UNIVERSITY FOR DEVELOPMENT STUDIES

EVALUATION OF COWPEA (*Vigna unguiculata L.*) GENOTYPES FOR RESISTANCE TO ANTHRACNOSE DISEASE CAUSED BY *Colletotrichum lindemuthianum* (Sacc. and Magn.)

BY

YAKUBU PAUL SEIDU

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THESIS SUBMITTED TO THE DEPARTMENT OF CROP SCIENCE, FACULTY OF AGRICULTURE, FOOD AND CONSUMER SCIENCES, UNIVERSITY FOR DEVELOPMENT STUDIES, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN CROP SCIENCE

JANUARY, 2025

DECLARATION

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere. Where other sources of information have been used, they have been duly acknowledged.

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Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

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DEDICATION

This work is dedicated to my late father, Daddy Kubadu Nembor.

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ABSTRACT

Anthracnose is a highly detrimental disease in cowpea which causes significant economic impact in cowpea production as well as health related diseases to cowpea consumers worldwide, especially in the sub-Sahara region. The study was conducted to screen twenty cowpea genotypes for resistance to anthracnose disease caused by *Colletotrichum lindemuthianum* under natural conditions. Under laboratory conditions, seeds from twenty cowpea genotypes were initially examined for the presence of seed-borne fungi and their impact on the field emergence of seedlings using the agar plate method. Field experiments were conducted in the 2022 cropping season, employing a randomized complete block design with three replications at two locations in Upper East Region - Ghana. Data on disease incidence and severity were collected. Agronomic and yield parameters, namely emergence percentage, days to 95% pod maturity, plant height at maturity, grain yield, hundred-seed weight, and biomass yield were also assessed. Pathogenicity test was conducted under screen house conditions, involving artificial inoculation on nine genotypes using a fresh culture suspension of *C. lindemuthianum* in a completely randomized design replicated thrice. Results of both field and screen house experiments revealed significant variations among cowpea genotypes in terms of disease severity and incidence. Anthracnose disease had significant impact on cowpea yield with susceptible genotypes, namely IT17K-2024-4 and IT17K-1367-2-1 displaying comparatively low yield. Based on the findings, two lines from the assessed genotypes, namely IT14K-1424-12 and IT14K-2030-2, exhibited significant resistance to anthracnose disease and demonstrated a high grain yield potential exceeding 2.0 t/ha. These are recommended for release to farmers as part of an integrated disease management approach. However, the most susceptible

genotypes such as IT17K-1367-2-1, and IT17K-2024-4, are not recommended for cultivation due to high susceptibility and low yield.

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LIST OF ACRONYMS

ANOVA	*Analysis of Variance*
CV	*Coefficient of Variation*
CL	*Colletotrichum lindemuthianum*
CSIR	*Council for Scientific and Industrial Research*
DAP	*Days after Planting*
D95PM	*Days to 95% Pod Maturity*
DI	*Disease Incidence*
DS	*Disease Severity*
IITA	*International Institute of Tropical Agriculture*
ISTA	*International Seed Testing Association*
LSD	*Least Significant Difference*
PDA	*Potato Dextrose Agar*
SARI	*Savanna Agricultural Research Institute*
SDW	*Sterile Distilled Water*
WHO	*World Health Organization*

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Cowpea (*Vigna unguiculata* (L) Walp), is a major leguminous crop cultivated globally (Weng *et al*., 2017; Molosiwa and Makwala, 2020). Cowpea serves as a crucial source of sustenance for small-scale farmers in developing nations and a valuable cash crop in advanced economies (Conner *et al*., 2020).

In Africa, Nigeria is leading as both the largest producer and consumer of cowpea, responsible for 61% of the continent's total cowpea production and 58% globally followed by Niger, Brazil and United State of America (Horn *et al*., 2022). The primary region for cowpea production on the continent is West Africa, particularly in the dry savanna and semi-arid agro-ecological zones (Abudulai, 2016). Key cowpea-producing nations in Africa include Nigeria, Niger, Senegal, Ghana, Mali, and Burkina Faso, as noted by Langyintuo *et al*. (2003).

In Ghana, cowpea holds a crucial role as a primary crop, and it is extensively grown in the savanna and transitional agroecological regions of the nation (Agyeman *et al*., 2014). In developing countries, cowpea plays a significant role especially to the low income-earners as it produces food as both leaves and grains, green manure, fodder and generates income (Kamara *et al*., 2018). The crop plays a pivotal role in tropical farming systems due to its ability to enhance marginal lands through nitrogen fixation and its use as a cover crop, as mentioned by Abayomi (2016). It has considerable adaptation to high temperatures and drought compared to other crops and at the same time thrives better in sandy, low-organic-matter soils, as highlighted by Singh *et al*. (1997). This makes cowpea the preferable crop for the dry savannah of sub-Sahara Africa (Boukar *et al*., 2013).

Cowpea grain yields when grown on farms in the savanna regions of West Africa are relatively low, often less than 0.5 tons per hectare as compared to its potential yield, exceeding 2.0 tons per hectare (Amoako *et al.*, 2020). The deficit in yield is attributable to various factors, including unavailability of resistant varieties, unfavorable environmental conditions, insect pests, and diseases. Emechebe and Florini (1997) reported that, cowpea anthracnose is one of the economically important fungal diseases that constraints maximum production of cowpea in tropical regions of Africa.

1.2 Problem statement

Cowpea anthracnose has been recognized as significant among biotic factors that limit yield in several cowpea production areas across the globe (Thangamani *et al.*, 2011, Ganesh *et al.*, 2022). In cowpea growing areas within West Africa, anthracnose disease is a widespread and devastating disease that affects all parts of the crop above the ground eventually posing a significant obstacle to the successful cultivation of cowpeas (Enyiukwu *et al.*, 2020).

Anthracnose has a significant impact on cowpea production, the repercussions of this pathogen extend beyond mere crop health, reaching into the realms of yield, seed quality, and ultimately, the marketability of the harvested produce (Ganiyu *et al.*, 2018). The interaction of these factors highlights the urgent need for effective management strategies to mitigate the detrimental effects of this pathogen on cowpea crops. In Ghana the disease is among the economically important ones (Gyasi *et al.*, 2022) since majority of the cultivars available to farmers are susceptible resulting in estimated grain yield losses of up to 100% (Horn and Shimelis, 2020; Lamini *et al.*, 2022).

Also, the use of chemical-based control measures has long been the primary approach to mitigate anthracnose, both during the cultivation phase in the field and in commercial packinghouses post-harvest (Ciofini *et al.*, 2022). Synthetic fungicides have demonstrated their efficacy in preventing and minimizing the damages caused by *Colletotrichum*, the fungus responsible for anthracnose. The effectiveness of synthetic fungicides in controlling anthracnose cannot be understated, offering a practical and efficient solution to a persistent agricultural challenge. However, despite their efficacy, it is essential to acknowledge the associated potential health risks, economic thoughts, and environmental consequences (Ali *et al*., 2016; Bordoh *et al*., 2020). In light of these challenges, the pursuit of sustainable and eco-friendly alternatives become increasingly crucial. The development of anthracnose-resistant cowpea cultivars, combined with other alternatives like biological control agents and cultural methods, presents viable substitutes for chemical treatments. This approach effectively combats anthracnose while aligning with the predominant objectives of sustainable agriculture, fostering harmony between crop protection and environmental conservation.

1.3 Justification

Anthracnose management methods employed are mostly skewed towards the conventional use of synthetic chemicals such as fungicides, although deleterious effects on human health and the environment may be derived (Martinez *et al.*, 2020). In cowpea production areas like Northern Ghana, the crop is extensively cultivated by peasant farmers who lack financial muscles to afford the cost associated with chemical control method, particularly during the growing season as reported by Abudulai (2016). The crop also sustained injury during the administration of the chemicals (Tettey *et al.*, 2018). The severity of these diseases sometimes calls for some farmers in the rural settings of Northern Ghana to use some non-recommended and highly hazardous agrochemicals of which they lack the requisite training in relation to mode of application and safety measures probably resulting in chemical poisoning (Demi and Sicchia, 2021).

The situation at hand is dire and calls for the use of alternative methods that are effective, cheaper, environmentally friendly and socially acceptable. Hence, the fundamental objective of this study is to identify resistant cowpea genotypes to anthracnose disease by way of screening. Evaluation of cowpea genotypes for resistance to disease will provide information for cowpea breeders on those characters of the genotypes that need further improvement to ensure social and environmental safety as well as food security under the rapid human population growth in Africa (Enyiukwu *et al.*, 2014).

1.4 Research objectives

The main goal of this research was to identify cowpea lines with enhanced resistance to anthracnose disease in open-field cultivation. The specific objectives of this study were:

i. to assess the seed health of the twenty cowpea genotypes and the impact on seed germination under field conditions.

ii. to determine the stage at which the anthracnose disease occurs under natural conditions.

iii. to determine the level of resistance to anthracnose disease among the cowpea genotypes and the impact of the disease on yield.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and diversity of cowpea

Cowpea (*Vigna unguiculata* (L.) Walp) is originated in Africa (Nkomo *et al.*, 2021; Osipitan *et al.*, 2021) and has since disseminated across all continents and is currently cultivated in numerous regions of Asia, Europe, the United States, as well as Central and South America (Lonardi *et al.*, 2019), though the exact place of domestication is not well known (Nkomo *et al.*, 2021). The exact place of domestication remains a debate among researchers and scholars, however, the area of cultivated cowpea domestication is located in West, East and Central Africa (Kouam *et al.*, 2012; Boukar *et al.*, 2020). According to Kouam *et al.* (2012) reviews, cowpea was domesticated from its wild progenitor, var. *spontanea*, in a region stretching from Senegal to Eritrea, with the domestication process occurring prior to 1500 BC, as evidenced by the discovery of unmistakable domesticated cowpea seeds in archaeological deposits dating to around 1500 BC in central Africa. It is a century's old human crop (Osipitan *et al.*, 2021; Abebe and Alemayehu, 2022) with wide global distribution, especially in tropical and semi-tropical regions (Molosiwa and Makwala, 2020).

Cowpea is mostly grown in the dry agro-ecologies of the tropics in Latin America, Africa and south Asia (Boukar *et al.*, 2018; Owade *et al.*, 2020; Omomowo and Babalola, 2021). Cowpea and its production play a vital role in the livelihoods of millions of people in the developing countries of the tropical and semi tropical regions (Kouam *et al.*, 2012; Lonardi *et al.*, 2019). Cowpea is well-adapted to arid and semi-arid regions, making it a resilient crop choice in areas prone to drought (Deshpande *et al.*, 2018; Yasin *et al.*, 2021), able to fix atmospheric nitrogen through its root nodules and well suited to intercropping with other crops (Ali and Dov, 2017; Deshpande *et al*., 2018; Molosiwa and Makwala, 2020).

2.2 Taxonomy of cowpea

Cowpea (*Vigna unguiculata* (L.) Walp) is a dicotyledonous self-pollinated plant (Yasin *et al.*, 2021), classified as a diploid legume with a chromosome number of 2n = 2x = 22, is cultivated primarily for its notable seed protein content (Lonardi *et al.*, 2019; Omomowo and Babalola, 2021; Ravelombola *et al.*, 2021). Cowpea holds a vital position among grain legumes, as well as in the context of fodder and cover crops, particularly within the countries of sub-Saharan Africa and belongs to the family *Fabacea*, tribe *Phaseoleae* and genus *Vigna* (Salifou *et al*., 2017; Boukar *et al*., 2018). Cultivated cowpea belonging to the subspecies *unguiculata*, encompasses five distinct cultivar groups (Boukar *et al*., 2018); [*Unguiculata* (domesticated cowpea group); Melanophthalmus and Sesquipedalis (primitive cultivar groups); Textilis and Biflora (wild progenitor groups)] (Kouam *et al.*, 2012).

2.3 Morphology and biology of cowpea

Cowpea is a warm-season crop that thrives in tropical and subtropical regions (Yasin *et al*., 2021) as well as an annual herbaceous plant that grows as a bush, that is typically more erect and compact or a climbing vine with long trailing stems, depending on the variety (Deshpande *et al.*, 2018). The leaves of cowpea are compound with three leaflets, arranged along the stem with variation in size and shape (Pottorff *et al.*, 2012) whereas the flowers are typically white or creamy in color, although some varieties may have purple or pink flowers. Cowpea reproduces through sexual reproduction, with flowers being pollinated by insects and after successful pollination, the flowers develop into pods containing seeds. It is cultivated in a variety of soil types but prefers well-drained, sandy loam soils with good organic matter content.

The growth cycle of cowpea is relatively short depending on the variety and growing conditions, cowpea under cultivation is an annual crop, and its improved varieties can be categorized as extra-early, maturing in just 60 days, early (65–75 days), medium (75–100 days), or late (over 100 days to mature) (Boukar *et al*., 2020), with symbiotic relationship with nitrogen-fixing bacteria (rhizobia) in its root nodules (Kyei-Boahen *et al*., 2017; Ravelombola *et al.*, 2017; Omomowo and Babalola, 2021).

2.4 Cowpea production

Cowpea is a crucial crop in Africa, it contribute significantly to food security and income generation and environmental sustainability (Olajide and Ilori, 2017; Ovalesha *et al*., 2017; Ayala *et al.*, 2020; Omomowo and Babalola, 2021). The primary cowpea-producing nations globally are situated in sub-Saharan Africa, specifically within the Sudano-Sahelian vegetation region (Boukar *et al.*, 2018; Omomowo and Babalola, 2021). Sub-Sahara Africa accounts for about 96% of the world's cowpea production (Osipitan *et al*., 2021; Nkomo *et al.,* 2022), with Nigeria being the largest producer (Boukar *et al.*, 2018; Omomowo and Babalola, 2021; Osipitan *et al.*, 2021) and consumer of cowpea, responsible for 61% of the Africa's production and 58% of the global total production (Nkomo *et al.*, 2021). The other countries such as Niger, Burkina Faso, Mali, Senegal, Cameroon and Ghana also contribute to the bulk production of cowpea in Africa (Kyei-Boahen *et al*., 2017; Boukar *et al.*, 2018; Haruna *et al.*, 2019; Omomowo and Babalola, 2021; Nkomo *et al.*, 2022).

In Ghana, cowpea holds significant importance to nutrition to both rural and poor resource urban households in Ghana as a staple crop (Tettey *et al.*, 2018), and it is extensively cultivated either as intercrop or relay crop in the savanna and transitional agroecological zones (Haruna *et al*., 2019; Tengey *et al*., 2021; Gyasi *et al*., 2022). Cowpea cultivation predominantly takes place in the dry savanna zones of Ghana characterized by a single rainy season (unimodal), with an annual rainfall range of 500 to 1200 millimeters (Lamini *et al.*, 2022; Karikari *et al.*, 2023). Cowpea cultivation is widespread throughout Ghana, covering various regions of the country, with the Northern Plains standing out as the primary hub for cowpea production, and this substantial yield in the Northern Plains greatly enriches Ghana's agricultural landscape (Herniter *et al.*, 2019).

Cowpea, a drought-tolerant crop well-suited for arid regions like Ghana's dry savannah areas, plays a crucial role in food security and income generation, particularly in the Northern and Volta regions (Asare-Bediako *et al.*, 2018; Tettey *et al.*, 2018). In Ghana, cowpea and groundnut are recognized as the two most important edible legumes (Agyekum *et al.*, 2023), with cowpea being the second most important legume crop after groundnut (Addy *et al.*, 2020; Akpo *et al.*, 2021; Lamini *et al.*, 2022). Northern Ghana, specifically, contributes around 85% of the nation's grain cowpea production; nevertheless, this output falls short of satisfying the country's overall demand (Karikari *et al.*, 2023).

Cowpeas play a vital role in Ghana by providing sustenance for both humans and livestock, while also serving as a valuable source of income for numerous farmers and grain traders (Addy *et al.*, 2020). Aside constraints like limited improved varieties, late maturity, pests and diseases susceptibility, low yields in cowpea production in the northern Ghana, poor seed quality, and germination issues are other challenges encountered by farmers in the production of cowpea (Karikari *et al.*, 2023).

