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EXPLORING FUSARIUM SPECIES AS POTENTIAL BIOLOGICAL CONTROL AGENT AGAINST STRIGA HERMONTHICA

\mathbf{BY}

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(UDS/MBT/0001/21)



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DECLARATION

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I, hereby declare that this thesis is the results of my original work and that no part of it has been presented for another degree in this University or elsewhere:

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I hereby declare that the preparation and presentation of the thesis was supervised following the guidelines on supervision of thesis laid down by the University for Development Studies.

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ABSTRACT

Striga species also known as witchweed are obligate root parasitic plants mostly found in sub-Saharan Africa (SSA). S. hermonthica, S. gesnerioides and S. asiatica parasitize most essential cereal crops leading to low yield and productivity. Available management efforts have been less or not effective at all due to the cost involved and the life cycle of the parasite. The development of biocontrol agents has shown to be one of the most effective ways in controlling Striga. This study was aimed at isolating and identifying of Fusarium isolates from diseased Striga leaves that have biocontrol potential against striga spp. The CTAB method was used to obtain DNA from isolated fusaria. Relying on sequences of translation elongation factor 1 alpha, the fusarium species were confirmed to be Fusarium duofalcatisporum (7), Fusarium incarnatum (1), Fusarium oxysporum (4), Fusarium fredkugeri (1) and Fusarium verticillioides (1). An in-vitro detached leaf assay was used to estimate the pathogenicity of the various Fusarium isolates. Lesions caused by the five Fusarium species on Striga hermonthica varied from 1.00 to 3.75 mm and 1.44 to 4.44 mm for week 1 and week 2 respectively. An analysis of variance indicated significant interactions between Fusarium isolates and the Striga leaves. Isolate F. oxysporum-C9 had the lowest lesion effect while F. fredkugeri-C10 exhibited the highest lesion on the Striga hermonthica leaves.



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DEDICATION

I dedicate this work to the Almighty Allah and all members of my family for their support.



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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Often referred to as witchweed, Striga species are root parasitic flowering plants that are widespread in sub-Saharan Africa, the Middle East, and portions of Asia. They severely restrict the output of crops including cereals and legumes. Striga has the capacity to adjust to a broad of range hosts and environmental situations, making it one of the most successful and widely distributed parasitic plants (Jamil et al., 2021). The degree to which Striga inhibits the growth of its host varies greatly and is impacted by several different factors, for instance the environment, infestation level, and genotype of the host plant's (Timko et al., 2012). High occurrence of Striga has been linked in several studies to low soil fertility, increased grain production, and intensification of land use through continuous cropping (Menkir et al., 2020). Developing striga roots cling themselves to the host plant's root (Tadesse, 2022). Striga has the ability to reroute water uptake from host plants and also drain the nutrients absorbed by these crops. This can cause stunted growth, low yields or reductions in productivity, and occasionally complete losses; in extreme cases, it can even lead to plant death under severe infection (Ohlson and Timko, 2020). Striga cannot exist on its own because prior to emerging from the earth, it is entirely dependent on its host (Jamil et al., 2021). Despite having the ability, they need other resources in order to photosynthesize and require a host to survive during the post-emergence phase of their life (Jamil et al., 2021).

Of the 42 known *Striga* species, *S. hermonthica*, *S. asiatica*, and *S. gesnerioides* are the very economically significant parasitic plants; these three different species vary regarding their host specificity. Its economic influence is seen in Africa in cereal and legumes production, which

are important to farmers since they serve as sources of food and fodder for low-income earners in the tropical and semiarid zones of cultivation (Arba, 2020). *Striga* susceptibility has been linked to yield losses of 29 to 58% in West Africa (Sadda *et al.*, 2021). It is a significant blow to Sub-Saharan Africa's cereal output, with yield losses estimated at US\$ 9 billion a year (Nchanji *et al.*, 2020). *S. hermonthica* has caused up to a 100% reduction in maize production in densely infested farms in Ghana, forcing farmers to quit their fields and some farmers have been compelled to switch from growing cereals to other less important crops in badly affected locations. *Striga's* effects are hard to quantify, however some estimates suggest that it affects the lives of over 300 million Africans (Jamil *et al.*, 2021). Every year, *Striga* species destroy crops, attacking crops, significantly lowering agricultural outputs including rice, cowpea, sorghum, sugarcane, finger millet, pearl millet, and maize (Mudereri *et al.*, 2020).

Because the parasitic weed is exclusively dependent on its host plant for nutrients and growth, farmlands with high invasion rates suffer significant crop damage, including chlorosis, wilted silk, thin stalks, decreased height, and total crop loss (Mbuvi *et al.*, 2017; Menkir *et al.*, 2020).

While several methods have been created and extensive research has been done on *Striga* management, the problem has continued and grown in scope despite these efforts. This parasitic weed has been managed in a way using a variety of techniques, including, using of resistant crop types, chemical, and biological approaches. Using the hand to pullout, rotational cropping, intercropping, push-pull technologies, and the increment of the soil fertility (nitrogen fertilization) are examples of techniques that have been tried to control *striga* on farms but all have not proven to be successful (Yali, 2022). To control striga, many chemical management techniques have been employed, such as weedicide, genetic control, suicidal germination, resistant crop types, biological control agents like bacteria and fungus, and certain insects (Naoura *et al.*, 2021). The prevalence of *S. hermonthica* has not been eliminated by any of these techniques.

Thus, the primary goal of this analysis is to clarify the difficulties and financial significance of *Striga* weed for crops and to examine environmentally friendly bioinoculants for weed control in Sub-Saharan Africa.

Fusarium species consists of both pathogenic and non-pathogenic strains, which are usually found in soils. The non-pathogenic ones are important and beneficial to be used in controlling infected crops (Joshi et al., 2013). According to Sajeena et al (2020) though they have similar characteristics as the disease-causing ones, many other strains differ in a way that they do not cause diseases (non-pathogenic), however they penetrate roots, hence making them essential, an example of such, that does not cause disease to cereal is F. oxysporum but which is specific to Striga species, and non-pathogenic to cereal crops (Elzein et al., 2006; Beed et al., 2007). Their tolerance nature gives them the advantage to be used as biocontrol agents against parasitic weeds that penetrates plant roots.



