strains of plant growth

promoting bacterium produces several antibacterial and antifungal substances that induce cytolysis, damage membrane structural integrity, impede mycelial development, and prevent spore germination (Xu *et al*., 2020).

5.3 Effects of *Bacillus amyloliquefaciens* on growth performance and covered kernel disease severity under greenhouse conditions

Our results displayed a notable synergistic interaction of genotypes and *Bacillus amyloliquefaciens* bacterium in enhancing plant growth and disease severity under controlled environments. Increasing the rates of bacterium inoculum was directly proportional to plant growth and increasing chlorophyll accumulation as compared to control treatments.

Increasing the rates of bacterium significantly increased chlorophyll a and chlorophyll b accumulation and this could be attributed to the fact that *Bacillus amyloliquefaciens* treated plants have more ACC-deaminase enzymes, which slow down the degradation of chlorophyll. Alternatively, it could be because of an increase in photosynthetic rate or the role that N nutrition plays in producing substances that promote growth and lead to more efficient nutrient absorption, which in turn produces more pigments that are the main components of photosynthetic pigments, increasing the amount of chlorophyll (Mohamed & Gomaa, 2012). These results are also in line with those of (Li *et al*., 2022; Vafadar *et al*., 2014), who recorded that; since nitrogen is one of the important nutrients in plants chlorophyll formation, plant growth promoting bacterium helps in nitrogen fixation in non-leguminous plants thereby increasing chlorophyll accumulation.

Gene sequencing done by (Xu *et al.*, 2020) revealed that *Bacillus amyloliquefaciens* strains

promotes plant growth through solubilization of phosphate and the formation of α-amylase, ammonia, cellulose, indole-3-acetic acid, pectinase, sideophores, and protease.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study established four foliar fungal diseases affecting sorghum production in Sega and Kibos sites of western Kenya namely: Anthracnose disease, leaf blight disease, leaf spot disease and covered kernel disease which varied in their intensities based on season and site. Additionally, among the sorghum genotypes screened Serena was found to be susceptible to all the foliar diseases of sorghum while N57 and Nyadundo 1 were the most tolerant. It was also concluded that anthracnose disease was more prevalent in Kibos than in Sega sites. Treatment of seeds with *Bacillus amyloliquefaciens* bacterium significantly suppressed disease incidence in both study sites. Sorghum foliar diseases were more severe during the short rains than during the long rains.

Bacillus amyloliquefaciens bacteria significantly inhibited mycelial growth rate of sorghum foliar disease-causing fungi under *in vitro* conditions. *Colletotrichum sublineola* was most inhibited while *Exserohilum turcicum* was least inhibited.

Increasing the rates of *Bacillus amyloliquefaciens* from 200 milliliters to 400 milliliters significantly reduced covered kernel disease severity.

6.2 Recommendations

Resistant sorghum varieties should be adopted by farmers in both sites to reduce disease pressures and improve crop yield. Proper timing of cropping seasons should be adopted to reduce disease severities. Studies should be done to establish causes of resistance of the identified genotypes at molecular levels. These genotypes should be screened under different environments to conclude universal results.

Bacillus amyloliquefaciens strain should be incorporated into integrated pest management systems to achieve potential crop yields.

Further studies should be done to ascertain the correct rates that are cost effective of *Bacillus amyloliquefaciens* bacteria that can be used to improve plant growth and also to reduce disease infections.

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APPENDICES

Appendix I: Anova tables and summarized table of means for performance of different sorghum varieties under different treatments

Variate: Anthracnose **Source of variation**	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Block stratum	2	0.2583	0.1292	0.25	
Variety	4	10.1521	2.538	4.93	0.027
Residual	8	4.1167	0.5146	1.05	
Treatment	3	94.3542	31.4514	64.33	<.001
Variety.Treatment	12	2.8229	0.2352	0.48	0.91
Residual	30	14.6667	0.4889	2.43	
Season	1	0.0375	0.0375	0.19	0.667
Site	1	0.0375	0.0375	0.19	0.667
Variety.Season	4	0.6187	0.1547	0.77	0.547
Treatment.Season	3	0.3208	0.1069	0.53	0.661
Variety.Site	4	0.9521	0.238	1.18	0.321
Treatment.Site	3	7.7542	2.5847	12.86	<.001
Season.Site	1	0.7042	0.7042	3.5	0.064
Variety.Treatment.Season	12	0.8562	0.0714	0.35	0.976
Variety.Treatment.Site	12	7.9229	0.6602	3.28	<.001
Variety.Season.Site	4	1.1187	0.2797	1.39	0.241
Treatment.Season.Site	3	1.5208	0.5069	2.52	0.061
Variety.Treatment.Season.Site	12	1.1562	0.0964	0.48	0.924
Residual	120	24.125	0.201		
Total	**239**	**173.496**			