2.5 Seed health test

Quality seeds are the most basic and important resources in the global sustainable agriculture (Caverzan *et al.*, 2018; Dadlani and Yadava, 2023), with their physiological condition being instrumental in crop establishment and productivity (Bagateli *et al.*, 2019; Moreno *et al.*, 2022). High-quality seeds yield uniform, productive seedlings, while poor-quality seeds hamper emergence and result in uneven crop growth, reducing yields (Caverzan *et al.*, 2018; Ebone *et al.*, 2020; Moreno *et al.*, 2022). Seed quality parameters encompass physical attributes, performance-related physiological qualities, genetic traits specific to seed varieties, and the health status regarding diseases in a seed lot (Dadlani and Yadava, 2023). The seed quality depends on the parent crop's genes, which affect how the crop responds to various biotic and abiotic factors and stresses, ultimately influencing its potential for yield (Njonjo *et al.*, 2019).

Healthy seeds are essential for robust crops, and their quality is assessed by detecting insect infestation and seed-borne diseases, which can contaminate disease-free regions and spread new ailments (Vishunavat *et al.*, 2023). Seed health is crucial for quality, as disease-free seeds are key to controlling 30% of seed-borne diseases unresponsive to fungicides or resistant varieties, safeguarding crops amid the rising challenges in seed trade (Vishunavat *et al.*, 2023), and seed health testing can pinpoint the types and quantities of pests and diseases within the seeds (Zhang *et al.*, 2023). Seeds are recognized as potential carriers of pathogens (Kumar *et al.*, 2021). Seeds transmit pathogens to both seedlings and mature plants, resulting in diseases, and since numerous diseases are seed-borne (Zhang *et al.*, 2023), conducting seed health testing plays a significant role in identifying the pathogens carried by seeds (Suhendar *et al.*, 2023).

2.6 Uses of cowpea

Cowpea crop plays a pivotal role globally in ensuring food security and population health due to its significant nutritional and nutraceutical attributes (Abebe and Alemayehu, 2022). The crop is a good source of plant protein, carbohydrates, vitamins, and essential nutrients for millions of people (Nkomo *et al.*, 2021). According to

Haruna *et al*. (2019) reviews, cowpea significantly contributes to food security with its high nutritional content, featuring 23-30% protein, 50-67% carbohydrates, 1.9% fat, 6.35% fiber, and essential B-vitamins and micronutrients, enhancing human nutrition and health.

In Africa, 52% of cowpea production is allocated for human consumption, 13% for animal feed, 10% for seed production, 9% for various other uses, and unfortunately, 16% goes to waste (Smale *et al.*, 2022). Cowpea in Ghana serves dual roles, primarily as a staple for household consumption and a cash crop, being cultivated for both leaves and seeds, catering to the needs of both humans and livestock (Haruna *et al.*, 2019). Cowpea plays a crucial role in livestock production, with its leaves and vines being dried and utilized as valuable fodder and feed supplements in livestock husbandry (Abebe and Alemayehu, 2022).

Cowpea is undeniably a multi-faceted crop, serving as a source of income for millions of smallholder farmers and also benefiting traders who profit from the sale of this nutritious grain (Omomowo and Babalola, 2021). Cowpea also contributes to soil fertility through nitrogen fixation and addition of phosphorus (Rego *et al.,* 2015; Nkomo *et al*., 2021), serving as a cover crop to control soil erosion and as a green manure crop (Ovalesha *et al.*, 2017), making it a valuable component of crop rotation systems. During cowpea growth and development, the average nitrogen contribution to the soil typically ranged from 40–80 kg $N.ha^{-1}$ and can occasionally reach as high as 200 kg $N.ha^{-1}$ (Meena *et al.*, 2015).

2.7 Common pests and diseases of cowpea

Cowpea production like other crops experience a number of biotic and abiotic stresses, biotic stresses are those caused by living organisms, such as pests and diseases, while abiotic stresses are non-living factors such as drought, salinity, temperature, soil infertility (Banla *et al*., 2018; Lobulu *et al.*, 2019; Baoua *et al.*, 2021; Osipitan *et al.*, 2021; Addae-Frimpomaah *et al.*, 2022). The effects of these biotic and abiotic stresses depend on the type and the degree of stress applied, the severity of pests and diseases, and the plant organs (Jayawardhane *et al.*, 2022; Karikari *et al.*, 2023). Globally, biotic stressors such as root and membrane pathogens significantly contribute to low agricultural productivity, poor-quality produce, and widespread food insecurity, resulting in substantial global monetary losses due to reduced crop yields (Savary *et al.*, 2019). The primary constraints on cowpea productivity due to biotic stress factors encompass a variety of organisms, including destructive pests, parasitic weeds, viral pathogens, bacterial pathogens, and fungal pathogens (Boukar *et al*., 2018; Baoua *et al*., 2021).

Insects pose a significant and formidable challenge to cowpea production due to it occurrence at pre flowering, post flowering, storage stages (Soulleymane *et al*., 2013; Mekonnen *et al.*, 2022), with the potential to cause complete yield losses of up to 100% in cases of severe infestations, particularly in the absence of effective control measures (Dhakal, 2019; Togola *et al*., 2017, 2023). Cowpea cultivation is often plagued by various common pests that can significantly impact crop yields (Omoigui *et al*., 2017; Karikari *et al*., 2023).

Approximately 21 insect species hold economic significance and are consistently found in cowpea-producing regions across the globe (Oyewale and Bamaiyi, 2013; Lal Choudhary *et al.*, 2017; Dhakal *et al.*, 2021; Togola *et al.*, 2023). The most prevalent and destructive insect species include the legume pod borer, *Maruca vitrata* Fabricius, the cowpea aphid, *Aphis craccivora* Koch., the flower bud thrips, *Megalurothrips sjostedti* Trybom, the pod-piercing bugs, *Clavigralla tomentosicollis* Stål, and the cowpea weevil *Callosobruchus maculatus* Fabricius (Oyewale and Bamaiyi, 2013; Lal

Choudhary *et al.*, 2017;; Dhakal *et al.*, 2019; Togola *et al.*, 2017, 2020, 2023). Cowpea is susceptible to various pests, including aphids, maruca (a pod-boring insect), and pod-sucking bugs, which collectively posed significant challenges (Omoigui *et al.*, 2017), Bacterial pathogens, particularly those from the Xanthomonas genus such *X. axonopodis* pv. *Vignicola,* causing bacterial blight, poses a significant constraint on cowpea yields, leading to substantial losses exceeding 70% in seed grain, pod, and fodder production (Shi *et al.*, 2016; Durojaye *et al.*, 2019; Omomowo and Babalola, 2021). Symptoms include water-soaked lesions on leaves, wilting, and necrosis. Lesions may have a V-shaped appearance and is spread through infected seeds, contaminated equipment, and water (Shi *et al*., 2016).

Bacterial blight can be controlled by planting disease-resistant cowpea varieties, crop rotation, seed treatment, and application of copper-based bactericides (Sundin *et al*., 2016). Root-knot nematodes are a significant cause of losses in cowpea production (Dareus *et al.*, 2021), hindering improvements by obstructing water and nutrient uptake and interfering with cell differentiation and auxin transportation pathways, with *Meloidogyne javanica* and *Meloidogyne incognita* being the predominant nematodes devastating cowpea (Oliveira *et al.*, 2012). *M. enterolobii* in recent times has emerged as a root-knot nematode (RKN) species with the ability to overcome resistance in various crops and cultivars that were previously considered resistant to RKN (Brito *et al.*, 2020; Dareus *et al.*, 2021).

Viral pathogens can substantially reduce cowpea yields, with some causing complete losses by decreasing essential Rhizobium populations for root nodulation (Taiwo *et al.,* 2014; Nsa and Kareem, 2015). Viral pathogens globally impact cowpea productivity negatively, with the highly destructive *cowpea aphid-borne mosaic virus* (CABMV) genus *Potyvirus*, cowpea mild mottle virus (CPMMV), and cowpea yellow mosaic virus (CYMV) genus *Comovirus* (Taiwo *et al*., 2014; Odedara and Kumar, 2017; Omomowo and Babalola, 2021).

Other viral diseases of cowpea prevalent in Ghana reported by Tettey *et al*. (2018) include; southern bean mosaic virus (SBMV), blackeye cowpea mosaic virus (BICMV) and bean common mosaic virus (BICM). Parasitic weeds severely threaten cowpea production, causing significant yield losses, and are challenging to eliminate due to their long dormancy in the soil (Omoigui *et al.*, 2017). *Striga gesnerioides* and *Alectra vogelii* are key hindrances to enhancing cowpea production in Africa (Omoigui *et al.*, 2017; Omomowo and Babalola, 2021).

Fungal pathogens are globally destructive, affecting crops in the field and post-harvest, causing up to 100% cowpea production loss (Fisher *et al.*, 2012; Omomowo and Babalola, 2021). Notable pathogens include *Rhizoctonia solani*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Sclerotium rolfsii* and *Colletotrichum* spp causing stem rot, fusarium wilt, macrophomina root rot, Southern blight disease and cowpea anthracnose (Pottorff *et al.*, 2014; Omomowo and Babalola, 2021). The growth and sporulation of many fungal pathogens is favored by high humidity, and warm temperature conditions (Emechebe and Florini, 1997).

2.8 Anthracnose disease of cowpea

Anthracnose disease is a significant fungal infection that affects cowpea, a widely cultivated legume crop in many parts of the world. Anthracnose, caused by a variety of fungal pathogens, exerts a detrimental influence on crop health and success, encompassing yield reduction, compromised seed quality, and diminished marketability (Ganiyu *et al.*, 2018). It is caused by the fungus *Colletotrichum* spp., specifically (*C. lindemuthianum)* which are the main pathogens associated with anthracnose in cowpea (Sawicka *et al.*, 2019). *Colletotrichum* spp. are among the world's top 10 most destructive plant pathogens, inflicting substantial economic losses in the agro-produce industry (Guarnaccia *et al.*, 2019; Enyiukwu *et al.*, 2020). Symptoms of anthracnose in cowpea typically appear on the leaves, stems, pods, and seeds of the plant (Cannon *et al.*, 2012). Anthracnose is favored by warm and humid conditions, making it prevalent in tropical and subtropical regions (Kamle and Kumar, 2016; Salotti *et al.*, 2022). The fungus can persist in infected plant debris and can also spread via seeds, while pathogen dispersal is enhanced by wind, rain, and the movement of infected plant material (Sawicka *et al.*, 2019).

2.9 Pathogenesis of anthracnose of cowpea

The anthracnose pathogens primarily enter the cowpea plant through natural openings such as stomata or wounds (Sawicka *et al.*, 2019; Li *et al.*, 2020; Batzer *et al.*, 2022). By forming specialized infection structures called appressoria (Li *et al.*, 2020), these fungi can penetrate host tissues, subsequently colonizing intercellular spaces, leading to tissue damage and disease symptoms (Thilini *et al.*, 2021).

2.10 Symptoms of anthracnose disease of cowpea

Anthracnose lesions on cowpea leaves initially appear as small, water-soaked spots that gradually enlarge and change from dark brown to black lesions (Falade, 2016; Ganiyu *et al.*, 2018). Seedling stems exhibit rust-colored flecks, while bean pods display circular to irregular, sunken lesions with tan to rust coloration, brown or purple borders, and seed coat browning or blackening, sometimes resulting in pod drying and poor filling during severe outbreaks (Dell'Olmo *et al.*, 2023).

2.11 Field and controlled environment inoculation methods in screening cowpea for anthracnose resistance

Field-based and controlled environment inoculation techniques are of utmost importance when assessing cowpea for its resistance to anthracnose. They play a crucial role in gauging how cowpea varieties perform in authentic agricultural settings, allowing the identification and subsequent selection of genotypes that exhibit resistance. The identified and most frequent used inoculation techniques in screening for anthracnose resistance crops or plants including spraying a spore suspension on seedling leaves (Miller-Butler *et al.*, 2018; Correa *et al.*, 2021), injecting a spore suspension into stems, and wrapping wounded seedling stems with inoculum meal (Adebitan *et al.*, 1992; Koima *et al.*, 2023).

2.12 Anthracnose pathogen

Cowpea's low productivity results from susceptibility to numerous pests and diseases, with the most devastating being anthracnose by *Colletotrichum lindemuthianum*, a cosmopolitan seed borne disease (Misal *et al.*, 2019; Sharma *et al.*, 2021), a hemi biotrophic fungus often causing complete crop loss (Pradhan *et al.*, 2018), and frequent in tropical and sub-tropical regions, especially in cool, humid conditions (Sharma *et al.*, 2021). The pathogen's seed-borne nature and widespread distribution pose challenges to its management, particularly when farmers repeatedly use their own seed for cultivation (Sharma *et al.*, 2021). *C. lindemuthianum*, stands as a significant threat to cowpea yield, seed quality, and marketability, with the potential for up to 95-100% yield loss when infected seeds are employed for cultivation under favorable weather conditions during the crop cycle (Ganiyu *et al*., 2018; Sujata *et al*., 2021).

Cowpea anthracnose disease, induced by the fungus *C. lindemuthianum*, is a significant fungal ailment affecting field-grown cowpea in Nigeria, with the potential to cause substantial yield reductions of up to 75% (Enyiukwu *et al.*, 2014; Falade, 2016). Fungal spore development displays a biphasic behavior, encompassing both saprophytic and biotrophic lifestyles, leading to the classification of the fungus as a hemibiotrophic (Mohammed, 2013). *C. lindemuthianum* is a filamentous fungus primarily undergoing asexual reproduction, with its sexual phase termed *Glomerella lindemuthiana*, and additional mechanisms for genetic material transfer, like the parasexual cycle, have been documented in the literature for this pathogen (Pinto *et al.*, 2012). Cowpea susceptibility to *C. lindemuthianum* persists from seedling to maturity, dependent on favorable environmental conditions for disease initiation and progression (Padder and Sharma, 2011).

2.13 Control of anthracnose disease

Plant disease management involves various methods (Modi and Tiwari, 2020), effective management of anthracnose in cowpea involves cultural practices (clean seeds and fields), host resistance, botanicals and chemicals with rotation, sanitation, and resistant cultivars key for reducing the disease (Enyiukwu *et al.*, 2014; Ganiyu *et al.*, 2018; Omoigui *et al*., 2018; Batzer *et al.*, 2022; Talekar, 2023). Combining these approaches can effectively control cowpea anthracnose (Modi and Tiwari, 2020).

2.13.1 Cultural control

This method seeks to limit the introduction of pathogen inoculum from neighboring disease-infected fields, reduce infection rates, and create unfavorable conditions for disease spread and development (Mohammed, 2013). Some cultural practices used to manage legume anthracnose include; crop rotation with a non-host crop where crops are rotated with non-host crops to break the disease cycle and avoiding the planting of legumes in the same field consecutively, as the fungus may persist in the soil (Vazin, 2015).

Adjusting planting times of legumes can significantly contribute to avoid conditions favorable for anthracnose development. For example, planting during periods of high humidity and warm temperatures may increase the risk of fungal infection (Alkemade *et al*., 2022; Salotti *et al*., 2022). Proper field sanitation can also be maintained by removing and destroying infected plant debris (Enyiukwu *et al*., 2021) to reduce the source of inoculum for the next growing season (Pandey *et al*., 2023).

Bean anthracnose is initiated by a seed-borne pathogen that can persist in varying soil conditions of dry and wet cycles (Tsedaley, 2015). Planting of healthy seeds, monitoring for early detection of anthracnose symptoms and expediting actions at the earliest sign of infection allows for more effective management strategies (Alkemade *et al*., 2022).

2.13.2 Chemical control

The widespread adoption of chemical treatment, driven by advances in chemical formulations, encompasses the application of fungicides, insecticides, nematicides, and rodenticides to seeds, effectively safeguarding seeds and emerging seedlings from diseases and strengthening crop health (Pandey *et al*., 2018; Lamichhane *et al.*, 2020). The widespread use of synthetic fungicides is the primary and widely accepted method for promptly and reliably controlling fungal diseases in legumes, thereby protecting crop yields from disease-related losses (Pandey *et al.*, 2018).

Chemical management strategies in agriculture involve the application of both protectant and systemic fungicides through various methods, including seed, soil, and foliar applications (Misal *et al*., 2019; Lamichhane *et al*., 2020; Pandey *et al*., 2018, 2023). Systemic fungicides are used either before or during disease development, whereas protectant fungicides are applied preventively before or at the onset of the disease (Pandey *et al*., 2023). Legume anthracnose is typically managed through the application of a combination of fungicides specially formulated to retard its progression, including Azoxystrobin, methyl benzimidazole carbamate fungicides such as carbendazim and thiophanate-methyl (Misal *et al*., 2019; Chatak and Banyal, 2020; Kumar *et al*., 2020; Pandey *et al*., 2023). Nonetheless, the utilization of chemicals for management of anthracnose has adverse effects on both soil health and the environment and continuous use leads to issues such as pathogen resistance and food contamination as well (Pinto *et al.*, 2012; Sharma *et al.*, 2021).