1.2 Problem Statement and Justification

Over half of Ghana's workforce is employed in the formal and informal sectors of the country's economy, which is dominated by agriculture (Peprah *et al.*, 2023). Improving food security, fostering supply-demand chain systems (such as the provision of raw materials to industry), and lowering poverty and inequality to promote shared prosperity, all depend heavily on the growth of agriculture. Ghana's agriculture industry is a major source of income for rural residents and provided over 21% of the country's GDP in 2022 (GSS, 2023) (Adzawla *et al.*, 2022; Giller, 2020). Regretfully, Ghana's agricultural potential not been fully realized. Farmers in Ghana are still the most impoverished people in society and key crop yields are still below potential (Darfour and Rosentrater, 2016). The infestation of *Striga* is one of the many

problems that have caused significant losses to agriculture in recent years (Dossa *et al.*, 2023). Food insecurity has increased as a result of the pace at which agricultural products and produce are impacted by, insects, pests and diseases, parasites, and other harmful organisms (Merem *et al.*, 2019). Food sovereignty in Ghana and other African nations, according to Raheem *et al.* (2021), depends on cowpeas, maize, millet, rice, and sorghum production. In addition to being a global issue, food insecurity is a significant barrier to Africa's achievement of sustainable development (Raheem *et al.*, 2021). When food is abundant, especially when social security is the primary concern, a country is partially secure.

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Poor soil fertility, sporadic droughts, and infestation by parasitic weeds like *Striga* are the main obstacles to producing large grain yields (Menkir *et al.*, 2020). The degree to which *Striga* inhibits the growth of its host varies greatly and is influenced by a number of variables, including the environment, infestation level, and genotype of the host plant. Numerous investigations have linked to the elevated occurrence of *Striga* to deficient soil fertility, increased land usage through continuous cultivation, and increased production of cereals (Begna 2021). According to reports, places with low soil fertility, little rainfall, and little to no fertilizer use have more severe infestations (Menkir, 2020).

Striga susceptibility has been linked to yield losses of 68% and 79% in Central and West Africa, respectively (Ndambi *et al.*, 2011). It is a significant blow to Sub-Saharan Africa's cereal output, with yield losses estimated at US\$ 9 billion a year (Nchanji *et al.*, 2020). S. hermonthica has caused up to a 100% reduction in maize production in densely infested farms in Ghana, forcing farmers to quit their fields (CSIR, 2011).

Berner et al. (2003) reported that *S. hermonthica*, *S. asiatica*, *S. aspera and S. Forbesii Benth* are the four *Striga* species that seriously harm cereal crops. According to Kim *et al.* (1991) and Koua (2011), *S. Hermonthica* is the most common species of *Striga* in SSA.

Numerous strategies have been employed to fight this weed parasite, such as Integrated Striga Management (ISM), which has been applied to a variety of crops in Africa. There have combine usage of resistant varieties, biological control agents, cultural practices, and chemical control to minimize the lost caused by the parasitic weed, although these methods have not totally eradicated this parasitic weed (Chitere and Omolo, 1993; Khan *et al.*, 2008; Hearne, 2009). African researchers have intensified their studies on Striga and how to eradicate it, more work is needed to create environmentally friendly and economically viable control options for low-income local farmers (Kanampiu *et al.*, 2003; Oswald, 2005; Kabambe *et al.*, 2008; Atera *et al.*, 2013). Fusarium species, which causes Fusarium wilt of Striga in Ghana, is thought to be a viable biological control agent for the weed in Western Kenya, according to Avedi *et al.* (2014).

According to research by Larkin and Fravel (2002); Benhamou and Garand (2001); Fravel et al. (2003), Fusarium oxysporum acts as a biocontrol agent because of its ability to compete with other pathogenic organisms for nutrients in the soil, slowing down their rate of germination. Fravel et al. (2003) found that Fusarium species can also compete at the site of infection on the root and can trigger the plant's defence mechanisms, resulting in systemic resistance; this can make the host crop's ability to prevent the parasite from attaching to its roots and going underground. These actions slow or decrease the rate of infection that the parasitic weed could have on cereal crops.

The creation of a biocontrol technique employing *Fusarium* spp. may enhance the crop's performance even in locations where *Striga* infestations exist, boosting output to satisfy the food demands of Ghanaians specifically, sub-Saharan Africans generally, and the global community at large.

Similar work has been done by Charles (2023) on Characterization and pathogenicity of *Fusarium* species isolated from *Striga hermonthica* and reported on how the *Fusarium* species

isolated were pathogenic and could be used as a biocontrol agent against *striga*. On the back of his study, we needed to research further to improve on the study as well as preserve the *Fusarium* isolates for future studies.

1.3 Main Objective

The research seeks to

1. To isolate and identify *Fusarium* species from *striga* leaves with potential biocontrol against *striga* species.

1.4 Specific Objectives

- 1. To isolate Fusarium species from diseased Striga leaves.
- 2. To molecularly characterize isolated *Fusarium* from the *Striga* leaves.
- 3. To determine the pathogenicity of the characterized Fusarium species from Striga species



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Sorghum- an overview

Sorghum is one of the top five grain crops grown worldwide, also known as Sorghum bicolor (L.) Moench (Mace *et al.*, 2009; Venkateswaran *et al.*, 2014). It makes up billions of people's basic diet and plays a crucial direction in the production of food worldwide (Mace *et al.*, 2009). A versatile crop, sorghum is used for its grain, sweet stem, fodder, and broomcorn.

Sorghum is an important crop to many of the world's poor, who live in fragile agro-ecological zones, as they depend on it for their food security and play a vital role in the battle against hunger and food insecurity. It is reportedly cultivated in large quantities in Africa, China, the US, Mexico, and India. One of the most important crops high in carbohydrates and also known as the "King of Millets" and provides a staple diet for millions of people living in semi-arid tropical regions (Cardoso et al., 2015). Sorghum is grown in over 100 countries worldwide (Rakshit et al., 2014), and it has become increasingly important, especially in Asia, Africa, and other semi-arid regions. Although sorghum is predominantly grown as a major feed grain for animal feeding in various western countries including the United States, Brazil, and Australia, it also serves as a staple diet for humans. Still, a lot of research has been done on sorghum for human consumption because of its enormous nutritional and functional potential (Rakshit et al., 2014). In addition, it provides alcoholic beverages, building materials, bioethanol, and fuel. While it is primarily used for fodder and animal feed in affluent nations, it is one of the most significant food crops in arid and semi-arid regions of the world (Hariprasanna and Patil, 2015; Venkateswaran et al., 2019). With an average annual global output of 50 megatons, the United States currently leads the world in sorghum production, followed by Nigeria, India, and Mexico (FAOSTAT, 2019).