VARIETY	TREATMENT	LONG RAINS		SHORT RAINS		**MEANS**
		KIBOS	SEGA	KIBOS	SEGA	
N57	Bacteria dressed fertilizer	3.2	3.7	3.2	2.8	
	Bacteria dressed seeds	1.7	2.3	1.5	2.2	2.9[b]
	Control	3.8	4.0	3.7	3.8	
	Fungicide dressed seed	3.2	2.7	3.7	2.2	
AV		3.0	3.2	3.0	2.8	
N68	Bacteria dressed fertilizer	3.2	3.3	3.3	3.0	
	Bacteria dressed seeds	1.7	2.3	2.2	2.0	2.9[b]
	Control	3.3	3.7	3.5	3.7	
	Fungicide dressed seed	2.8	2.7	3.0	2.0	
AV		2.8	3.0	3.0	2.7	
Nyadundo 1	Bacteria dressed fertilizer	3.5	3.0	3.5	3.2	
	Bacteria dressed seeds	1.8	2.2	1.7	1.8	3.0[b]
	Control	4.2	3.5	4.0	3.8	
	Fungicide dressed seed	3.0	3.2	3.0	3.0	
AV		3.1	3.0	3.0	3.0	
Nyadundo 2	Bacteria dressed fertilizer	3.7	2.7	3.3	2.8	
	Bacteria dressed seeds	1.8	2.2	2.0	2.0	2.9[b]
	Control	3.3	3.7	3.5	3.7	
	Fungicide dressed seed	2.7	2.8	3.2	2.5	
AV		2.9	2.8	3.0	2.8	
Serena	Bacteria dressed fertilizer	2.2	2.8	2.2	3.0	
	Bacteria dressed seeds	1.2	1.7	1.0	2.0	2.4[a]
	Control	2.8	3.0	3.0	3.7	
	Fungicide dressed seed	3.0	2.3	3.3	1.8	
AV		2.3	2.5	2.4	2.6	
MEANS		2.8	2.9	2.9	2.8	**2.8**

Variate: Leaf spot Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Block stratum	2	1.2	0.6	7.97	
Variety	4	1.57292	0.39323	5.22	0.023
Residual	8	0.60208	0.07526	0.64	
Treatment	3	5.975	1.99167	16.92	<.001
Variety.Treatment	12	1.61875	0.1349	1.15	0.363
Residual	30	3.53125	0.11771	2.12	
Season	1	0.00417	0.00417	0.08	0.785
Site	1	0.81667	0.81667	14.7	<.001
Variety.Season	4	0.13125	0.03281	0.59	0.67
Treatment.Season	3	0.22083	0.07361	1.33	0.269
Variety.Site	4	0.48542	0.12135	2.18	0.075
Treatment.Site	3	0.89167	0.29722	5.35	0.002
Season.Site	1	0.00417	0.00417	0.08	0.785
Variety.Treatment.Season	12	0.39375	0.03281	0.59	0.846
Variety.Treatment.Site	12	1.13958	0.09497	1.71	0.073
Variety.Season.Site	4	0.02708	0.00677	0.12	0.974
Treatment.Season.Site	3	0.1375	0.04583	0.83	0.483
Variety.Treatment.Season.Site	12	0.33125	0.0276	0.5	0.913
Residual	120	6.66667	0.05556		
Total	**239**	**25.75**			