2.13.3 Genetic resistance

Developing anthracnose-resistant legume cultivars is the most cost-effective and efficient long-term strategy for mitigating economic losses caused by the disease. Screening legume germplasm for anthracnose resistance involves diverse methods, including detached leaves, artificial inoculations in controlled environments like screenhouses, and evaluations in natural disease conditions; field screening is particularly valuable in high disease-pressure regions, while artificial inoculation with virulent pathogen isolates can provide valuable insights (Pandey *et al*., 2023).

Cowpea varieties with bush growth habit display diverse degrees of anthracnose resistance, whereas those with climbing or vining growth habit are considerably more susceptible to the anthracnose disease (Pradhan *et al.*, 2018). Cowpea anthracnose resistance sources have been identified, with the primary reservoirs of anthracnose resistance in cowpea being predominantly located in Nigeria (Amusa et al., 1994; Pandey *et al*., 2023). Cowpea variety VBN 3 (VCP 09-013), Arimbra Local (trailing-type vegetable cowpea) Kanakamony (bush-type cultivar) have been reported to possess resistance to anthracnose disease caused by *C. lindemuthianum* (Shiny *et al.*, 2015; Ganesh *et al.*, 2022).

2.13.4 Integrated disease management

Integrated Disease Management (IDM) is a multifaceted approach that combines various strategies, including crop rotation, site preparation, resistant cultivars, altered planting practices, environmental adjustments, and, when needed, targeted fungicides use (Dania and Gbadamosi, 2019; Forghani and Hajihassani, 2020; Deguine *et al.*, 2021; Sikandar *et al.*, 2023). Planting cowpea varieties that demonstrate resistance or tolerance to anthracnose is a key component of IDM, as it reduces the likelihood of disease development (Ahmad *et al*., 2018).

Cowpea crops can be rotated with non-host plants to disrupt the disease cycle. This helps in reducing the buildup of anthracnose inoculum in the soil (Chatak and Banyal, 2020). When necessary, apply fungicides following recommended guidelines. Fungicides can be used as a preventive or curative measure, but their application should be based on disease severity and the specific stage of crop development.(Chatak and Banyal, 2020; He *et al*., 2021). Incorporating organic matter into the soil can be a good measure, as it can enhance soil health and microbial activity. Healthy soils contribute to a more robust plant defense system against diseases (Ravelombola *et al*., 2021).

IDM also involves monitoring environmental conditions, disease forecasting, and setting economic thresholds, all within the framework of an ecologically and economically mindful pathogen control strategy (Scortichini, 2022). An integrated approach, combining chemical and non-chemical methods, incorporating fungicides, botanical treatments, and bio-agents, was executed in field conditions to manage cowpea anthracnose caused by *C. lindemuthianum*, resulting in a notable reduction in the disease intensity under these field conditions (Ahmad *et al.*, 2018; Dania and Gbadamosi, 2019).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Source of planting materials

A total of twenty (20) early maturing cowpea genotypes, were used for the study. The seeds were sourced from the Cowpea Improvement Program of the CSIR-Savanna Agricultural Research Institute (SARI), Nyankpala as shown in **Error! Reference source not found.**

Table 3.1: Cowpea genotypes for assessment in an open field under rain-fed

No.	Genotype	Testa color	No.	Genotype	Testa color
1	IT17K-1704-5	White	11	Padiyuya	White
2	IT17K-1707-2-2	White	12	IT17K-1403-1-1	White
3	IT16K-1970-1	White	13	Kirkhouse-Benga	White
4	IT17K-2024-4	Brown	14	IT10K-837-1	White
5	IT14K-1424-12	White	15	IT16K-1966-1	White
6	KVX782-1	White	16	SONGOTRA	White
7	IT14K-2030-2	White	17	IT17K-1802-1	White
8	Wangkae	White	18	IT17K-1367-2-2	White
9	IT17K-1809-4	White	19	IT17K-1095-2-2	White
10	IT17K-849-2-1	White	20	MOUSA +1	White

3.2 Seed health test

3.2.1 Sample collection

Cowpea genotypes samples were collected from CSIR-SARI and transported to the Spanish laboratory complex, University for Development Studies (UDS), Nyankpala where seed health test was conducted in the microbiology laboratory using a Completely Randomized Design layout as per the procedure adopted by Baysah (2013). Four hundred and fifty (450) seeds were randomly taken from each cowpea genotype as a working sample.

3.2.2 Seed treatment and medium preparation

Seed borne fungi were detected using the agar plate method. As adopted by (Narasimha *et al*., 2022), the collected cowpea seeds were treated with a 10% sodium hypochlorite solution for 1 minute. Seeds were then rinsed three times with Sterile Distilled Water (SDW) and blotted dry on sterile tissue papers in accordance with Vazin (2015). As described by Ekhuemelo *et al*. (2019), Potato Dextrose Agar (PDA) was prepared by adding 39 g in 1.0 L SDW in a conical flask. The flask, containing the prepared medium, was autoclaved at 121 °C for 15 minutes at a pressure of 15 psi. The medium was then allowed to cool. Streptomycin Sulphate was added at the rate of 0.2 g/L to inhibit bacterial growth. The prepared medium was poured on to sterilized Petri dishes and allowed to solidify.

3.2.3 Setup of Petri dishes

Following the procedure used by Khare *et al*. (2016) three hundred (300) seeds, fifteen randomly selected from each genotype, was used for the seed health test. Five disinfected seeds were placed at equal distance on each 9 cm PDA Petri dish. The treatment was replicated thrice to give a total of sixty (60) experimental units.

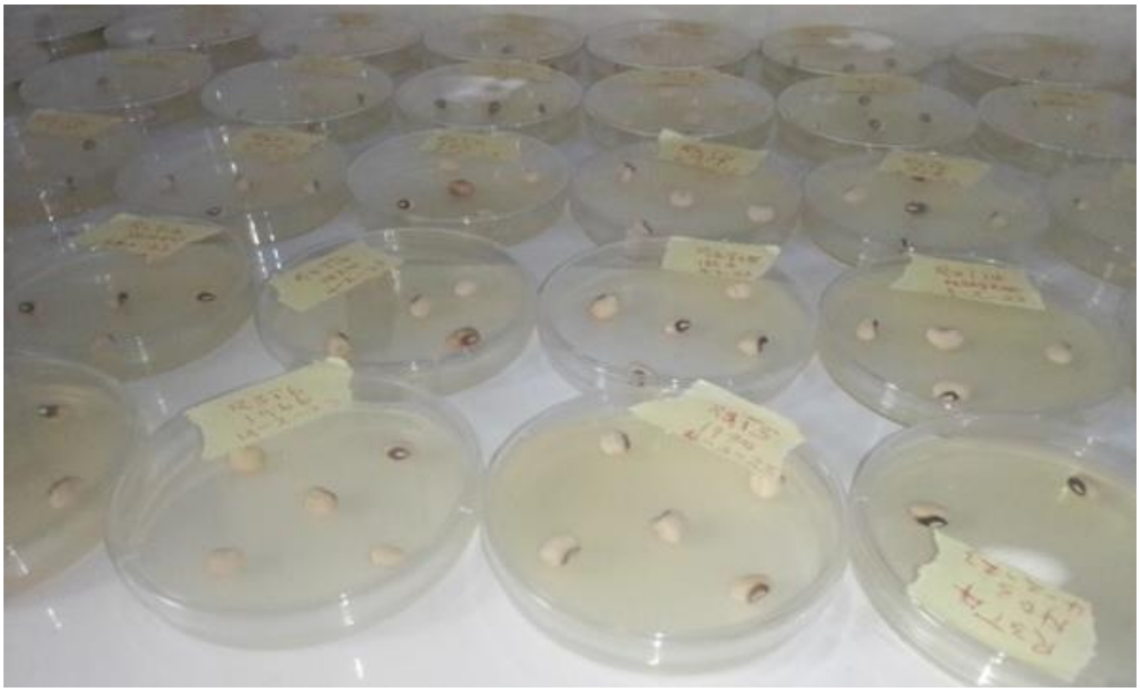

Figure 3.1 Disinfected cowpea seeds plated on culture medium (PDA) for incubation

3.2.4 Incubation

The plated seeds were incubated for seven days in the laboratory at room temperature under continuous artificial light for 12 hours and 12 hours in the dark according to the method adopted by Bolanle *et al.* (2019).

3.2.5 Pathogen growth observation

During the incubation period, the seeds were observed on daily basis for any signs of pathogen growth.

3.2.6 Subculturing and pathogen identification

After seven days of incubation, pure cultures were subsequently, obtained from the fungal colonies that grew around each seed on potato dextrose agar (PDA) for identification of the various fungal species. Seven days period was again allowed for the pure culture to mature. Little portion of the hyphae containing spores was then taken from pure culture using a sterile needle and placed on sterile glass slide stained with lactophenol blue and examined under the microscope for fungal structures as adopted by Vazin (2015). Identification was based on colony characteristics, morphology of fruiting bodies and spores using a compound microscope and compared with reference manual per the procedure followed by Tsedaley (2015).

3.2.7 Data collection on seed health test

Incubated seeds in each Petri dish were examined for the presence of various fungi species. The degree of infection on each genotype seed was assessed using the equation: Fungal Infection (%) = (total incidence / total number of tested seeds) x 100, as per the methodology outlined by Mahmoud *et al*. (2013).

3.3 Description of field study area

The field study was conducted in two locations in the Sudan savannah agro-ecological zone of Ghana in 2022 cropping season. Tilli (N 10°51'0.23436", W 0°34'4.26756) and Gumyoko (N 10°59'16.64916, W 0°20'22.7562) fall within the Sudan Savanna Agro-ecological zone and are characterized with sandy loam soil types as stated by Kusi *et al*. (2019). Both places experience a single-peaked rainfall trend starting in May and concluding in October. Average rainfall at Gumyoko in 2022 was 996.4 mm, while that of Tilli in the same year was 949 mm (SARI, 2022).

3.4 Experimental design

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replicates consisting of three blocks each with twenty plots of 4 m x 2 m ($8m^2$) (Okeleye *et al*., 1998). Each plot consists of four rows of cowpea plants.

3.5 Field layout and planting

Planting was done in July to allow the cowpea genotypes to get exposed to the peak of rainfall which builds up the incidence of fungal diseases (Conner *et al.*, 2020). As described by Ajeigbe *et al*. (2008), two seeds were sown per stand at a planting distance of 20 cm × 60 cm. The plots were separated by 1 m alley and replicates separated by 1.5 m alleys (Baidoo and Mochiah, 2014). Total field size was 70 m x 20 m (0.14 Ha).

3.6 Crop management

A pre-emergence herbicide, Pendimethalin, was applied at the rate of 1 kg /ha to protect the crop from germinating weeds, which are killed by the herbicide in accordance with Amoako *et al.* (2020). Seven days after planting, Supplying was carried out making sure there were two plants per stand (Ibrahim, 2010). Manual weeding was done at two weeks after planting using hoe, and subsequent weed control carried out when necessary to ensure a clean farm.

In order to boost vegetative growth at the early stages, NPK fertilizer 15-15-15 was applied at the rate of 100 kg/ha after two weeks of planting. Insecticide, LION EC (Lambda-cyhalothrin 15g/L and 300g/L dimethoate) was applied at vegetative and flower initiation stages based on the manufacturer's recommended rate of 35 mL per 15 L of water using a knapsack sprayer according to Kusi *et al*. (2019).

3.7 Data collection

Data were collected on; emergence percentage, disease incidence and severity, plant height at maturity, days to 95% pod maturity, 100 seed weight, grain yield per hectare and plant biomass yield.

3.7.1 Percentage emergence of seedlings

After five days of planting, the number of seedlings emerged on each plot was determined and then expressed as a percentage (Amoako *et al.*, 2020).

% Emergence = (Number of seedlings emerged)/ (Total number of seeds planted) × 100

3.7.2 Disease incidence (DI)

The natural field environment engineered the pathogen to build up for fungal infection (Enyiukwu *et al.*, 2014). As reported by Asare-Bediako *et al*. (2018), disease incidence for anthracnose was assessed by counting the number of infected plants in relation to the total number of plants per plot expressed as a percentage at 5, 8 and 11WAP corresponding to vegetative, flowering and pod stages.

%DI = (Number of infected plants)/ (Total number of plants per plot) × 100%

3.7.3 Severity of disease per plot (DS)

Disease severity (DS) for anthracnose was taken at 5, 8 and 11 weeks after planting corresponding to pre-flowering, flowering and pod stages, according to Eno *et al*. (2016) study. Assessment of severity was done by considering different plant parts which encompassed; leaf, peduncle, stem and pod of eighty plants per plot (Narasimha *et al.,* 2022).

The plant parts were visually assessed and scored according to the 0-5 severity scale based on the percentage covered by necrotic lesions on the plant organ as adopted by Khare *et al*. (2016). In this approach, the scale used was; 0-No visible anthracnose symptoms, 1-few discrete non-coalescing lesions on the leaf surface, 2-many lesions on the leaf surface occasionally coalescing, 3-coalescing lesions on the leaf surface that are continuous more than 40% but less than 61%, 4-coalescing lesions on the leaf surface that are continuous on more than 60% but less than 81%, 5-collapse of affected part, fall of leaflet, buckling or fall of petiole, death of stem.

As adopted by Eno *et al*. (2016), data collected on each replication were pooled and the average of the three replications taken. Disease severity was expressed using the formula described by Eno *et al.* (2016) as follows:

DS= (Sum of individual scores)/ (Total No. of plant examined)

3.7.4 Anthracnose disease resistance rating

The reaction of the Cowpea genotypes to anthracnose disease was evaluated using a modified rating scale adopted from Khare *et al*. (2016). 0.0-1.4= highly resistant, 1.5-

2.4 = moderately resistant, 2.5-3.0 = moderately susceptible, more than 3.0 = highly susceptible.

3.7.5 Days to 95% pod maturity

This was conducted by recording per plot, the number of days cowpea genotype takes from planting to when 95% of them attain harvest maturity as adopted by (Sakariyawo *et al*., 2016).

3.7.6 Plant height and plant biomass

As adopted by Ansoba (2016), plant height was measured at harvest maturity. In this approach, the respective heights of eighty plants were taken from the base of the plant to the tip using a metallic measuring tape. Then, the average for each cowpea genotype determined. At harvest, the biomass of eighty plants of each genotype in all the replications excluding the roots was taken using a balance, after oven-dried at 65 °C for 72 hours (Abdou, 2021).

3.7.7 Grain yield per hectare

The pods obtained from the plants in the two central rows after harvesting were threshed. Their grains were dried under optimal moisture conditions at 65 °C for 72 hours. The dry weights were taken and then extrapolated to estimate the grain yield per hectare using the procedure specified in Baysah (2013) guidelines.

Grain yield (kg/ha) = [Grain yield (kg)] / [Harvested Area(m^2)] × 10000m^2

3.7.8 Hundred seed weight

As per the method applied by Nkoana *et al*. (2019), all the pods were threshed on plot basis and their seeds removed. The seeds from each treatment were then put in brown envelopes and oven dried for 72 hours at 65 °C. One hundred (100) seeds were counted from each envelope in the laboratory and weighed on an electronic balance and the weights recorded in grams.

3.8 Isolation and identification of fungi from anthracnose infected field plants

3.8.1 Field observation of infected leaves

Following regular field observation, disease lesions on cowpea leaves were seen with symptoms similar to those reported by Muimba-Kankolongo (2018). In effect, rust-colored specks appear on petioles, leaves, and leaf veins showing brick-red to yellowish or black lesions (Figure 3.2). The infected cowpea genotypes were observed to possess symptoms of anthracnose per the description by Muimba-Kankolongo (2018).