2.1.1Origin of Sorghum

The Sahara, where hunter-gatherers lived circa 7500 BC, is where the oldest evidence of using wild sorghum as food comes from (Venkateswaran *et al.*, 2019). Similar to this, a 2017 research work by Winchell et al. uncovered that Neolithic people in Sudan from the fourth millennium BC are home to the oldest cultivated sorghums. There is disagreement on the precise place and time of sorghum domestication (De Wet *et al.*, 1970; Venkateswaran *et al.*, 2019); however, archaeological data points to eastern Sudan circa 3000 BC as the likely domestication site (Fuller and Stevens, 2018). According to some research, there might have been multiple domestication events, which could account for the origin of the more recent group guineamargaritiferum of the genus Sorghum (Kimber, 2000; Mace *et al.*, 2013). Rowley-Conwy *et al.* (1997) put out 3 theories regarding the domestication of sorghum. Based on research by Murdock (1959), who detailed an autonomous nuclear Mande center in West Africa, the first theory was developed. The next theory suggests that sorghum originated in the eastern Sahara between 9700 and 6200 BC (Ehret, 2014), while the last theory is based on proof that the race Durra existed in India around 4000 BC.



2.1.2 Economic Importance of Sorghum

Using average data over three years, 2014, the top producing nations of sorghum are the US, Nigeria, China, Brazil, Australia, Ethiopia, Mexico, Sudan, India, and Niger (FAOSTAT, 2017). Together, these nations account for over 76% of global output of sorghum and 65% of its harvested land. Sorghum covers 7.5 million hectares (m ha) in Asia (Yemen, Saudi Arabia, Thailand, China, India, Myanmar, and Pakistan)

and 28.5 million hectares (m ha) in Africa (mostly in Ethiopia, Mali, Nigeria, and Sudan). Even though more than 100 countries are known to cultivate sorghum, only eight of them have more

than one million hectares under cultivation; these eight nations collectively account for more than 60% of the world's sorghum production. Grain is a vital component of animal and bird feed in affluent nations like the US, Japan, and Australia, as well as in certain developing nations like China and Mexico. Sweet sorghum is a unique crop with several benefits as food, feed, fodder, fuel, and a source of fiber. Lately, it has become a significant biofuel crop. Sorghum stems are an excellent source of energy for the production of biofuels, bioenergy, biogas, and bioethanol because they are high in soluble sugars (such as glucose, sucrose, and fructose) and insoluble carbohydrates (such as cellulose and hemicellulose). Similar to sugarcane, sweet sorghum with sugary liquid, both bagasse and ethanol are employed in the stem. While the leftover material after crushing cane, known as bagasse, is utilized in a variety of ways, such as the production of wood composite, the sweet juice from the stem is fermented to produce ethanol, which is used as fuel (Mathur *et al.*, 2017; Wright *et al.*, 2017).

2.1.3 Nutritional Composition and Health Benefits of Sorghum

Numerous functional and physiological benefits are provided by sorghum. It has potential health advantages and is a good source of vitamins, proteins, carbohydrates, and phytochemicals. Protein (10%), fat (2%), carbs (68%), and dietary fiber (10%) are all included in it (Awika and Rooney, 2004). Sorghum takes pride in being a "healthy cereal" due to its greater amount of dietary fiber, micronutrient content, complex carbohydrate makeup, and phytochemicals that have positive health effects. It is simple to turn into food products via micronization, steam-flaking, extrusion. Its pale hue and neutral, occasionally sweet flavor make it versatile in a range of recipes. It works well in a variety of baked goods that are free of gluten as a wheat flour alternative. The nutritional and physiological benefits of sorghum, as well as the various food products that may be made from it, are well documented in rural areas and supported by science.

De Morais Cardoso and Pinheiro (2015) talked about the composition of this grain and its health advantages in particular. The nutritional value and chemical makeup of whole sorghum grains are comparable to those of rice, wheat, and maize. The primary components of sorghum are polysaccharides, which include proteins, lipids, and starch as well as non-starch. A 100 g portion of sorghum grains has between 296.1 and 356.0 kcal of caloric content. Zinc, potassium, and phosphorus are among the essential minerals found in sorghum, while the precise amounts vary depending on the growing region (Martino *et al.*, 2012). Studies conducted suggested that soluble complexes found in sorghum, such as polycosanols and phenolic complexes, were shown to balance or stabilize the gut microbiota and factors linked to cancer, diabetes, obesity, oxidative stress, inflammation, dyslipidemia, and hypertension in vitro and in animals. Furthermore, it was noted that most sorghum varieties have substantial levels of phenolic compounds, specifically 3-deoxyanthocyanidins and tannins.

Patients with celiac disease are advised to avoid sorghum because it is gluten free (Kasarda, 2001; Pontieri *et al.*, 2013). The small intestine's mucosa is harmed in celiac disease due to consumption of specific wheat proteins, particularly gliadins and glutenins, as well as comparable proteins found in rye and barley (Fasano and Catassi, 2001). The sole known cure is to completely avoid gluten for the rest of one's life, with an estimated 1:266 global prevalence (Fasano and Catassi, 2001). Patients with celiac disease can choose from a variety of foods as an alternative because sorghum makes a wonderful foundation for gluten-free breads and other baked goods (such cakes and cookies), snacks, and spaghetti.

Because both the protein and the starch in sorghum are generally poorly digested, the grain has a lot of explores for beating down weight and obesity. Due to its slower digestion and lower glycemic index, sorghum starch remains in the digestive system longer than starches from other

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grain flours or flour substitutes. For the goal of managing obesity, there are sorghum cultivars that are high in polyphenols, particularly condensed tannins, and act as natural antioxidants (Dykes and Rooney, 2006; Moraes *et al.*, 2017).

Minerals and vitamins are among sorghum's other essential components. Due to its numerous nutritional benefits, sorghum grain is now being produced and used more effectively as a diet for humans (Zhu, 2014).

2.2 Cowpea- Overview

The warm-season legume cowpea (*Vigna unguiculata* [L.] Walp) (Fabaceae) is primarily eaten as a grain but can also be eaten as a vegetable in the form of young pods and leaves (Boukar *et al.*, 2018). Cowpeas are a highly adaptable crop that can withstand heat and drought (Boukar *et al.*, 2018). An area of more than 14.5 million hectares is planted with cowpeas annually, with 6.2 million metric tons of crop produced worldwide. Over the course of 30 years, the average rate of growth in global cowpea production was 5%, with annual increases in area of 3.5% and yield of 1.5%; the expansion of the area accounted for 70% of the total growth during this period (Boukar *et al.*, 2016). Africa accounts for more than 80% of cowpea production worldwide, accounting for 84% of the global cowpea producing area and 83.4% of total cowpea production. In Africa, cowpea was produced on an estimated 12.3 million hectares in 2014, according to FAOSTAT (2016). The majority of the crop was produced on 10.6 million hectares in West Africa, primarily in Nigeria, Niger, Burkina Faso, Mali, and Senegal. Despite the fact that cowpea variants are centered in Ethiopia (Beshir *et al.*, 2019).