VARIETY	TREATMENT	LONG RAINS		SHORT RAINS		**MEANS**
		KIBOS	SEGA	KIBOS	SEGA	
N57	Bacteria dressed fertilizer	1.0	1.0	1.0	1.0	1.0[a]
	Bacteria dressed seeds	1.0	1.0	1.0	1.0	
	Control	1.0	1.3	1.0	1.3	
	Fungicide dressed seed	1.0	1.0	1.0	1.0	
AV		**1.0**	**1.1**	**1.0**	**1.1**	
N68	Bacteria dressed fertilizer	1.0	1.3	1.0	1.2	1.2[ab]
	Bacteria dressed seeds	1.0	1.0	1.0	1.0	
	Control	1.7	1.5	1.7	1.7	
	Fungicide dressed seed	1.0	1.0	1.0	1.0	
AV		**1.2**	**1.2**	**1.2**	**1.2**	
Nyadundo 1	Bacteria dressed fertilizer	1.0	2.0	1.0	1.3	1.3[c]
	Bacteria dressed seeds	1.0	1.0	1.0	1.0	
	Control	1.3	1.7	1.3	2.0	
	Fungicide dressed seed	1.2	1.2	1.0	1.0	
AV		**1.1**	**1.5**	**1.1**	**1.3**	
Nyadundo 2	Bacteria dressed fertilizer	1.0	1.0	1.0	1.0	1.1[ab]
	Bacteria dressed seeds	1.0	1.0	1.0	1.0	
	Control	1.0	1.0	1.0	1.0	
	Fungicide dressed seed	1.0	1.0	1.0	1.0	
AV		**1.0**	**1.2**	**1.0**	**1.1**	
Serena	Bacteria dressed fertilizer	1.0	1.0	1.0	1.0	1.1[a]
	Bacteria dressed seeds	1.0	1.0	1.0	1.0	
	Control	1.0	1.2	1.2	1.5	
	Fungicide dressed seed	1.0	1.0	1.0	1.0	
AV		**1.0**	**1.0**	**1.0**	**1.1**	
MEANS		1.1	1.2	1.1	1.2	**1.1**

Variate: Leaf blight Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Block stratum	2	1.9562	0.9781	1.29	
Variety	4	2.4333	0.6083	0.8	0.558
Residual	8	6.0854	0.7607	2.57	
Treatment	3	63.0281	21.0094	71.02	<.001
Variety.Treatment	12	7.175	0.5979	2.02	0.058
Residual	30	8.875	0.2958	0.8	
Season	1	0.876	0.876	2.36	0.127
Site	1	7.526	7.526	20.26	<.001
Variety.Season	4	0.9208	0.2302	0.62	0.649
Treatment.Season	3	2.0115	0.6705	1.8	0.15
Variety.Site	4	1.9583	0.4896	1.32	0.267
Treatment.Site	3	1.2448	0.4149	1.12	0.345
Season.Site	1	0.7594	0.7594	2.04	0.155
Variety.Treatment.Season	12	0.8375	0.0698	0.19	0.999
Variety.Treatment.Site	12	7.5	0.625	1.68	0.079
Variety.Season.Site	4	0.0792	0.0198	0.05	0.995
Treatment.Season.Site	3	0.5115	0.1705	0.46	0.712
Variety.Treatment.Season.Site	12	1.1292	0.0941	0.25	0.995
Residual	120	44.5833	0.3715		
Total	**239**	**159.491**			