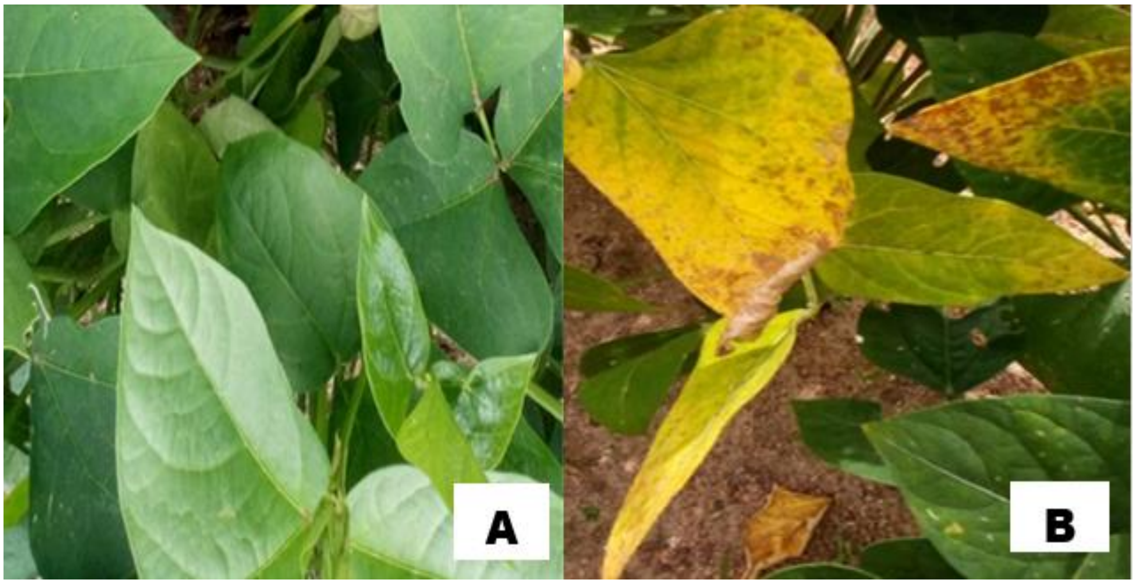

Figure 3.2: **A.** Healthy (uninfected) cowpea growing in the field. **B.** Infected cowpea plant observed to possess symptoms of anthracnose (yellowish or black irregular lesions on leaves of the crop)

3.8.2 Sample collection

In August 2022, leaves samples of infected plants with more than three anthracnose lesions, were collected in a zigzag manner from the above-ground parts viz; leaves, stems and pods of sampled cowpea genotypes in both locations following the procedure adopted by Hamim *et al*. (2014). The samples were placed in hermetically sealed bags and transported to the Spanish Microbiology Laboratory of the University for Development Studies, Nyankpala where they were stored at 4 °C until further use.

3.8.3 Isolation of fungi from cowpea plants

The laboratory procedures for isolating fungi from field samples were conducted at the University for Development Studies (UDS) in the Microbiology Laboratory of the Spanish Laboratory.

Samples from organs and the plant tissues were first washed in sterile distilled water and surface sterilized in 10% Sodium hypo chloride for one minute and then in 70% alcohol each for one minute (Narasimha *et al.*, 2022). Small sections (3-5mm) were cut from the edges of infected parts to contain both diseased and healthy tissues (Zainab and Shinkafi, 2016). The tissues were surface-sterilized for 1 minute in 10% Sodium hypo chloride solution after which they were rinsed in three changes of Sterile Distilled Water (SDW) and blotted dry on sterile tissue papers (Rana *et al.*, 2013).

Potato Dextrose Agar (PDA) was prepared per the procedure adopted by Ekhuemelo *et al*. (2019). The cut sections with the lesions were carefully placed on the culture medium under aseptic condition in a lamina flow. The plates were then incubated on the laboratory bench at ambient conditions of light and temperature (30± 2^{o}C) for three to seven days. Pure culture was obtained by sub culturing unto fresh PDA plates. Microscopic examination was done by examining the spore characteristics.

3.8.4 Identification of fungi isolates obtained from cowpea plants

Little portion of the hyphae containing spores was taken using a sterile needle and placed on sterile glass slide stained with lactophenol cotton blue, examined under the microscope for fungal structures and compared with reference manual as per procedure adopted by Bolanle *et al*. (2019).

3.9 Pathogenicity test of the organism associated with anthracnose disease

Pathogenicity test was studied in pot culture by artificial inoculation under screen house conditions using the isolated fungi in accordance with Koch's postulate as per a method adopted by (Thio *et al*., 2017). A sample of both resistant and susceptible cowpea genotypes seen in the field experiment was subjected to this test under screenhouse conditions to confirm the cause of the disease.

3.9.1 Sources of seeds and experimental site

Genotypes subjected to the pathogenicity test were selected from the sample used for the previous field trial, obtained from the Cowpea Improvement Program at CSIR-Savanna Agricultural Research Institute, Nyankpala. Genotypes were selected based on their level of resistance seen in the field experiment into; highly resistant, moderately resistant, moderately susceptible and highly susceptible as shown in **Error! Reference source not found.**

Table 3.2: List of selected genotypes for pathogenicity test

Genotypes/ Level of Resistance			
Highly resistant	Moderately resistant	Moderately susceptible	Highly susceptible
IT14K – 2030-2	IT14K -1424-12	IT17K-1809-4	IT17K-1367-2
MOUSA+1	Wangkae	IT14K-849-2-1	IT17K-1707-2-2
-	-	-	PADITUYA

The pathogenicity test was conducted in July, 2023 in the Cowpea Improvement Screenhouse at the CSIR-Savanna Agricultural Research Institute Nyankpala. CSIR-SARI, Nyankpala is located in the Northern region of Ghana with coordinates 9.3965° N, 0.9892° W within the Guinea savannah zone.

3.9.2 Preparation of inoculum

Inoculum was obtained by preparing a fresh *C. lindemuthianum* culture of 7 days old from stored cultures of the pathogen isolated from field infected plants.

3.9.3 Soil Sterilization

Soil sterilization was done according to a modified procedure adopted by Kankam *et al*. (2019). Topsoil (sandy loam) was thoroughly mixed using a shovel and sterilized with steam. The steam sterilization was done using a metallic barrel with a fitted iron mesh to about one-third its length starting from the bottom. The wire mesh served to separate the water from the soil.

The barrel was then filled with water to the point of the fitted wire mesh and a jute sack was laid on top of the wire mesh to prevent water from mixing with the soil. About two-thirds of the metallic barrel's volume was filled with the soil to be used for the experiment. The set up was left on the fire using fire wood until water started to boil. The steam generated by the boiling water was allowed to pass through the soil for about two hours to heat-up the soil to sterilize it. This process was repeated several times until there was enough sterilized top soil to fill the perforated plastic pots to be used for the experiment. Sterilized soil was allowed to cool before used to fill the pots.

3.9.4 Layout of pot experiment and preparation of test cowpea materials

This experiment was arranged in a completely randomized design (CRD) with three replications. Fifty-four Plastic pots (10 L), control inclusive, were filled with the sterilized top loamy soil. Seeds were sterilized and manually sown at 2.5 cm depth on 1st August 2023 at the rate of 15 seeds per pot (Thio *et al.*, 2017), and watered with tap water using watering can. The seedlings were later thinned to 10 after emergence. Pots were placed on iron tables in the Cowpea Improvement Program Screenhouse for two weeks.

3.9.5 Preparation of spore suspension and inoculation of pathogen

The inoculation procedure was done as described by Jarek *et al.* (2018) beginning with the preparation of a spore suspension obtained from fresh *C. lindemuthianum* fungi culture that had been grown for seven days. A 60 cm^2 piece of the culture was taken into a beaker containing 200ml of sterile distilled water. The mixture was then strained through four layers of sterile cheesecloth to remove any agar and mycelial mesh. The resulting suspension was then subjected to centrifugation for 10 minutes.

The spore suspension was standardized to a concentration of 105 spores/ml using a hemocytometer counting slide, as outlined in Enyiukwu *et al*. (2020). At two weeks old of seedlings, the *C lindemuthianum* suspension was evenly applied abaxially and adaxially to each plant by spraying, following the method described by (Thio *et al.*, 2017). The plants were then incubated to allow symptoms to develop. The control experimental cowpea plants were sprayed with Sterile Distilled Water (SDW).

Figure 3.3: Two weeks old cowpea seedlings in the screenhouse freshly inoculated with *C. lindemuthianum*

3.10 Data collection on pathogenicity test

Data was collected on disease incidence and disease severity at the seedling stage at 1, 2 and 3 weeks after inoculation.

Figure 3.4: Anthracnose disease incidence on susceptible genotype

3.10.1 Disease incidence (DI)

Foliar disease incidence in the canopy was rated on ten seedlings of each genotype about a week after inoculation. As per the procedure adopted by Asare-Bediako *et al*. (2018), disease incidence for anthracnose at the seedling stage was assessed by counting the number of infected plants in relation to the total number of plants per pot expressed as a percentage at 1, 2 and 3 weeks after inoculation.

%Incidence = (No of plants affected by anthracnose)/ (total No of plants assessed) × 100

3.10.2 Disease severity (DS)

Severity of disease was assessed weekly on ten seedlings of each genotype about a week after inoculation. Disease severity assessment was rated on a 1-5 scale adopted from Adebitan *et al*. (1992). DS = (sum of individual scores)/ (Total No. of plant examined)

3.11 Data analysis

Data obtained was subjected to analysis of variance (ANOVA) using GenStat 18 edition at confidence level of $P < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Seed health test

Five seed borne pathogens namely *Colletotrichum lindemuthianum, Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer* and *Fusarium oxysporum* were observed growing on the various cowpea genotypes in the PDA medium and subsequently isolated.

Among the genotypes, IT17K-1809-4 exhibited the highest fungal infection with 86.7% of its seeds being infected by fungi. Following closely were IT17K-1802-1, IT17K-1403-1-1 and IT17K-849-2-1, all of which displayed a 73.3% seed infection rate. In contrast, IT17K-1704-5, IT16K-1966-1 and Songotra were the least affected, each showing a 26.67% infection rate.

The various cowpea genotypes assessed, also exhibited differences in fungal isolation frequencies, as shown in Table 4.1. *C. lindemuthianum* was isolated from all the cowpea genotypes except for, IT14K-2030-2, with highest frequency of 20.67% followed by *Aspergillus niger* (8.83%). *Fusarium* spp. recorded the third highest frequency of 8.65%. *A. flavus* obtained the least frequency of 4.99%.

Table 4.1: Fungi isolated from seeds of cowpea genotypes

Genotype	Incidence of Fungi					Total Incidence	%Fungi Recovered
	CL	*A. niger*	*A. flavus*	*Rhizopus* sp	*Fusarium* sp		
IT16K-1966-1	0.67	0.33	0	0	0.33	1.33	26.67
SONGOTRA	1	0	0	0	0.33	1.33	26.67
IT17K-1704-5	1	0	0	0	0.33	1.33	26.67
IT14K-2030-2	0	0.67	0	0.33	0.67	1.67	33.33
Kirkhouse-Benga	1	0.33	0	0.33	0	1.67	33.33
Padituya	1	0.33	0	0	0.33	1.67	33.33
IT10K-837-1	1	0	0.33	0.33	0.33	1.67	46.67
IT14K-1424-12	1.33	0.33	0	0.33	0	2.33	46.67
IT17K-2024-4	1.33	0	0	0	0.67	2	40
IT17K-1707-2-2	0.67	0.33	0.33	0.67	0.33	2.33	46.67
KVX782-1	0.67	0.67	0.67	0.33	0	2.33	46.67
MOUSA +1	0.33	0.67	1	0	0.33	2.33	46.67
IT17K-1095-2-2	1	0.67	0.33	0.33	0.33	2.67	53.33
Wangkae	1	0	0	0	2	4	60
IT16K-1970-1	1.67	1	0	0.67	0	3.33	66.67
IT17K-1367-2-1	1.67	1	0.33	0.33	0	3.33	66.67
IT17K-849-2-1	1	0.67	0.33	0.67	1	3.67	73.33
IT17K-1403-1-1	1.33	00.5	0.33	1	0.67	3.67	73.33
IT17K-1802-1	1	1	0.67	0.67	0.33	3.67	73.33
IT17K-1809-4	2	0.33	0.67	0.67	0.67	4.33	86.67
Total Frequency	20.67	8.83	4.99	6.66	8.65	50.66	1006.68

CL = *Colletotrichum lindemuthianum*, A = *Aspergillus*

4.2 Cultural characteristics of isolated fungal species from cowpea seeds

A total of five genera were isolated from the infected cowpea seeds. Cultural characteristics of fungi isolated from cowpea seed are presented in plates as shown in Plate 4. 1 to Plate 4.6.

4.2.1 *Colletotrichum lindemuthianum*

Mycelia growth of the fungus was observed 'normally' on PDA medium after seven days of incubation. The fungus exhibited rapid growth rate, initially appearing white or cream colour and subsequently became pigmented with time. At maturity, pure colonies on PDA developed dark pigments (Plate 4. 1A) whiles on the reverse, isolate showed more darker colour as shown in Plate 4. 1B. Spores were observed under microscope to be cylindrical/fusiform in shape, tapered and septate.

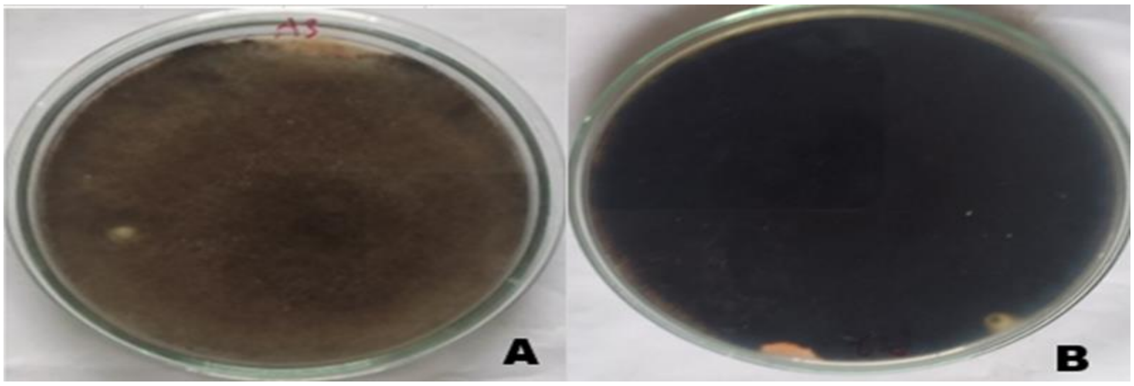

Plate 4. 1: Cultured *Colletotrichum lindemuthianum* in Petri dish after seven days of incubation; 'Normal view' (A) and 'Reverse view' (B) from the seed of IT17K-1809-4.

4.2.2 *Aspergillus niger*

Seven days Mycelia growth of the fungus was observed visibly on the PDA medium after five days of incubation (Plate 4.2*)*. The mycelium was dark green to black pigment. The reverse side of the colony appeared pale to yellow. Colony surface was woolly in texture.

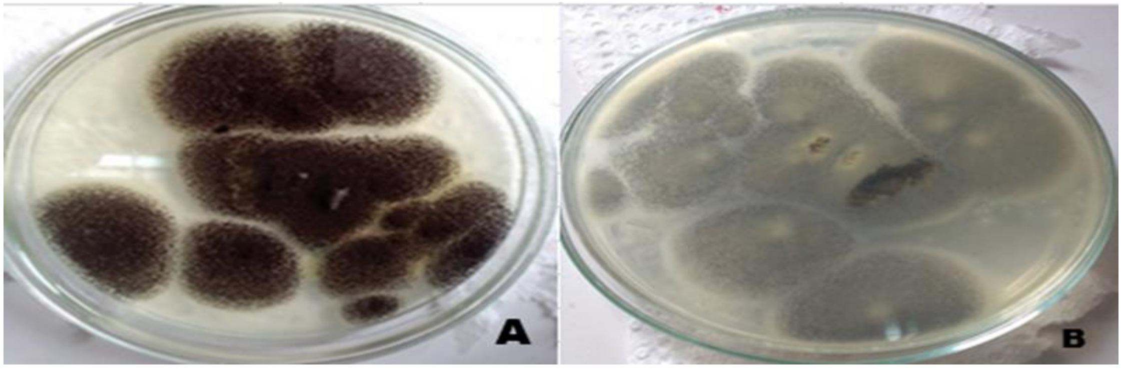

Plate 4.3: Cultured *Aspergillus niger* in Petri dish after seven days of incubation; 'Normal view' (A) and 'Reverse view' (B) from the seed of IT16K-1970-1.