In Ghana, cowpeas rank as the second most important type of legume. In terms of acreage under cultivation, annual production, and consumption, it is surpassed only by groundnut. Ghana's cowpea crop reached its maximum size in 2003, at 190,400 hectares (MOFA, SRID, 2011). Nonetheless, the area planted to cowpeas has somewhat decreased since then, reaching 163,700 acres in 2010. Between 2004 and 2010, the annual production of cowpea grains

increased from 142,300 MT to 219,300 MT, despite the area loss. This is a sign of increased yields, which could result from the use of a mix of elements such as higher yielding varieties and improved agronomic techniques. In Ghana, cowpea consumption exceeds production. In 2010, the nation produced 219,300 MT of cowpea grains, which were supplemented by 3,380 MT of imports. Nonetheless, compared to the 1990 deficit of 113,000 MT, this represents a significant improvement (Owusu *et al.*, 2020). There are several reasons why Ghana cannot produce enough cowpea to feed its people. According to Owusu *et al.*, (2020), the primary cowpea production region in Ghana is the Guinea Savanna zone. According to MOFA and SRID (2011), the Upper West and Northern areas produced 75,969 and 105, 841 MT of cowpea in 2010 respectively. Unfortunately, owing of the extended drought in these areas, output is limited to a brief period of time each year. Varietal preference is another important element that can be influencing cowpea production and consumption in Ghana (Langyintuo *et al.*, 2003). Reporting by Quaye *et al.* (2009), cream seeded cowpea is preferred by Ghanaians.



2.2.1Origin of Cowpea

It was proposed that cowpea originated in Africa (Richard *et al.*, 1847). Since wild cowpea plants have been discovered in Madagascar and tropical Africa, where they were most likely domesticated after the Neolithic era, this notion has not been questioned (Vanderborght *et al.*, 2001). Pasquet (2000) proposed that *V. unguiculata* ssp. unguiculata var. spontanea is the most likely origin of the domesticated cowpea.

Numerous researches have been conducted in recent decades to identify the specific domestication site and cowpea diversity centers, but it has been challenging to come to a definitive conclusion. The domestication of cowpeas has been attributed to a number of places,

including Ethiopia, West Africa, Eastern Africa, and Southern Africa. Using morphologic data and amplified fragment length polymorphisms (AFLPs), Coulibaly *et al.* (2002) deduced that the wild species originated in Eastern Africa. In this instance, the plant should have been domesticated in Northeastern Africa and then most likely spread to Western Africa. Padulosi (1997) assert that West Africa seems to be the epicenter of cultivated form variety. It was also proposed that there was a "diffuse" domestication on the African savanna following the spread of grains. The last theory was put out by Harlan (1971), who believed that the African Non-Center was where the cowpea was domesticated. Cowpeas are ancient legumes that were domesticated by African farmers, gatherers, and cultivators from their wild forms in Africa as early as the Neolithic era, regardless of where the domestication occurred. India, which was formerly thought to be a secondary center of cowpea genetic variety, was first exposed to the cowpea during the Neolithic era (Narayana and Angamuthu, 2021).

2.2.2 Economic Importance of Cowpea

In less developed tropical nations where animal protein is hard to get by for the family, cowpeas perform a vital part in the livelihoods of comparatively impoverished people. Cowpeas are a strategically important crop for enhancing population health and food security across all continents. It has various agronomic, environmental, and economic benefits, is extensively consumed throughout many countries, and possesses exceptional nutritional and nutraceutical qualities. It also aids in food security and environmental preservation (Carreiro da Silva and associates, 2018). Apart from its nutritional content, ability to improve soil, and fodder value, cowpea is an important source of money for farmers; the grain and leaves can be sold in nearby marketplaces. (Alemu *et al.*, 2016). One of the most popular grains and legumes in nearly every local market, particularly in Sub-Saharan Africa, is cowpea. For farmers and small- and medium-sized business owners, it is a commodity that generates income (Timko and Singh,

2008). Ngalamu *et al.* (2015) state that trading in fresh fruit, processed foods, and cowpea leaves gives people in both rural and urban areas, especially women, the chance to make a little money. Exchange of cowpea haulms for feed to ruminants, big and tiny, can also yield financial benefits. In all cowpea-producing areas, grain marketing took precedence over the cultivation of cowpeas, according to a survey by Beshir *et al.* (2019). Sixty-one percent of Ethiopian farm households said they sold at least some of their cowpea goods from the previous harvests on the local market. About the portions of the cowpea that are commercialized, there are notable differences throughout the regional states. Especially in Gambella and the southern regions of the nation, the marketing of fresh cowpea leaves is very crucial. Furthermore, majority of farmers (73.3%) expressed satisfaction with cowpea market pricing due to their similarity concerns the cost of common beans, a popular legume in Ethiopia and a component of the commodity exchange market (Beshir *et al.*, 2019).



2.2.3 Nutritional Composition and Health Benefits of cowpea

Cowpeas are a native African grain legume that are highly nutritious and are grown in tropical and subtropical regions of the world. Cowpeas have edible parts that are strong in protein, carbs, vitamins, and minerals, such as fresh leaves, immature pods, and grains (Alemu *et al.*, 2019). Carneiro da Silva *et al.* (2018) state that for millions of people living in poverty—an estimated 38 million families, or 194 million individuals—cowpeas are an important source of protein that may be added to low-protein cereals and tuber crops to improve their nutritional value. Because of this, cowpeas are a multipurpose crop that may be used to generate hay, silage, pasture, green manure, concentrate for farm animals, and food for humans and cattle (Alemu *et al.*, 2019). The grain, which includes 22–23% protein (as opposed to 2% in cassava and 10% in maize) and a considerable amount of thiamine (vitamin B1), riboflavin (vitamin

B2), and niacin (vitamin B3), is the most useful part of the cowpea plant for human consumption. Its iron and calcium content are also higher than that of cereals (Ngalamu *et al.*, 2015). By increasing the given types and quantities of vitamins and proteins found in cereal grains, cowpea grains can also be used to enhance human diets. Cowpea grains, for instance, are a good source of folic acid, a vitamin that is essential for everyone, but especially for expectant mothers, as it helps to prevent neural tube problems in babies, such as spina bifida. During the "hungry period," fresh pods and leaves, as well as dry, fresh grains of early-season cowpea cultivars, are frequently a significant source of nourishment (Kebede and Bekeko, 2020).