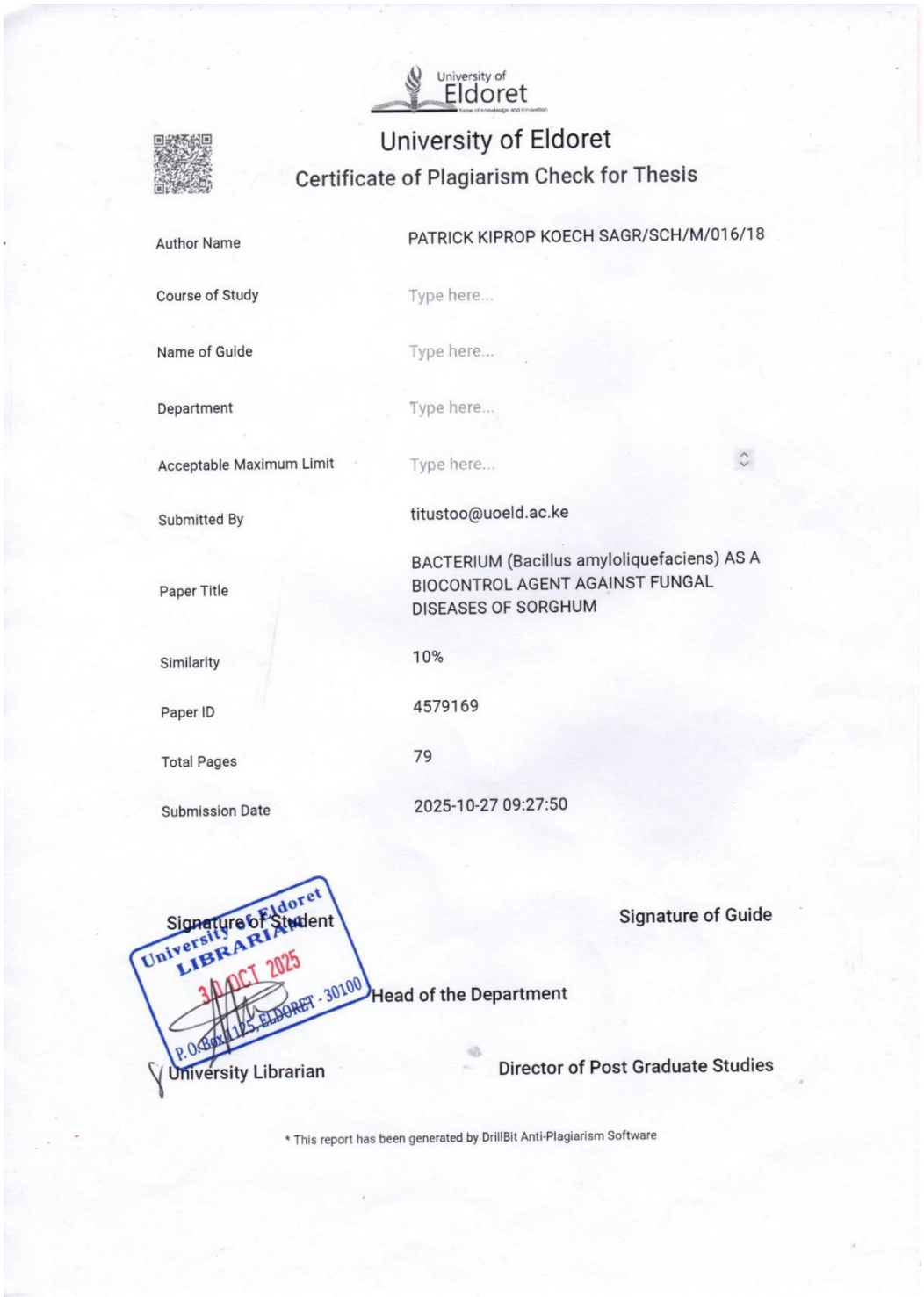
VARIETY	TREATMENT	LONG RAINS		SHORT RAINS		**MEANS**
		KIBOS	SEGA	KIBOS	SEGA	
N57	Bacteria dressed fertilizer	2.3	1.5	2.3	1.2	2.2[a]
	Bacteria dressed seeds	1.7	1.3	1.7	1.3	
	Control	3.0	2.8	4.0	3.2	
	Fungicide dressed seed	2.0	2.2	2.0	2.2	
AV		2.3	2.0	2.5	2.0	
N68	Bacteria dressed fertilizer	2.3	1.2	2.3	1.2	2.1[a]
	Bacteria dressed seeds	1.3	1.5	1.3	1.5	
	Control	3.3	2.7	3.7	2.5	
	Fungicide dressed seed	2.2	2.0	2.3	1.7	
AV		2.3	1.8	2.4	1.7	
Nyadundo 1	Bacteria dressed fertilizer	1.5	1.5	1.5	1.5	2.0[a]
	Bacteria dressed seeds	1.7	1.5	1.8	1.2	
	Control	2.8	2.3	3.8	2.2	
	Fungicide dressed seed	2.3	1.8	2.0	1.8	
AV		2.1	1.8	2.3	1.7	
Nyadundo 2	Bacteria dressed fertilizer	2.0	1.3	2.2	1.5	2.2[a]
	Bacteria dressed seeds	1.8	1.7	1.7	1.5	
	Control	2.7	3.0	3.2	3.2	
	Fungicide dressed seed	2.3	2.2	2.5	2.0	
AV		2.2	2.0	2.4	2.0	
Serena	Bacteria dressed fertilizer	1.8	2.0	2.2	2.2	1.9[a]
	Bacteria dressed seeds	1.7	1.2	2.0	1.7	
	Control	2.0	2.2	2.7	2.8	
	Fungicide dressed seed	1.5	1.7	1.8	1.5	
AV		1.8	1.8	2.2	2.0	
	MEANS	2.1	1.8	2.4	1.9	**2.1**

Appendix II: ANOVA table for in vitro assessments of % inhibition of bacterium on isolated sorghum pathogens

Variate: %_Inhibition_Index

Source of variatio	d.f.	s.s.	m.s.	v.r.	F pr.
DISEASE	3	2494.38	831.46	12.93	<.001
Residual	32	2057.41	64.29		
Total	35	4551.79			

Appendix III: Similarity Report



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