4.2.3 *Rhizopus stolonifer*

Pure colonies demonstrated rapid growth rate after two days of incubation on PDA typically exhibiting cottony or fluffy texture. Mycelium gave white, gray appearance at the 'normal view' (Plate 4.4A). on the 'reverse', stoloniferous growth was observed (Plate 4.4B)

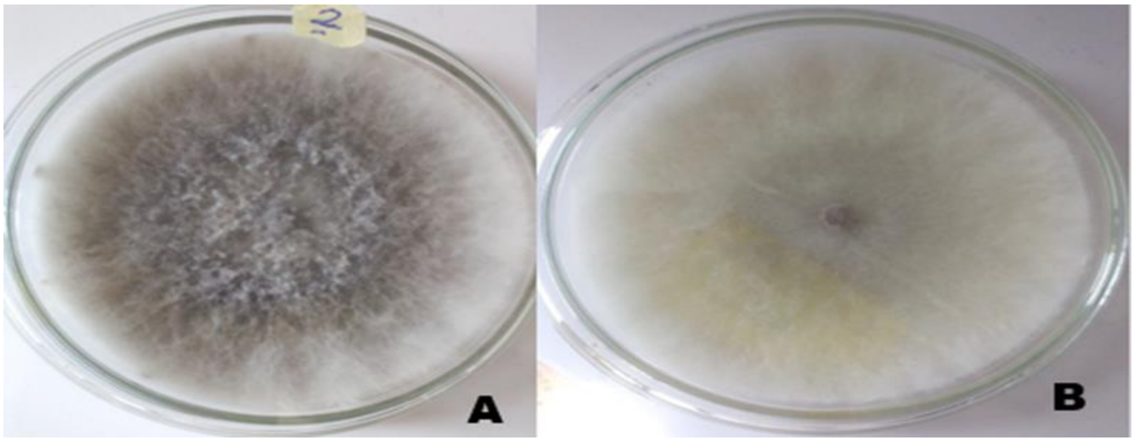

Plate 4.4: Cultured *Rhizopus stolonifer* isolated from the seed of IT17K-1403-1-1 in Petri dish after seven days; 'Normal view' (A) and 'Reverse view' (B).

4.2.4 *Aspergillus flavus*

Colonies of *A. flavus* after seven days of incubation on PDA developed compact yellowish-green mycelia and became green as it matured in concentric circles (Plate 4.5A). The reverse side of the plate initially was creamish-yellow and gradually became yellow with time and age (Plate 4.5B).

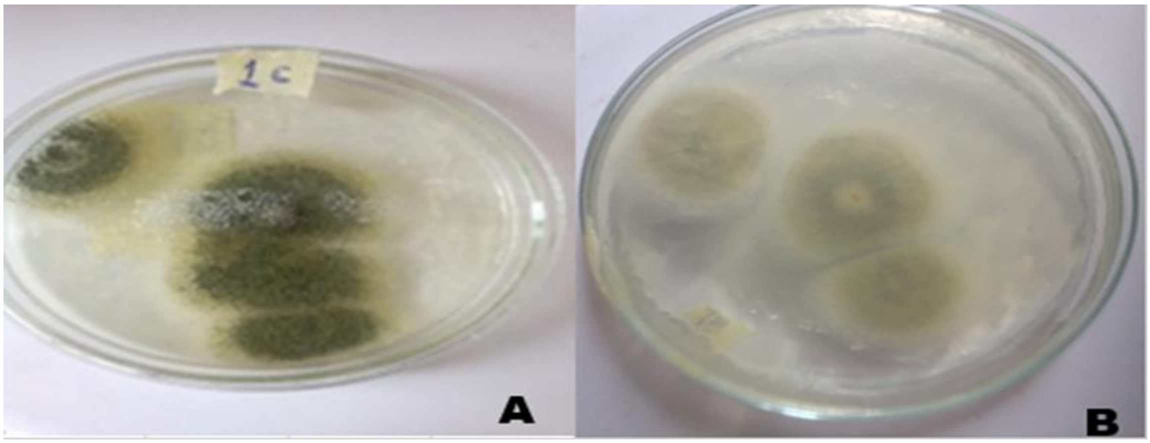

Plate 4.5: Cultured *Aspergillus flavus* in Petri dish after seven days of incubation; 'Normal view' (A) and 'Reverse view' (B) from the seed of MOUSA +1.

4.2.5 *Fusarium oxysporum*

After seven days of incubation, the observable development of fungus mycelia was noted on the PDA medium. The mycelium exhibited a dense, cottony surface with shades of pink and white (Plate 4.6A). On the reverse side, a dark-purple color was evident (Plate 4.6B).

Plate 4.6: Cultured *Fusarium oxysporum* in Petri dish after seven days; 'Normal view' (A) and 'Reverse view' (B) from the seed of Wangkae.

4.3 Isolation and identification of anthracnose pathogen from infected cowpea plant parts

The seven days old culture of *Colletotrichum lindemuthianum* showed that the mycelia growth rate was rapid, initially appearing white or cream colour and subsequently became pigmented with time. At maturity, pure colonies on PDA developed dark pigments (Plate 4.7A). Spores were viewed under microscope and appeared to be fusiform in shape, tapered and cross- walled (Plate 4.7B).

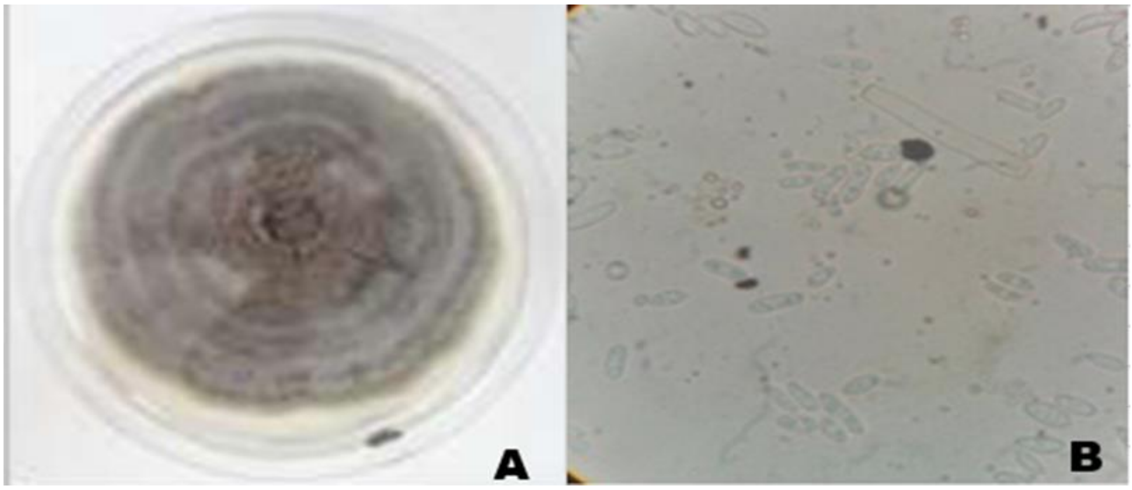

Plate 4.7: Mycelial growth of fungal pathogen associated with anthracnose from infected cowpea plant parts after nine days (A) and microscopic view (× 400 LPCB) (B)

4.4 Percentage of seedling emergence

There were significant ($P < 0.05$) differences among the genotypes in percentage emergence. The genotype IT17K-1704-5 had the highest germination emergence with 93.7% but was however not significantly different ($P > 0.05$) from the genotypes IT16K-1966-1 (93.0%), Songotra (91.8 %), Kirkhouse-Benga and IT10K-837-1 with each recording 90.8 % respectively. The least germination occurred in the genotype IT17K-1809-4 with 74.8 % and was followed by the genotypes IT17K-1802-1 (75.4 %), IT17K-1403-1-1 (78.5 %), and IT17K-1367-2-2 (79%) in increasing emergence percentage. Generally, there were varied significant differences among the genotypes as shown in Figure 4.1 below.

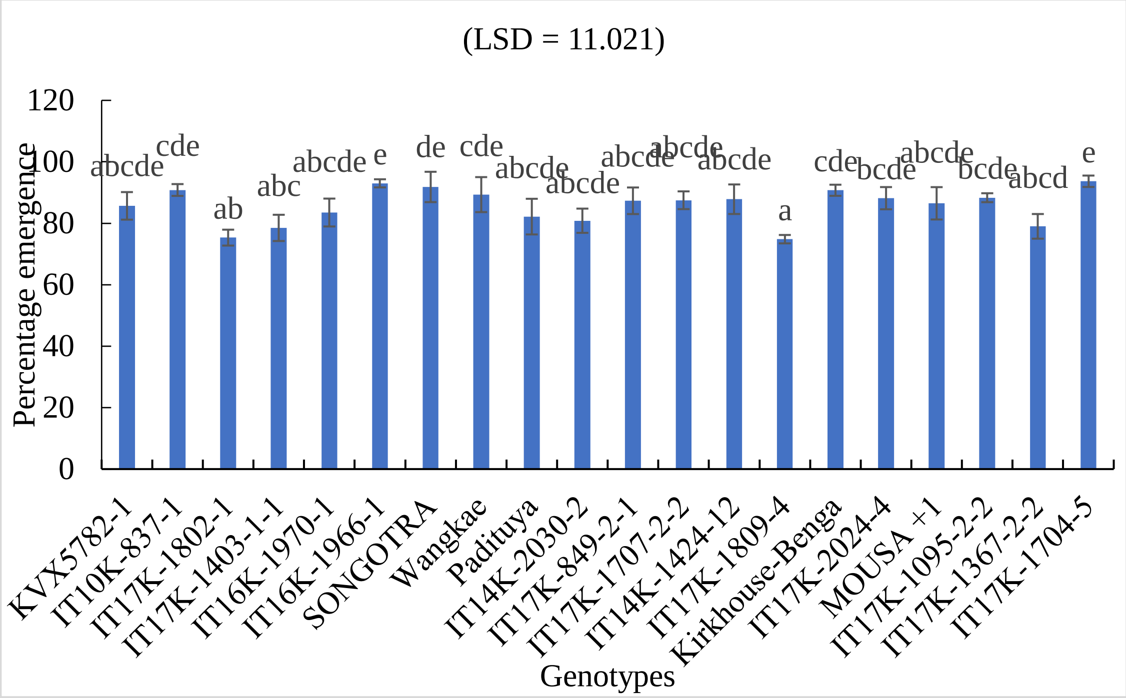

Figure 4.1: Mean emergence percentage of cowpea genotypes screened for anthracnose. Genotypes with different letters indicate significant difference (P < 0.05) between them while genotypes with the same letters are not significantly different (P > 0.05).

4.5 Disease incidence (DI)

Mean incidence (%) of anthracnose disease at three stages of growth on twenty cowpea genotypes are shown in Figure 4.*2*, Figure 4.*3* and Figure 4.*4*. Anthracnose disease occurred at both locations and in all the three growth stages but was more pronounced in the reproductive stages. Generally, all the 20 genotypes reacted differently to the disease in all the three stages.

In the pre flowering stage, the ANOVA showed significant difference (P < .001) in mean disease incidence among genotypes. Anthracnose disease incidence among genotypes at this stage was significantly lower than in the flowering and pod stages. Genotype IT17K-1707-2-2 attained the highest mean anthracnose incidence of 3.84% but did not differ significantly (P < 0.05) from 2.92 and 2.73 recorded for genotypes, IT17K-849-2-1 and IT14K-1966-, as second and third highest respectively. Genotype ITI4K-2030-2 had zero disease incidence (0.00%) followed, by MOUSA +1 (0.37%) in the pre-flowering stage.

The flowering stage, shown in Figure 4.3, indicated significant (*P* = 0.001) difference in mean disease incidence among the genotypes. The study showed that, anthracnose disease incidence was progressive with increasing weeks after planting. Incidence of disease was highest on Padituya (12.9%) but was not significantly higher than IT17K-1707-2-2 (9.6%) and IT17K-1367-2-2 (9.23%) that obtained the second and third highest respectively. ITI4K-2030-2 virtually maintained no disease incidence of 0.33% with the next two genotypes, IT14K-1424-12 and Wangkae recording 2.62% and 2.72% respectively. All the cowpea genotypes at the flowering stage were moderately resistant except ITI4K-2030-2 which was rated highly resistant.

Results at the pod stage revealed that the general incidence of anthracnose disease on cowpea genotypes progressed significantly. IT17K-1802-1 had the highest mean disease incidence of 40.02%, classified as moderately susceptible, but was not significantly different from the next highest genotype, IT17K-1367-2-2 attaining 37.32%. Genotypes, ITI4K-2030-2 and MOUSA+1 had the least incidence of 2.95% and 7.6% respectively and fall within the moderately resistant class with; IT14K-1424-12(12.67%), Wangkae (18.2%), IT16K-1970-1(18.32), Kirkhouse-B (23.8%), and KVX782-1(24.65%).