2.3 Maize- an overview

Together with rice and wheat, maize (*Zea mays* L.) is one of the most popular cereal grains in the world (FAOSTAT, 2014). The main goal of maize breeding has been to increase yield stability and potential under various environmental circumstances. Nonetheless, numerous projects to breed genotypes with enhanced quality have been started in response to the need for maize that is healthier and more nutritious (Berardo *et al.*, 2009).

While there are significant national variations in the processing and consumption of maize, two of the most widely used products are flour and meal (USAID, 2002). Because of wastage, their usage in nonfood products, and the removal of some of the bran—the outer layers of the grain that is typically used as animal feed—during milling, The true amount of these cereals consumed by humans is slightly less than the estimated figures. Since the majority of micronutrients in maize are primarily found in the grain's outer layers, as they are in all cereals, most vitamins and minerals are lost during the milling process.

Around the world, several varieties of maize are grown, and one notable distinction is color. Kernels of maize can be any color—white, yellow, red, or black, for example (Zilic *et al.*, 2012). While most American maize is yellow, people in Africa, Central America, and the



southern United States prefer white maize. Yellow maize is not commonly consumed in Africa due to social status views; it is thought to be connected to food aid programs and is only consumed by the underprivileged. Additionally, yellow maize is largely used by the feed sector to make animal feed (Ranum et al., 2014).

2.3.1Origin of Maize

One of the crop plants that have been studied the most in terms of agronomy, cytology, genetics, and its evolutionary history under domestication is maize (Zea mays). Nevertheless, opinions on where it originated continue to differ (Staller et al., 2006). The terms "corn" and "maize" are interchangeable in the West. This is because, in the early days of British and American trade, all grains were referred to as corn, and since maize being the most widely used grain in trade, the name was kept for it (Ranum et al., 2014). While there is some debate on the word's etymology, it is widely agreed upon that the Arawac tribes of the indigenous people of the Caribbean are the source of the word. Linnaeus added the name as a species epithet in the scientific classification Zea (Zea mays L.) based on this common name.



Between 7000 and 10,000 years ago, maize is said to have been one of the first crops that farmers grew. Evidence of maize being used as food can be seen in some archaeological places in Mexico with tiny maize cobs that date back more than 5000 years were discovered in caves (Mangelsdorf et al., 1964). It is supported by the finding of cave corncobs and fossil pollen in ancient sites that maize originated in Mexico. According to some theories, maize originated in Bolivia, Ecuador, and Peru's high Andes; this is corroborated by the discovery of popcorn in South America and the extensive genetic diversity of Andean maize, especially in Peru's highlands (Grobman, 2008). Alternatively, it developed in the Asian Himalayan region as a consequence of a hybrid between some Andropogoneas (probably Sorghum species) and Coix spp., both of which have five pairs of chromosomes in their parents.

With the application of sophisticated techniques and genetic research, biological and archeological methods to pinpoint the location and time of maize's first domestication are constantly developing. While some scholars believe that maize originated from a wild grass called teosinte, which is very different from modern maize, others propose that a hybrid of two wild grasses—a species of Tripsacum and a perennial subspecies of Zea diploperennis—formed. Over several millennia, Native Americans changed maize into a more nutritious crop by methodically gathering and growing the plants most suitable for human use. The result was a plant bearing more rows of kernels and larger cobs. This provides them with enough food to cover most of their meals for a whole year, enabling them to spend a considerable amount of time in one place.

2.3.2 Economic Importance of Maize

The manufacture of ethanol fuel, or ethyl alcohol, which is the same kind of alcohol as in alcoholic beverages, is a significant aspect of maize farming. It is mostly used as a biofuel additive for gasoline in motor vehicles (Taneja *et al.*, 2021). The main feedstock used to make ethanol is maize. Prices for maize have risen due to the strong demand for ethanol production, which has also encouraged farmers to expand their maize fields (Kocak *et al.*, 2022). The development and use of biofuels raise a number of social, economic, environmental, and technical concerns, such as the "food versus fuel" argument and the impact of declining oil prices.

The world's greatest producer of maize and the leader in the global maize trade is the United States. The demand for American maize is accounted for by exports to the tune of about 15% (Tigchelaar *et al.*, 2018). Experts believe that because there is little demand for exports, the



supply and demand dynamics in the American market mostly set maize prices, with the rest of the globe typically adjusting to reflect these prices. World corn commerce is heavily influenced by the United States' supply of maize. After learning the size of the U.S. harvest, Argentine farmers plant their maize, enabling a prompt, supply-oriented reaction to the short U.S. crop. When crops were abundant or international prices were favorable, a number of nations includes South Africa, Brazil, Romania, and the Ukraine, exported a sizable amount of maize. China has been a significant contributor to the volatility of the world market for maize, fluctuating between importing and occasionally being the second-largest exporter. Due to the fact that Chinese prices are typically higher than those on the international market, the government's export subsidies and tax breaks play a major role in China's maize exports (Yang et al., 2008). Mexico is a major producer of maize, but it imports yellow maize to feed its cattle in order to maintain rising meat output (Zahniser et al., 2019). White maize is processed into food products for humans.

2.3.3 Nutritional composition of maize and Health Benefits

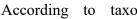
With around 72% carbohydrates, 10% protein, and 4% fat, maize contains less protein than rice and wheat but nevertheless has an energy density of 365 kcal/100 g (Nuss and Tanumihardjo, 2010). Along with fiber, maize is a good source of many B vitamins and critical minerals. However, it is generally low in calcium, folate, and iron, and it is deficient in several other nutrients, such as vitamin C and B12. Some foods or dietary components, like vegetables, tea (for example, oxalates), coffee (for example, polyphenols), eggs (for example, phosvitin), and milk (for example, calcium), might block the absorption of iron, especially the nonheme iron found in maize (Ranum et al., 2014). To enhance micronutrient consumption and avoid iron insufficiency, iron and other vitamins and minerals have been added to maize flour and cornmeal in nations where anemia and iron deficiency are regarded as moderate to serious public health issues.