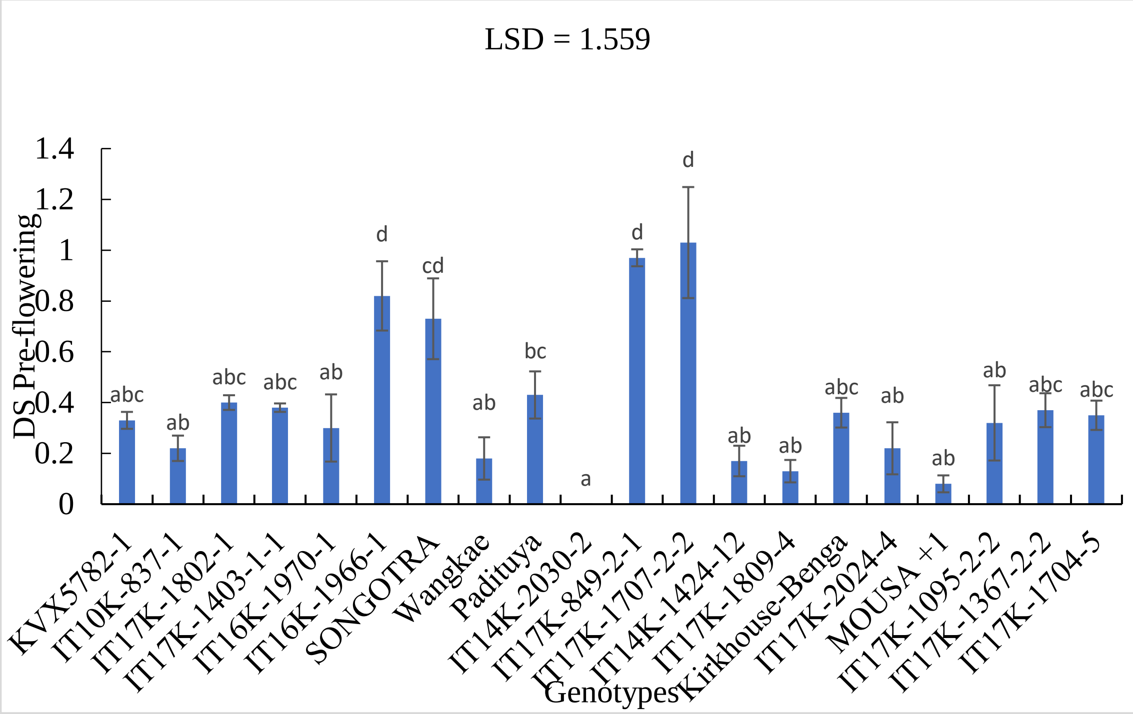

Figure 4.2: Anthracnose disease incidence at pre-flowering stage

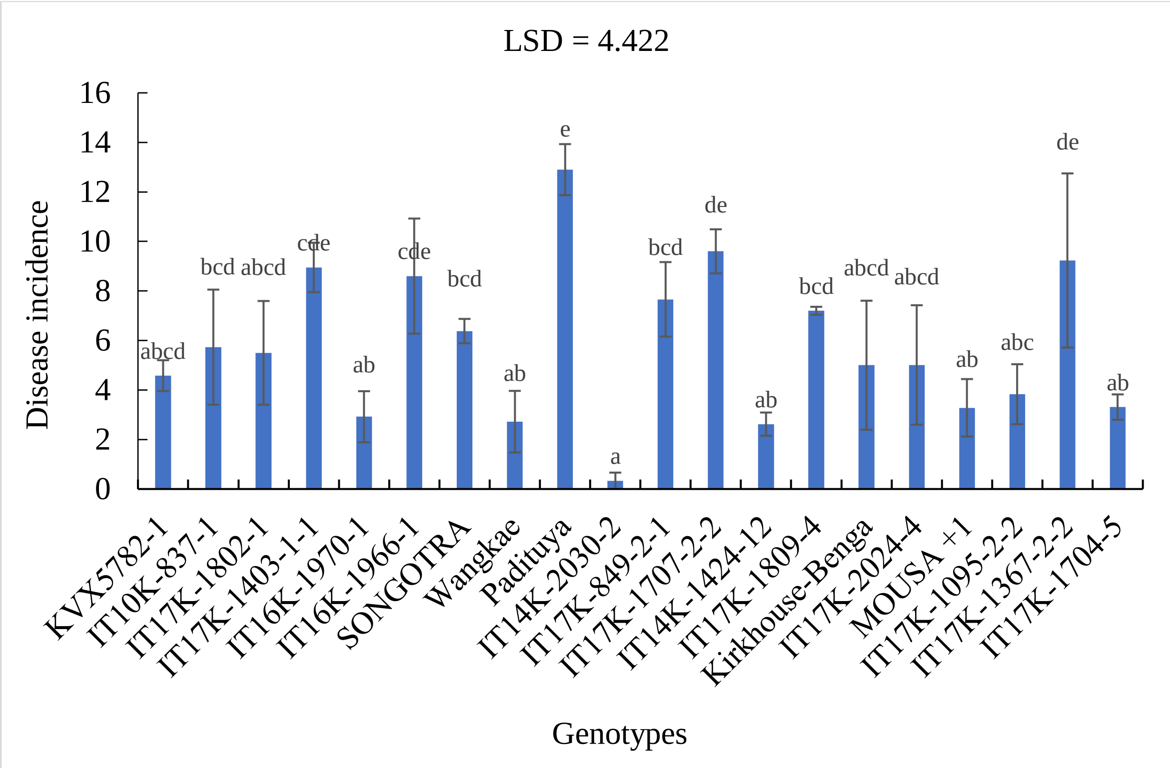

Figure 4.3: Mean disease incidence of anthracnose at flowering stage

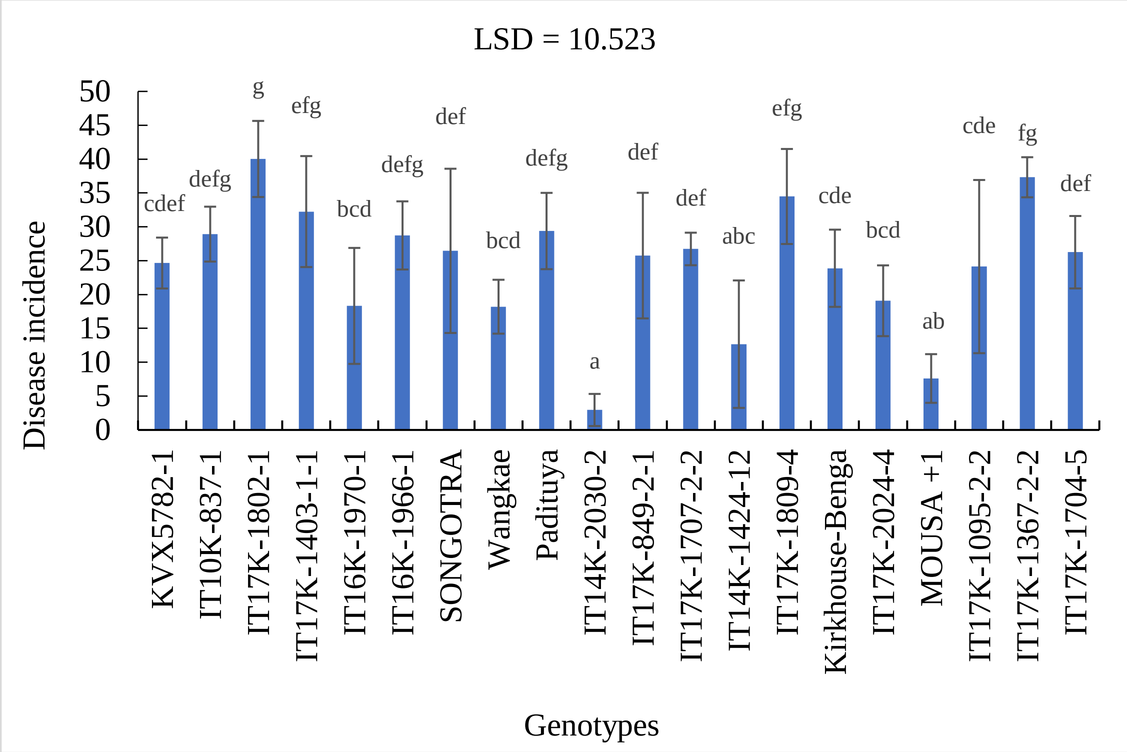

Figure 4.4: Anthracnose disease incidence at pod stage genotypes with different letters indicate significant difference (P < 0.05) between them while genotypes with the same letters are not significantly different (P > 0.05).

4.6 Disease severity

The mean severity of anthracnose disease on twenty cowpea genotypes at three growth stages is presented in Figure 4.5. The severity of anthracnose disease was significantly (P = 0.001) different among genotypes at the pre- flowering stage (Figure 4.6). Cowpea genotype IT17K-1707-2-2 had the highest symptom disease severity score of 1.033 not significantly (P < 0.001) higher than IT17K-849-2-1(0.97) and IT16K-1966-1(0.82). IT4K-2030-2 visibly did not show any symptom of disease. The rest of the genotypes ranged from 0.083 to 0.967 of the disease severity score (all resistant). Averagely, all the genotypes across the two locations at the pre-flowering stage were resistant to anthracnose disease, even though they significantly exhibited varying levels of disease severity (P = 0.001).

Significant differences ($P = 0.001$) were observed among the genotypes for disease severity (Figure 4.*6*) at the flowering stage. Padituya followed by IT17K-1707-2-2 recorded the highest mean disease severity score of 2.533 (moderately susceptible) and 2.383 (moderately resistant) respectively. IT14K-2030-2-2 (0.1) and IT14K-1424-12(0.833) recorded the lowest disease severity and were both rated as highly resistant according to the disease severity score index. IT10K-837-1(1.3), IT17k-1704-5(1.183), IT17k-1802-1(1.233), IT17k-2024-4(1.117), Kirkhouse (1.425), MOUSA + 1 (1.25) and Wangkae (1.25) were also seen to be highly resistant.

Genotype × location interaction, shown in Table 4.*2*, had significant difference ($P = 0.001$) at the pod stage. Anthracnose disease severity of cowpea genotypes was progressive with advancement in stages. The average disease severity of the twenty cowpea genotypes varied between 1.03 to 5.0 at Gumyoko and 0.47 to 4.867 at Tilli. The worst performance, in terms of disease severity, was recorded by cowpea genotype IT17K-1367-2-2 (5.0) at Gumyoko and IT17K-1704-5 (4.867) at Tilli. IT14K-2030-2 produced the least disease severity score of 1.03 at Gumyoko whereas 0.47 was recorded as the minimum severity for same genotype, IT14K-2030-2 at Tilli. On average, 3.803 was recorded at Gumyoko as the disease severity while that of Tilli was 3.767.

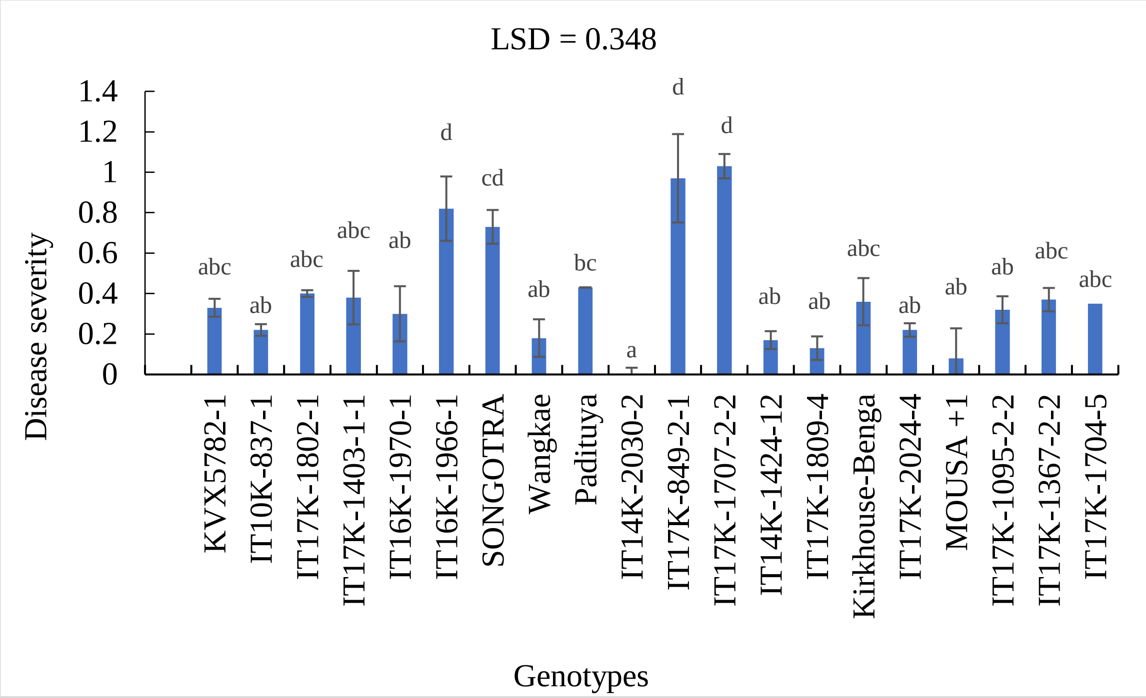

Figure 4.5: Mean severity of anthracnose disease at pre-flowering stage

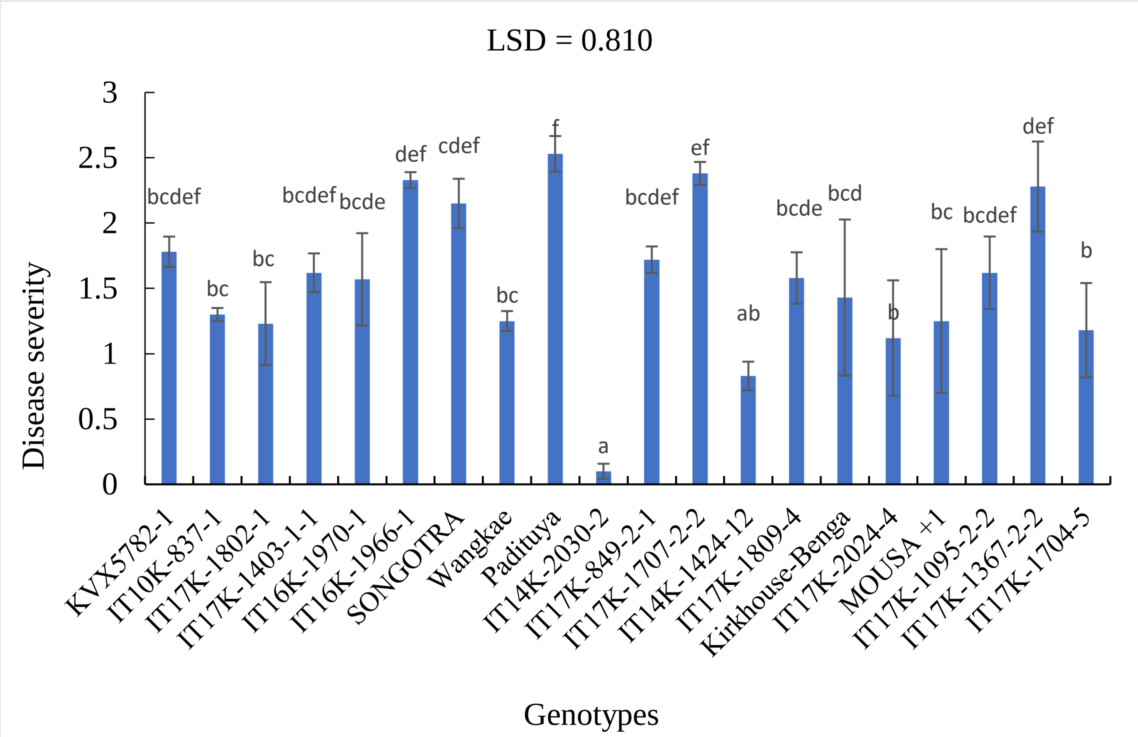

Figure 4.6: Anthracnose disease severity of cowpea genotypes at the flowering stage. Genotypes with different letters indicate significant difference ($P < 0.05$) between them while genotypes with the same letters are not significantly different ($P > 0.05$).

Table 4.2: Severity of anthracnose disease on twenty cowpea genotypes in Gumyoko and Tilli at pod stage.

Genotype	Disease severity			
	Gumyoko	Reaction Class	Tilli	Reaction Class
KVX5782-1	3.8(0.15) cdefghij	HS	2.967(0.29) bcdef	MS
IT10K-837-1	4.9(0.39) ijk	HS	2.93(0.74) bcde	MS
IT17K-1802-1	4.2(0.1) efghijk	HS	4.43(0.18) ghijk	HS
IT17K-1403-1-1	4.03(0.03) defghij	HS	3.03(0.18) bcde	MS
IT16K-1970-1	2.27(0.72) b	MR	4(0.21) defghij	HS
IT16K-1966-1	4.92(0.33) jk	HS	4.07(0.20) defghij	HS
SONGOTRA	3.9(0.31) cdefghij	HS	4.83(0.07) hijk	HS
Wangkae	2.67(0.44) cdefghi	MS	2.4(0.48) bcdef	MR
Padituya	4.43(0.3) ghijk	HS	4.43(0.29) ghijk	HS
IT14K-2030-2	1.03(0.03) a	HR	0.47(0.03) a	HR
IT17K-849-2-1	3.0(0.15) bcdefg	MS	2.9(0.15) bcd	MS
IT17K-1707-2-2	4.98(0.27) jk	HS	4.33(0.12) fghijk	HS
IT14K-1424-12	2.4(0.28) bc	MR	2.3(0.78) bc	MR
IT17K-1809-4	3.1(0.15) bcdefg	HS	2.7(0.90) bcd	MS
Kirkhouse-Benga	3.833(0.42) cdefghij	HS	4.5(0.06) ghijk	HS
IT17K-2024-4	3.7(0.23) cdefghi	HS	3.5(1.07) bcdefgh	HS
MOUSA +1	1.1(0.67) a	HR	1.3(0.26) a	HR
IT17K-1095-2-2	3.4(0.21) bcdefg	HS	4.43(0.32) ghijk	HS
IT17K-1367-2-2	5.0(0.34) k	HS	4.233(0.57) efghijk	HS
IT17K-1704-5	4.73(0.16) ghijk	HS	4.867(0.09) hijk	HS
P Value < .001	LSD = 1.143		CV% = 18.4	

HR: Highly Resistant = 0.0-1.4 MR: Moderately Resistant = 1.5-2.4
MS: Moderately Susceptible = 2.5-3.0 HS: Highly Susceptible = > 3.0

4.7 Days to 95% pod maturity

The number of days to 95% pod maturity (D95PM) of 20 cowpea genotypes is shown in Figure 4.7. The cowpea genotypes screened had their mean D95PM ranging from

70.67 to 74. The first to reach 95% harvest maturity was IT14K- 2030-2 recording 70.6 mean number of days followed by IT17K -849-2-1 and Songotra both took 70.83 days to reach harvest maturity. MOUSA+1 attained 95% pod maturity in 74 days later than the rest of the genotypes making it significantly different from IT14K- 2030-2.

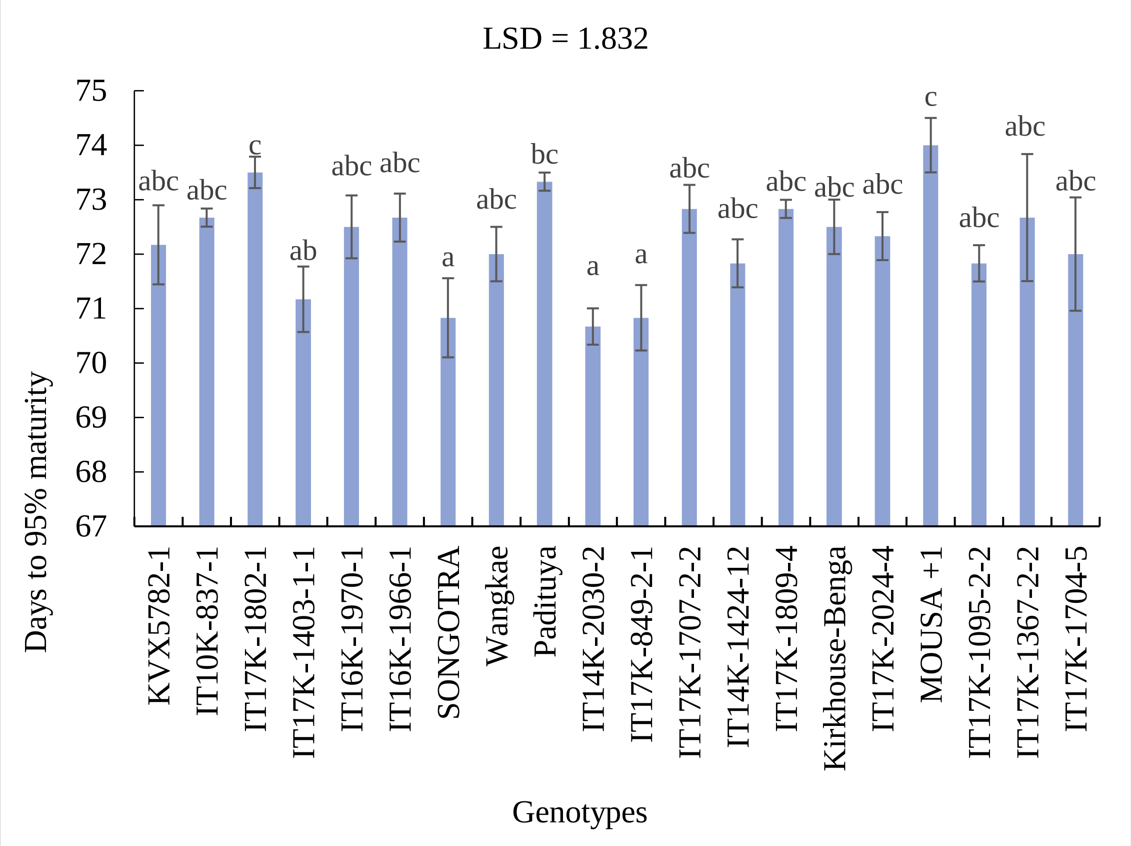

Figure 4.7: Mean Number of days to 95 pod maturity among cowpea genotypes. Genotypes with different letters indicate significant difference ($P < 0.05$) between them while genotypes with the same letters are not significantly different ($P > 0.05$).

4.8 Plant height at maturity

Results of plant height are presented in Figure 4.*8*. The results show that plant height differed significantly ($P = 0.001$) among the various cowpea genotypes. Plant heights ranged from 10cm to 28.07 cm across the two locations with a mean of 22.99cm. ITI7K-1707-2-2 was the tallest with mean height of 28.07cm but did not show significant ($P < 0.05$) difference in comparison with IT14K-2030-2 (27.83cm) and IT17K-849-2-1 (27.28) as the second and third best performing genotypes in terms of plant height. MOUSA+1 recorded significantly the lowest plant height with a mean of 10cm. The mean plant height at Gumyoko (27.28cm) was significantly higher than that of Tilli (18.71cm).

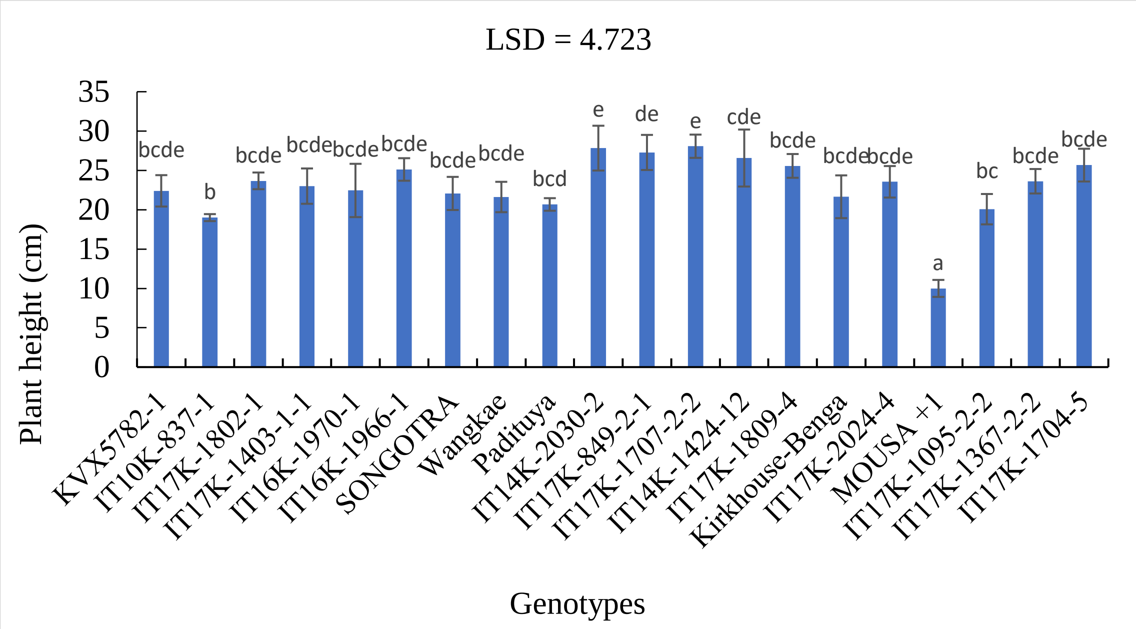

Figure 4.8: Mean plant height among cowpea genotypes. Genotypes with different letters indicate significant difference ($P < 0.05$) between them while genotypes with the same letters are not significantly different ($P > 0.05$).

4.9 Hundred seed weight

Significant differences ($P = 0.016$) were observed on the interaction effect for hundred seed weight (**Error! Reference source not found.**). Hundred seed weight ranged from 15.13 g to 24.4 g with a mean of 18.882 g at Gumyoko and 12.4 g to 20.3 g at Tilli. The highest 100 seed weight was obtained from IT14K-1424-12 at both Gumyoko (24.4g) and Tilli (20.43g) with significant difference between them**.** The trend was replicated for the minimum seed weight with Songotra attaining 15.13g at Gumyoko and 12.4g at Tilli. The second highest genotypes in 100 seed weight were IT14K-2030-2 (23.97g) and IT17K-1809-4 (20.3g) for Gumyoko and Tilli respectively.

Table 4.3: Variation in 100 seed weight of cowpea genotypes at Gumyoko and Tilli

	Locations	
Genotype	Gumyoko	Tilli
IT10K-837-1	18.4abcdefgh	16.6abcdef
IT14K-1424-12	24.4h	20.43defgh
IT14K-2030-2	23.97gh	18.97bcdefgh
IT16K-1966-1	16.47abcdef	16.37abcde
IT16K-1970-1	16.47abcdef	17.1abcdef
IT17K-1095-2-2	16.87abcdef	15.7abcde
IT17K-1367-2-2	18.17abcdefg	13.43ab
IT17K-1403-1-1	17.23abcdef	15.4abcde
IT17K-1704-5	16.97abcdef	14.37abcd
IT17K-1707-2-2	20.83efgh	19.43bcdefgh
IT17K-1802-1	16.27abcde	18.6bcdefgh
IT17K-1809-4	20.17cdefgh	20.3defgh
IT17K-2024-4	19.87cdefgh	15.17abcde
IT17K-849-2-1	16.8abcdef	14.13abc
Kirkhouse-Benga	19.2bcdefgh	15.9abcde
KVX782-1	22.43fgh	16.27abcde
MOUSA +1	17.07abcdef	14.63abcd
Padituya	20.83efgh	18.7bcdefgh
Songotra	15.13abcde	12.4a
Wangkae	20.07cdefgh	15.73abcde
P value < .001	LSD = 2.976	CV % = 12.3

Means with different letters indicate significantly different ($P < 0.05$) among genotypes while means with the same letters are not significantly different ($P > 0.05$).

4.10 Biomass yield of genotypes

Genotypes significantly interacted ($P < 0.05$) in both locations on biomass yield. The genotype IT14k-1424-12 recorded the highest biomass yield with 5333 kg/ha in Gumyoko while 3022 kg/ha was recorded by the genotype IT14k-2030-2 in Tilli as the highest biomass yield among the genotypes. The genotypes MOUSA+1 and IT17k-1707-2-2 recorded 2244 kg/ha and 1339 kg/ha as the lowest in biomass yield in

Gumyoko and Tilli respectively. Averagely, genotypes in Gumyoko produced more biomass yield with 4132.25 kg/ha as compared to 2076.75 kg/ha produced by the genotypes in Tilli as shown in **Error! Reference source not found.** below.

Table 4.4: Biomass yield (kg/ha) of cowpea genotypes at Gumyoko and Tilli

Genotypes	Gumyoko	Tilli
KVX5782-1	4003(3.44) ^{p}	2978(40.06) ^{mn}
IT10K-837-1	3176(27.31) ^{n}	2004(3.56) ^{de}
IT17K-1802-1	4279(40.84) ^{qr}	2474(70.35) ^{ghij}
IT17K-1403-1-1	3556(98.76) ^{o}	2056(33.35) ^{de}
IT16K-1970-1	2941(82.69) ^{lmn}	2063(75.30) ^{de}
IT16K-1966-1	2978(177.78) ^{mn}	2361(14.69) ^{fgh}
SONGOTRA	2756(44.44) ^{klm}	996(6.19) ^{a}
Wangkae	5067(33.33) ^{s}	1967(33.33) ^{d}
Padituya	3956(44.44) ^{p}	2456(22.22) ^{ghi}
IT14K-2030-2	4978(88.89) ^{s}	3022(48.43) ^{n}
IT17K-849-2-1	4144(11.11) ^{pq}	2572(33.79) ^{hijk}
IT17K-1707-2-2	4978(29.39) ^{s}	1339(5.56) ^{b}
IT14K-1424-12	5333(38.49) ^{t}	1422(48.43) ^{bc}
IT17K-1809-4	5067(69.38) ^{s}	2711(44.44) ^{jkl}
Kirkhouse-Benga	4356(80.12) ^{qr}	1606(96.38) ^{c}
IT17K-2024-4	5211(122.22) st	1450(41.94) ^{bc}
MOUSA +1	2244(22.22) ^{efg}	1917(72.65) ^{d}
IT17K-1095-2-2	5022(29.23) ^{s}	2635(185.69) ^{ijk}
IT17K-1367-2-2	4400(250.16) ^{r}	2150(76.38) ^{def}
IT17K-1704-5	4200(167.77) ^{pqr}	1356(11.11) ^{b}
P value < .001	LSD = 228.7	CV % = 4.5

Means of biomass yield ± SE in brackets. Means with different letters indicate significantly different ($P < 0.05$) among genotypes while means with the same letters are not significantly different ($P > 0.05$).

4.11 Grain yield per hectare

There was significant interaction ($P < 0.05$) among the genotypes in both locations in grain yield as displayed in table 4.5. The highest grain yield was recorded at 2406 kg/ha in both Gumyoko and Tilli by the genotypes IT14K-2030-2 and IT14K-1424-12 respectively. In Gumyoko, the genotypes IT17K-2024-4 and IT17K-1095-2-2 recorded the same yield of 300 kg/ha as the lowest while the genotype IT17K-849-2-1 recording 872 kg/ha as the least yield in Tilli. Averagely, the genotypes in Tilli produced 1407.85 kg/ha grain yield as compared to 1084.75 kg/ha produced by same genotypes in Gumyoko.

Table 4.5: Grain yield kg/ha of cowpea genotypes at Gumyoko and Tilli

	Location	
Genotype	Gumyoko	Tilli
KVX5782-1	533 (136.54) ^{ghi}	1667 (210.12) ^{j}
IT10K-837-1	1667(384.9) ^{ghi}	972(294.38) ^{cdef}
IT17K-1802-1	1478(190.11) ^{fghi}	1017(277.11) ^{cdef}
IT17K-1403-1-1	872(56.38) ^{bcde}	1306(175.2) ^{defghi}
IT16K-1970-1	1794(65.50) ^{hi}	1606(339.34) ^{ghi}
IT16K-1966-1	1228(105.56) ^{defgh}	1317(18.39) ^{defghi}
SONGOTRA	1317(330.96) ^{defghi}	1203(68.65) ^{defg}
Wangkae	1277(33.72) ^{defghi}	1228(184.56) ^{defgh}
Padituya	956(105.56) ^{cdef}	1200(144.13) ^{defg}
IT14K-2030-2	2406(120.31) ^{j}	1794(115.18) ^{hi}
IT17K-849-2-1	1411(219.50) ^{efghi}	872(99.38) ^{bcde}
IT17K-1707-2-2	1722(111.11) ^{ghi}	1478(331.01) ^{fghi}
IT14K-1424-12	1728 (66.67) ^{abc}	2406 (668.39) ^{ghi}
IT17K-1809-4	489(56.38) ^{abc}	1828(455.52) ^{i}
Kirkhouse-Benga	811(200.30) ^{abcd}	2106(381.91) ^{efghi}
IT17K-2024-4	300(91.79) ^{a}	972(294.39) ^{cdef}
MOUSA +1	561(164.52) ^{abc}	956(184.56) ^{cdef}
IT17K-1095-2-2	300(48.11) ^{a}	1017(277.11) ^{cdef}
IT17K-1367-2-2	356(72.86) ^{ab}	1306(175.20) ^{defghi}
IT17K-1704-5	489(155.85) ^{ab}	1606(339.34) ^{ghi}
P value = 0.001	LSD = 464.8	CV% = 23.4

Means of grain yield ± SE in brackets. Means with different letters indicate significant different (P < 0.05) among genotypes while means with the same letters are not significantly different (P > 0.05).

4.12 Pathogenicity test of the pathogen associated with anthracnose disease

Symptoms of anthracnose disease appeared on susceptible cowpea genotypes within the first week after seedling inoculation with cowpea genotypes; Padituya, IT17K-1707-2-2 and IT17K-1367-2-2 being the first to show symptoms of infection by anthracnose. Black lesions appeared on the lower leaf surface along the veins at the trifoliate leaf stage. The symptoms of anthracnose disease progressed acropetally. The incidence and severity worsened with increasing number of days after inoculation.

Disease severity (DS) scores as a result of the artificial inoculation with *Colletotrichum lindemuthianum* ranged from highly resistant to highly susceptible for the nine genotypes assessed at the seedling stage. IT17K-1707-2-2 (3.2) IT17K-1367-2-1 (5), including the susceptible check, Padituya (3.1), all rated highly susceptible according to the disease severity score adopted from Adebitan *et al*. (1992). DS on IT17K-1367-2-2 was highly significant compared to other genotypes resulting in death due to severe damage of the genotype seedlings as shown in Figure 4*.9*. IT14K-2030-2 (1.2), IT14K-1424-12 (1.5), Wangkae (1.3) and the resistant check, MOUSA +1, emerged as highly resistant as the DS observed on such genotypes were minimal. DI ranged from 5% on MOUSA+1 to 37.6% on IT17K-1367-2-2. In the control (uninoculated) experiment, cowpea genotypes IT17K-1707-2-2, IT17K-1367-2-2, IT14K-2030-2 and IT14K-849-2-1 displayed symptoms of anthracnose infection, even though, the effect of *Colletotrichum lindemuthianum* was not severe.