2.4 Millet-an overview

Millets belong to the family of cereals that are found worldwide and have varying cultural and regional significance. A diverse group of cereal crops known as millets consistently produce tiny seeds. Numerous genera, comprising several dozen species, have their origins in Africa and Asia and were domestically produced by small-scale farmers. Millets stand out due to their high nutritional content, minimal input requirements, and capacity to adapt to a range of agroecological settings. In dry, infertile, and marginal soils, millets are an important plant genetic resource for the agriculture and food security of low-income farmers (Garí, 2002). Their cultivation takes place in a variety of challenging conditions, especially in arid, semi-arid, to sub-humid agricultural ecosystems that are prone to drought (ICRISAT/FAO, 1996; Garí, 2002). Known as one of the most vital food grains for human nutrition, millet is a vital crop for the worldwide food and nutrition industry. About one-third of the world's population grows millet, which can be grown as a cash crop by small farmers and can tolerate marginal soil (Ashoka et al., 2020). According to Ushakumari et al. (2004), millets' capacity to withstand a wide range of agroclimatic conditions makes them crucial for food security as well. Ranking sixth (6th) among cereal crops in terms of worldwide agricultural production, one of the most significant crops resistant to drought is millet. These are significant crop species in underdeveloped nations. They provide resilience compared to major cereals, it is less resilient to pests and diseases, has a shorter growth season (producing grain only 60 days after sowing), and is more productive during dry spells (rice and wheat) (Devi et al., 2011; Popovic et al., 2020).

2.4.1 Origin of Millet

According to taxonomy, millets and sorghum are included in the subfamilies Andropogonoideae and Panicoideae, respectively, of the family Poaceae (formerly known as



Gramineae) (Amadou et al., 2011). About 500 species of millet are known to exist worldwide, but only a small number are grown for food crops. These species include foxtail millet (Setariaitalica, also known as Panicumitalicum), proso/broomcorn/common millet (Panicummiliaceum), finger millet (Eleusine coracana), and pearl millet (*Pennisetumglaucum*). Pearl millet is the type of millet that is most frequently produced in Africa and India.

Africa is home to a large number of millets' origins, varieties, and growing centers. Africa is home to extensive cultivations of both the West African millets (fonio, black fonio, and guinea millet) and the global millets (pearl millet and finger millet). Numerous cultivars of millets that have been adapted to difficult agro-ecological circumstances are among the huge genetic diversity of millets that African farmers care for (Garí, 2002).

Neolithic China is where millet, notably pros and foxtail millet first appeared. In China, the Neolithic era started at 10,000 B.C. But its exact start points and starting time have long been disputed (Lu et al., 2009). While millet is believed to have originated in Neolithic China, it is uncertain which species was domesticated first, foxtail millet or common millet. We do not yet know where they distributed or when they became domesticated (Lu et al., 2009).

2.4.2 Economic importance of Millet

The two most often grown millets worldwide are finger millet (Eleusinecoracana) and pearl millet (Pennisetumglaucum). Both finger millet and pearl millet are native to sub-Saharan Africa; finger millet comes from the sub-humid uplands of East Africa. Because they are cultivated in a range of climates, produce and trade the bulk of millet worldwide, and have profited from most millet research and agricultural initiatives, they are referred to as "global" millets. (Garí, 2002). Millions of sub-Saharan Africans work as small-scale farmers and depend on them as vital staple crops, and their production is widespread there. In addition, they are widely grown in rural parts of Asia, particularly in China, India, and Nepal. Consequently, two African millet cultivars that have proliferated and achieved notoriety globally are finger millet



and pearl millet (Garí, 2002). The majority of African nations place a high importance on the production and consumption of traditional fermented beverages made from cereals, which offer health, socioeconomic, recreational, and medical benefits (Amadou *et al.*, 2011; Aka *et al.*, 2014). Tropical cereals that are commonly used alone or in combination to create a range of traditional fermented beverages include maize (*Zeamays*), pearl millet (*Pennisetumglaucum*), finger millet (*Eleusinecoracana*), sorghum (Sorghumbicolor), and fonio (*Digitariaexilis*) (Aka *et al.*, 2014; Misihairabgwi *et al.*, 2018).

In Africa, grains can be consumed uncooked to create various of novel foods and drinks, including porridges, dumplings, baked items, and alcoholic and non-alcoholic beverages (Raheem, 2006). In sub-Saharan Africa, cereal grains—sorghum or guinea corn, maize, rice, and millets—are the main ingredients in fermented foods. Around the world, cereal porridge is a common breakfast dish. In tropical African nations, a variety of fermented and unfermented cereal meals are regularly ingested and constitute a significant portion of the diet (Hesseltine, 1979). Many African nations like eating porridge, both fermented and non-fermented (Amadou et al., 2011; Misihairabgwi and Cheikhyoussef, 2017). They are usually served with soup and vegetables and can be creamy, thick liquids or thick solids. Foods made from millet are made commercially or locally. Strong enzyme activity is used in the soaking, germination, and drying stages of the malting process to generate malt, a food or beverage that is produced locally or historically (Amadou et al., 2011). Many people in West Africa frequently eat spicy millet porridge or "Koko" as it is known in the Ghanaian dialect, for breakfast, lunch, or snack on a daily basis. Processed locally from germinated single cereal grains like millet or a combination of cereal grains, "pito" and "burukutu" are fermented and unfermented malts that are processed into a brownish suspension or liquor (Iwuoha and Eke, 1996; Badau, 2006). In Sub-Saharan Africa, burukutu is a popular alcoholic beverage (Amadou et al., 2011). African beers are typically sourer, lower in carbonation and devoid of hops than their Western counterparts.

According to Almadou *et al.* (2011), unprocessed African beers are consumed along with unfermented substrates and microorganisms. Weaning meals, drinks, beer, and biscuits and confections are a few of the growing principal applications of sorghum and millet as raw materials. Cereals such as millet, sorghum, and corn can be found more and more readily as grits, flour, and meals. Soft biscuits and cookies are made from sorghum, maize, and wheat; research is also being done on cakes and non-wheat breads (Amadou *et al.*, 2011). The ability to produce enough food limits the advancement of the infant weaning foods business. In addition to sorghum and maize, several commercially available beers contain sizable amounts of local millet. Grain storage quality, nutritional losses during processing, high import equipment costs, and cultivar diversity are some of the obstacles to increasing millet use in developing nations (Charalampopoulos *et al.*, 2002; Floros *et al.*, 2010; Amadou *et al.*, 2011).

2.4.3 Nutritional Composition and Potential Health Benefits of Millets

Millets are a precursor to nutrition that is necessary for human health (Kate and Singh, 2021). They are regarded as one of the most significant cereal grains in terms of health. According to Amadou et al. (2013), they are vital source of food and a good source of energy in arid and semi-arid regions of the world. Additionally, according to Jahan (2021), they are a good source of minerals like potassium, iron, manganese, phosphorus, magnesium, and niacin. They are rich in lecithin, fiber, protein, and vitamin E (Aishwarya and Jahan, 2021). They are also low in methionine, an important amino acid. Protein content of proso millets (12.5%) and foxtail millets (12.3%) is higher than that of wheat (11.8%) and rice (6.8%). Compared to wheat (41 mg/100 gm) and rice (10 mg/100 gm), finger millets have substantially greater calcium content (344 mg/100 gm), which is even three times more than that of milk.

According to Jahan (2021), millet has three to five times the nutritional value of the most extensively utilized grains, rice and wheat combined, despite the fact that both offer food security.



Furthermore, according to Mal and Padulosi (2010), crops in this group have a great deal of potential to increase genetic diversity, which will increase food security. Veena (2003) states that these millets offer numerous health advantages in addition to being highly nutritious. Jahan (2021) provided evidence for this in a study demonstrating the nutritional and health benefits of millets. According to the study's findings, they also help manage conditions including hyperlipidemia and diabetic mellitus.

2.5 Striga

2.5.1 Overview of Striga

The Latin word "striga" implies "witch." It is thought to have originated in the high mountains of Semien of Ethiopia and Sudan in the Nubia hills. It is an obligatory hemi-parasitic weed or plant (Mohamed et al., 2001). Ejeta (2007) states that significant agricultural crops, such as millet and sorghum, which are also prone to witchweed infection, are known to originate from these places. According to Kountche and Haussmann (2016), Striga is widely dispersed throughout Australia, Asia, the Middle East, and Africa which all have tropical and semi-arid climates. At least 44 African nations are home to them (Rodenburg et al., 2016). Striga are most prevalent in West Africa, infesting 64 percent of available ground used for grain production, and in East and Central Africa, 23 percent of the land (Gressel et al., 2004). Striga results in considerable yield losses, which have a major financial consequence. An estimated \$117 million worth, or 293,000 tons, of milled rice are lost each year due to striga infection in rain-fed rice (Rodenburg et al., 2016). High losses are also seen in sorghum and millet, which combined have an annual loss of 8.6 million tonnes, and maize, which is expected to lose 2.1 million tonnes annually (Gressel et al., 2004). In countries that grow cereals, factors including inadequate soil fertility and prolonged monocropping significantly contribute to the growth and infestation of Striga (Emechebe et al., 2004). According to studies, Striga has been found in agricultural fields in over forty (40) nations worldwide; sub-Saharan Africa and India have the greatest infection rates (Ejeta, 2007). In regions with poor fertility and erratic water/rainfall

patterns, *Striga* species have spread like wildfire, devastating grain and legume yields. The species is most prevalent in western Africa, infesting 17 million hectares, or 64% of the territory used to grow cereals. In semi-arid and sub-humid tropical zones, the genus may cover approximately 100% of the area (Gressel *et al.*, 2004).

2.5.2 Types of Striga

The predominant *Striga* species that parasitize grains and legumes consist of the following: *S. asiatica, S. angustifolia, S. forbesii Benth, S. laterica Vatke, S. multiflora Benth, S. parviflora Benth, S. aspera, S. densiflora, S. passargei Engle, and S. curviflora Benth.* The main hosts of *S. gesnerioides* are cowpea and wild legume species, whereas *Striga hermonthica and S. asiatica* mainly infect cereals in the Poaceae family. (Sadda *et al.*, 2021)

For the purpose of this research, we are limiting the study to the three common striga types in this part of the world, these are; *S. hermonthica, S. gesnerioides* and *S. asiatica*.









S.gesniorides





S. asiatica

Figure 2. 1: shows the common types of *striga* species

2.5.2.1 Striga hermonthica

One of the primary biological obstacles to sub-Saharan Africa's food production is *Striga hermonthica*, also referred to as "purple witchweed." It is a root-parasitic plant (Rodenburg *et al.*, 2016). *Striga hermonthica*, which negatively impacts human sustenance and drastically increases food insecurity and poverty, is a significant biotic obstacle to the production of cereals in Sub-Saharan Africa (SSA) (Dawud *et al.*, 2017). This plant has forced farmers to part with their land. By developing superior breeds that can withstand abiotic stresses like drought and low nitrogen levels as well as bioinoculants to fight *Striga* infestation, researchers have tried to lessen its impact on agriculture (Tofa *et al.*, 2021). Massive yield losses occur

when Striga infests cereals including upland rice, pearl millet, sorghum, and maize (*Zea mays*). It can result in total crop failure, which would have a substantial negative impact on the over 300 million people's access to food and have an annual economic impact of about seven billion US dollars (Atera *et al.*, 2012). *Striga* is thought to be present on 50 million hectares of arable land in Africa (Gressel *et al.*, 2004; Ejeta, 2007). According to Jamil et al. (2012), *Striga* is distinguished by its extraordinary fertility, which results in thousands of seeds per plant and a 10- to 15-year soil life span. This remarkable fertility causes *Striga* to accumulate one of the main obstacles to the long-term treatment of the disease is the presence of significant seed repositories in affected fields (Kountche *et al.*, 2019). *Striga* is still a significant issue for agriculture in sub-Saharan Africa, despite the use of several mechanical, cultural, biological, and chemical management methods by local farmers (Jamil *et al.*, 2021). Therefore, control methods that can reduce the number of stored seed in infected soils or particularly prevent Striga seeds from germinating are desperately needed (Kountche *et al.*, 2019).

In every aspect, *Striga hermonthica* poses a greater threat than *Striga asiatica*. It is a larger plant that rarely grows higher than 50 cm in West Africa but can reach 1 m in Eastern Africa, causing significantly more damage to agriculture over much larger areas in several nations. The majority of incidences occur in northern Africa (East and West), with minimal occurrences south of the equator. The range of crops affected is similar, but the locations where it is most detrimental are oddly different. Only Africa and the Arabian Peninsula are home to it. According to a comprehensive study conducted in Western Kenya, crop losses can reach 100%. Typically, maize losses fall into one of two categories: 80% of cases are classified as "severe" infestations and 50% as "moderate" infestations (Manyong *et al.*, 2007). Areas of maize crops estimated to be contaminated range from 20 to 30 percent in Guinea, Ethiopia, Cameroon, Cote d'Ivoire, and Togo; 30 to 40 percent in Mali, Nigeria, and Togo; and 65 percent in Benin. According to Teka, (2014), 25 African nations reported having an infestation of Striga in 2005.