Figure 4.9: Anthracnose susceptible genotype severely infected after artificial inoculation

Table 4.6: Incidence and Severity of anthracnose and susceptibility class of nine cowpea genotypes inoculated with *Colletotrichum lindemuthianum*

Genotype	**Disease Severity**		**Disease Incidence**		**Susceptibility Class**
	ICL	**UI**	**ICL**	**UI**	
MOUSA+1	0.367a	0.00^{c}	5.00^{a}	0.00^{c}	Highly resistant
IT14K-2030-2	1.167b	0.67^{b}	13.00^{b}	6.00^{b}	Highly resistant
Wangkae	1.300b	0.00^{c}	15.33^{c}	0.00^{c}	Highly resistant
IT14K-1424-12	1.533c	0.00^{c}	21.67^{d}	0.00^{c}	Moderately resistant
IT14K-849-2-1	2.600d	1.40^{a}	26.33^{e}	5.67^{b}	Moderately resistant
IT17K-1809-4	2.600d	0.09^{c}	29.33^{f}	0.00^{c}	Moderately resistant
Padituya	3.133e	0.00^{c}	31.00^{f}	0.00^{c}	Highly susceptible
IT17K-1707-2-2	3.217e	1.20^{a}	33.67^{g}	10.67^{a}	Highly susceptible
IT17K-1367-2-2	5.000f	1.00^{ab}	37.67^{h}	11.00^{a}	Highly susceptible
Over all mean	2.324	0.47	23.67	3.70	
LSD (5%)	0.16	0.41	1.69	4.59	

Average of three replicates. 0.0-1.4= highly resistant, 1.5-2.4 = moderately resistant, 2.5-3.0 = moderately susceptible, more than 3.0 = highly susceptible. ICL=inoculated with *Colletotrichum lindemuthianum,* UI=uninoculated plants

Figure 4.10: Control treatment of cowpea genotypes to distilled water under screenhouse condition (uninoculated)

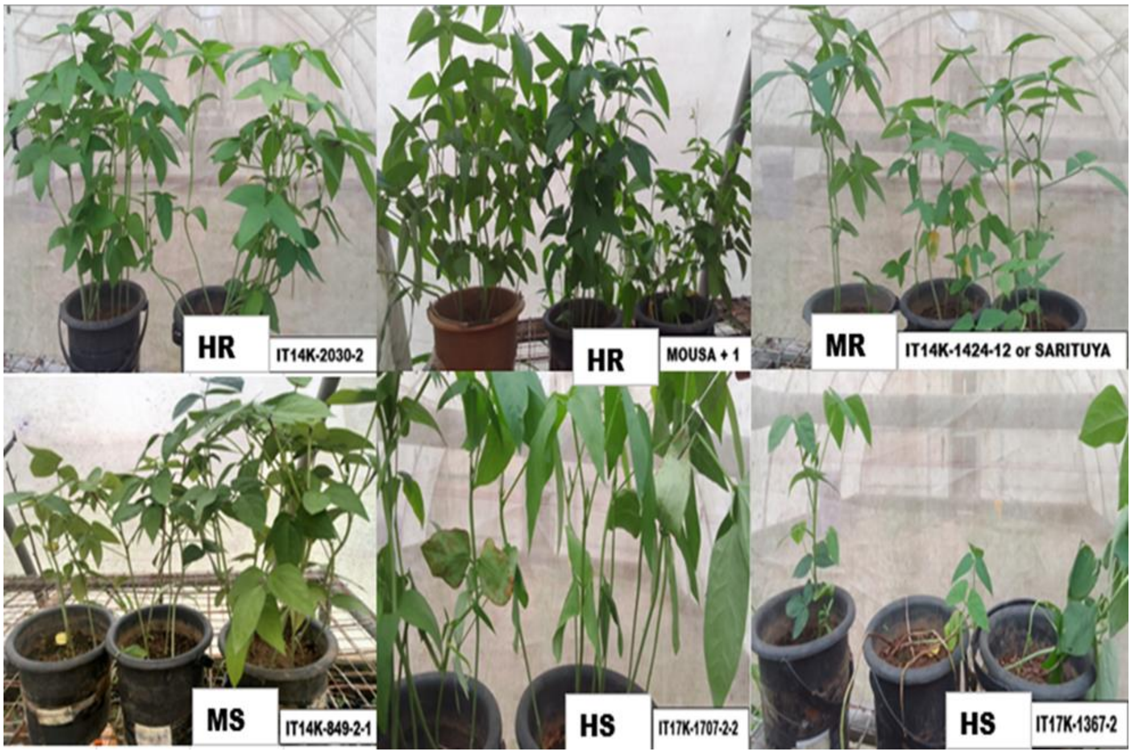

Figure 4.11: Reaction of cowpea genotypes to *Colletotrichum lindemuthianum* planted and inoculated same day. HR= highly resistant MR = moderately resistant MS = moderately susceptible HS = highly susceptible

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Seed health and field emergence percentage

The study revealed inverse relationship between seed borne fungal infection and emergence percentage. The seed with the highest fungal infection in the seed health test, IT17K-1809-4, recorded the minimum germination percentage. Conversely, IT17K-1704-5, which had the least infection, achieved the highest percentage emergence.

The low germination percentage recorded on IT17K-1809-4, as well as other genotypes such as Padituya, IT17K-1367-2-2, Wangkae, IT14K-849-2-1 and IT17K-1095-2-2 was probably pathogenic. This agrees with Baysah (2013) who reported that, seed borne fungi associated with cowpea affect seed germination. Assessing seeds for their health before planting is crucial as healthy seeds contribute to an elevated germination rate and higher crop yield. Many economic diseases of plants caused by fungi are reported to be seed borne (Mahmoud *et al*., 2013). A pathogen present either on or within a seed as a contaminant can lead to various detrimental effects, including seed abortion, rot, necrosis, and a reduction or elimination of germination capacity (Tsedaley, 2015).

Additionally, seedling damage may occur, ultimately leading to the development of disease at later stages of plant growth through systemic or local infection. *Colletotrichum lindemuthianum* was isolated from almost all the genotypes' seeds and consequently the plants grown from such seeds expressed symptoms of anthracnose. This presupposes that the cowpea anthracnose was seed borne with the pathogen (*C. lindemuthianum*) carried by the seed and subsequently caused infection to the plant at seedling or later growth stages. This assertion is in line with Vazin (2015), describing anthracnose disease of bean as the one that is basically seed borne.

5.2 Disease severity (DS) and disease incidence (DI)

Cowpea anthracnose was observed to be widespread in both of the two examined locations, Gumyoko and Tilli, where genotypes displayed varying degrees of resistance and susceptibility. However, the disease was less prevalent in Tilli, with a comparatively lower incidence and severity than in Gumyoko. Although weather data is not available, Gumyoko experienced higher rainfall and lower temperatures compared to Tilli due to seasonal differences. The increased precipitation and decreased temperatures in Gumyoko may have led to higher relative humidity, as indicated by Pandey *et al*. (2023) in their research findings. This, coupled with reduced light intensity, resulting in the increased accumulation of cowpea anthracnose fungi spores, leading to a higher intensity of infestation and spread in Gumyoko.

The higher relative humidity likewise implies prolonged periods of leaf surface wetness, a condition known to promote the development and sporulation of fungal diseases, as observed in studies by Kadege *et al.* (2022). These findings support the conclusion that environments within humid agro-ecological regions are more conducive to the growth and development of pathogens causing fungal diseases, as reported by Enyiukwu *et al.* (2014) and Adegbite and Amusa (2008).

Disease incidence and severity were generally lower in highly resistant genotypes namely IT14K-2030-2 and MOUSA+1 and moderately resistant genotype IT14K-1424-12, however, susceptibility was notably higher in most genotypes, particularly in IT17K-1707-2-2, IT17K-1367-2-2, and Padituya, all of which were rated highly susceptible in both Gumyoko and Tilli. The cowpea genotypes that exhibited low disease incidence rates also tended to have low disease severity rates. This phenomenon could be attributed to the genetic constitution of the various genotypes, which demonstrates varying levels of resistance to anthracnose disease, aligning with the findings of Ekhuemelo *et al*. (2019) on the assessment of cowpea leaf spot disease. Although anthracnose disease occurred in all three stages (Pandey *et al*., 2023), it was more severe during the reproductive stages, significantly impacting grain and fodder yields. The incidence and severity of anthracnose disease might have contributed to the disparity in biomass and grain yields among genotypes as shown in the negative correlation of disease severity and yield. This finding is in agreement with Vazin (2015) who reported that, yield was low as a result of limited photosynthetic area due to anthracnose infection. Also, the theory of disease tolerance suggests that certain genotypes can maintain high yields despite being infected by pathogens (Pagán and García-Arenal, 2018). This phenomenon is likely responsible for the high grain yields recorded in susceptible genotypes such as IT17K-1707-2-2 and Songotra.

The recorded disease severity at the reproductive stages determined the resistance levels of the genotypes. The study identified three superior genotypes namely IT14K-2030-2, IT14K-1424-12, and MOUSA +1 in terms of resistance to anthracnose disease, while several genotypes, including Padituya, IT17K-1707-2-2, and IT17K-1367-2-2, were highly susceptible.

5.3 Days to 95% pod maturity

Cowpea genotypes IT14K-2030-2, IT17K-849-2-1 and Songotra attained pod maturity at 70 days after planting and these were classified as extra early according to Baidoo and Mochiah (2014) whereas the rest of the test genotypes reached pod maturity within 71 to 74 days after planting. The study showed that IT14K-2030-2 exhibited high superiority by combining early maturity, high grain yield, substantial fodder yield, and resistance to anthracnose disease. Early maturing genotypes could be a panacea for improving cowpea yield on farmers' fields as they can escape adverse conditions or stresses including pests and diseases which can reduce yield up to 80% (Abdou, 2021).

According to Salifou *et al*. (2017), early maturing varieties are climate fast and can be recommended to areas with short rainfall period where rain fed agriculture is practiced. It must however be noted that, late season rains may pose challenges regarding the quality and viability of seeds, hence farmers are advised to secure storage facilities before adopting such varieties (Abdou, 2021).

5.4 Plant height

The finding revealed that, plant height was significant among the cowpea genotypes. Cowpea genotypes namely IT17K-1707-2-2, IT14K-2030-2, IT17-849-2-1, IT14K-1424-12 and IT17K-1704-5 recorded the highest plant height among the genotypes. The variation in plant height among genotypes may be attributed to their genetic composition as observed by Agyeman *et al*. (2014). A positive correlation between plant height and grain yield for cowpea genotypes namely IT14K-1424-12 and IT14K-2030-2 was detected in the present work. This observation is in line with Abdou (2021) who observed that, plant height positively correlates with grain yield for some genotypes. This observation, however, contradicts the case of MOUSA +1, which achieved the lowest plant height and biomass but exhibited a comparatively higher grain yield. Despite MOUSA +1 being a short cowpea variety, it should be noted that it belongs to the spreading cowpea type. This implies that its growth habit allows it to produce numerous vines, favoring more reproductive growth and translating into a higher grain yield.

An inverse relationship between plant height and grain yield for IT17K-849-2-1 was observed at Tilli. These results are in agreement with the previous study in cowpea by Yahaya *et al.* (2016) who reported that, the taller plants at maturity produced increased biomass yield at the expense of grain yield. The influence of plant diseases on plant height among genotypes is evident, as seen in the case of Padituya and IT10K-837-1, which were among the shortest genotypes and also among the most severely affected by anthracnose disease. This observation aligns with the findings of Enyiukwu *et al.* (2021), who, in their assessment of histological aberrations and damage modes in cowpea caused by *C. destructivum*, reported similar results.

5.5 Plant biomass yield

Biomass yield was significantly higher at Gumyoko than Tilli. The highest fodder yield was obtained by IT14K-1424-12 which was significantly different from the maximum yield obtained from IT14K-2030-2 at Tilli. MOUSA +1 and Songotra recorded the lowest biomass yield for Gumyoko and Tilli respectively. This implies that, there was varying levels of biomass yield obtained among genotypes between the two study locations. The biomass yield variation among genotypes may be due to the inherent differences in their genetic constitution as observed by Agyeman *et al*. (2020). Again, it is probably the case that the inherent traits of IT14K-1424-12 enabled it to produce taller plants and vegetative growth as opposed to MOUSA +1 which by virtue of its genetic nature produced shorter plants. The variation observed in relation with the interaction effect implied that environmental factors and soil conditions influenced biomass yield of the test genotypes. This finding again agrees with the report of Agyeman, *et al*. (2020).

It is possible that the low grain yield at Gumyoko was being compensated with fodder yield as postulated by Abdou (2021) that, grain and fodder yields have inverse relations. This assertion deviated with the findings on the performance of IT14K-2030-2 and IT1424-12 as they produced higher yields of both biomass and grains.

The heavy rainfall recorded at the flowering stage might have favored more vegetative growth at the expense of reproductive growth, hence, the reason for the observed high fodder yield at Gumyoko as compared to that of Tilli. This assertion is in accordance with the findings of Abdou (2021).

5.6 Grain yield per hectare

The study revealed that grain yield varied significantly among genotypes and location × genotype interaction. Generally, grain yield obtained from Tilli was significantly higher as compared to an average grain yield from Gumyoko. The cause of low grain yield recorded at Gumyoko could be attributable to the high average disease severity at the flowering stage compared to severity at Tilli. Anthracnose disease might have interfered with the normal physiological processes and affect optimal flowering and pod formation. This finding is in conformity with the report of Eno *et al*. (2016) who stated that, anthracnose disease reduced cowpea yield significantly at the reproductive stages if intervention is not made, causing pods to develop poorly and cotyledons to deteriorate. Again, the flowering stage at Gumyoko coincided with the peak of rainfall in September, 2022 which caused inflorescence to drop translating to low grain yield. This assertion is consistent with the one associated with Asare-Bediako *et al*. (2018) on screening cowpea genotypes for resistance to viral diseases.

The study unveiled that IT14K-2030-2 and IT14K-1424-12 demonstrated significantly higher grain yields compared to other genotypes, and concurrently, these top-yielding genotypes exhibited resistance to anthracnose disease. IT17K-1095-2-2 and IT17K-849-2-1 obtained the lowest mean grain yield for Gumyoko and Tilli respectively. This performance is probably due to the inherent genetic potentials of the individual genotypes in yield or disease tolerance.

5.7 Hundred seed weight

Hundred seed weight is one of the important parameters usually employed in assessing cowpea productivity (Agyeman, 2014). Gumyoko exhibited a higher 100-seed weight compared to Tilli, and this variation may be attributed to differences in the genetic makeup of cowpea genotypes, the impact of diseases, and variations in climatic and soil conditions. This observation aligns with the findings of Sujata *et al*. (2021), who reported a negative and significant correlation between 100-seed weight and both mean disease incidence and disease severity.

5.8 Pathogenicity test of the pathogen associated with anthracnose disease

In this study, the fundamental symptoms observed on innoculated cowpea leaves were sunken necrotic lesions, consistent with the findings of Pandey *et al*. (2021) regarding field-relevant new sources of resistance to anthracnose.

IT17K-1707-2-2, IT17K-1367-2-1, along with the susceptible check, Padituya, were all classified as highly susceptible based on the disease severity score adopted by Adebitan *et al*. (1992), reaffirming their susceptibility as previously observed in the field trial. IT14K-2030-2, Wangkae, and the resistant check, MOUSA +1, demonstrated high resistance, with all three genotypes maintaining their levels of resistance recorded from the field trial, except for Wangkae, which displayed moderate resistance to anthracnose in the field experiment. The case of Wangkae might have been occasioned by the different inoculation methods employed in the two cases agreeing with the finding associated with Kadege *et al*. (2022) on pathogenicity and approaches for management of anthracnose. The combined analysis of morphological characteristics of the pathogen, field symptoms, and pot experimental results provided additional confirmation that the isolated pathogen was *Colletotrichum lindemuthianum*, and the same organism infected the cowpea genotypes in the field conditions. Among the uninoculated genotypes, namely IT14K-849-2-1, IT14-2030-2, IT17K-1707-2-2, and IT17K-1367-2-2, mild symptoms of anthracnose disease were still observed at a later stage. This suggests the possibility that the source of inoculum may have originated from the seeds of these genotypes, given that anthracnose disease is known to be seed-borne.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From the study, the following conclusions were made;

i. The current investigation has validated the predominant seed transmission of anthracnose, significantly impacting seed germination. This disease stands out as a prominent economic threat to cowpea crops in farmers' fields, and preventive measures, such as rigorous seed health assessments, are crucial for sustaining the productivity and overall health of the crop.

ii. Also, the study reveals that cowpea anthracnose occurred throughout all growth stages of the plant, with heightened severity and incidence particularly during the reproductive stages. Therefore, cowpea farmers should prioritize selecting suitable planting dates or opting for early maturing varieties to escape the disease and its effects on the crop.

iii. The study showed that the cowpea genotypes evaluated ranged from highly resistant to highly susceptible to anthracnose disease. According to the findings, two lines among the evaluated genotypes, namely; IT14K-1424-12 and IT14K-2030-2 demonstrated significant resistance to anthracnose disease as well as high grain yield potential exceeding 2.0 tons per hectare. Majority of the test genotypes including IT17K-2024-4, IT17K-1367-2-1, and IT17K-1704-5 were found to be highly susceptible to anthracnose disease coupled with low yield.

6.2 Recommendations

Based the conclusions made above, the following recommendations were made;

i. Cowpea farmers in northern Ghana are advised to prioritize seed health testing before planting to proactively mitigate the risks associated with anthracnose.

ii. Cowpea genotypes IT14K-2030-2 and IT14K-1424-12 are recommended for release as varieties to farmers for cultivation as part of an integrated disease management approach by virtue of their dual potentials of disease resistance and high yield. This precautionary measure aims to prevent cowpea farmers from over-relying on cultivars highly susceptible to anthracnose, which often results in lower productivity.

iii. The study could be replicated in other agro-ecological zones of Ghana in multi-locational trials to find facts on the impact of the environment on the present observation.

iv. Future researchers are advised to consider conducting quality test on the harvested cowpea grains to assess the impact of anthracnose disease on grain quality.

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