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In northeastern Nigeria, a survey found that 85% of the fields were poisoned (Dugje et al., 2006). The proportion of infestation in sorghum and pearl millet is probably comparable. Estimates for all grains varied in 1991 from 40 to 50% in Nigeria, Ghana, and Cameroon to over 70% in Benin and the Gambia (Koltai and Prandi, 2019), and there has not been any indication that the issue has gotten any better since (Parker, 2009).

2.5.2.2 Striga gesnerioides

The life cycle of the autogamous parasite *S. gesnerioides* is comparable to that of other Striga species that are important to agriculture. According to Berner and Williams (1998), its extensive host range consists of tobacco (*Nicotiana tabacum* L.), Ipomea spp., cowpea and groundnut (*Arachis hypogaea* L.), as well as numerous other legumes (*Alysicarpus* spp., Euphorbia spp., Indigofera spp., and Tephrosia spp.). One of the ways that Striga species have adapted to parasitism is by their ability to produce large numbers of microscopic seeds with prolonged viability and distinct germination requirements (Siame *et al.*, 1993). Straw plants are classified as trap crops because seeds will only germinate when exposed to strigolactones, which are exogenous germination stimulants produced by root exudates of host and commonly non-host plant species (Bouwmeester *et al.*, 2007; Yoneyama *et al.*, 2009).

According to Gomez-Roldan *et al.* (2008), strigolactones are plant hormones that control the architecture of plant shoots and roots in response to environmental stimuli. They also serve as indicators of host recognition for arbuscular mycorrhizal fungi and rhizobia (Akiyama *et al.*, 2005) and parasitic weed seeds (Foo and Davies, 2011). According to Parker and Riches (1993), a significant factor in cowpea production losses in sub-Saharan Africa, India, and Southeast Asia is *Striga gesnerioides*. Each plant can produce up to 200,000 seeds, making the parasite hard to control and adding to resistant seedbanks that can last for decades (Bebawi *et al.*, 1983). 153 cowpea fields dispersed across six West African countries were surveyed, and *S. gesnerioides* was found in 40% of the places analyzed. Up to 81% of the cowpea fields in

northeastern Nigeria were infested (Dugje *et al.*, 2006). Complete yield loss could happen in cases of severe *striga infection* (Emechebe *et al.*, 1991). Striga infestation varies in severity according on cropping system and soil fertility; in monocrop systems with limited crop rotations, impoverished, sandy soils tend to have the worst infestations (Tignegre, 2010).

2.5.3 Biology, Morphology and Life Cycle of Striga

2.5.3.1 Biology and Morphology of Striga

other plants in order to survive (Spallek *et al.*, 2013). Its complex developmental process is intimately associated with its host. A single *Striga* plant can yield up to 500,000 seeds under optimum conditions, and those seeds can remain in the soil for 20 years (Lobulu *et al.*, 2019). It combines the life cycles of a hemiparasite as an emerging green plant with chlorophyll and a holo-parasite during the seedling stage (Mohamed *et al.*, 2001). These species have opposing, hairy green leaves on hairy stems. The leaves range in size from linear-lanceolate to lanceolate, measuring 2.5–7.5 cm in length and up to 2 cm in width. Larger and usually pink with a few white blooms, the inflorescence is a much larger raceme with many more flowers. The foliage is similar to *S. asiatica*, but more robust. This plant produces asymmetrical blooms with a corolla tube that is slightly twisted in the middle. Although there are numerous variations, the majority of flowers are brilliant pink; on rare occasions, white, red, and yellow flowers have also been seen (Parker, 2012).

Striga species' annual roots are photosynthetically active, hemiparasitic plants that feed on



2.5.3.2 Life Cycle of Striga

Mechanisms that balance the host and parasitic plant lifecycles are necessary for the Striga lifespan to function concurrently with that of its host. Its life cycle typically entails the following processes: vascular connection penetration and establishment, germination, host attachment, haustoria growth, nutrient accumulation, flowering, and seed production (Yacoubou et al., 2021). Only strigolactones, which are chemical signals derived from the host that typically precede a pre-conditioning period requiring warm weather and moist soil, can cause these seeds to germinate (Wood, 2020). The parasite attaches itself to the host plant and forms a haustorium. This facilitates the transfer of nutrients and water amid the host plant and the Striga plant causing harm to the latter. But for development and life, this parasite is totally reliant on the host plant. It is unable to thrive on its own at any stage (Solomon et al., 2015). After egression, *Striga* spp. take around 10 weeks to finish their life cycle, and this completion usually takes place following the harvest of the host plant. (Yacoubou et al., 2021). Striga hermonthica has notable variety within a given area and is a high crossover species (Mrema et al., 2017). Its distinctive dispersal feature has further enhanced its genetic alteration. The dispersion of the parasitic plant has been facilitated by the availability of dispersion agents (Menkir et al., 2020). For host-specific activity, Striga hermonthica is the population with the highest genetic diversity (Mandumbu et al., 2019). The resistivity potential during the accommodating stage of the parasite is explained by the variation process that takes place within it. A common instance is observed in a specific variety of crops cultivated with species S. hermonthica. The aforementioned is a list of some of the traits that make the parasite so harmful to the plant host.



2.5.3.3 How Striga detects or finds its host; the role of strigolactones

Striga plants yield a large number of tiny seeds, which can remain dormant in the soil for decades until they are stimulated to germinate by substances found in the host root, such as strigolactones. A lot of non-parasitic plants release strigolactones to draw in mycorrhizal fungi that work in symbiosis. Striga spp. employ this signal to identify nearby hosts. Indeed, Striga genomes contain a large number of strigolactone receptor genes, which facilitate the identification of hosts and the detection of various strigolactone derivatives. The processes governing the chemotaxis by which striga roots grow a few millimetres in the direction of the host root are unknown (Mutuku and Shirasu, 2019). Chemotaxis is a transmembrane receptor-mediated sensing process that is frequently utilized by migratory organisms to monitor changes in environmental circumstances and steer migration to more favorable areas, according to Keller and Segel (1971) and Stock and Baker (2009). The haustorium, a multicellular structure created by the Striga root upon reaching the host root, allows the host to be invaded. Haustorium production is stimulated by chemicals produced from hosts called haustorium-inducing factors (HIFs). Mutuku and Shirasu (2019) use 2,6-dimethoxy-p-benzoquinone (DMBQ), a chemical generated from oxidized lignin, as an example.

2.5. 4 How Striga Infest their Host

Without a host, *striga* cannot develop or germinate; germination only occurs when a suitable host is present, which is identified by strigolactones released by the host roots (Kgosi *et al.*, 2012). The *Striga* radicle elongates and differentiates into the haustorium, a specialized organ that develops in reaction to the host's chemical stimuli after germination (Yoshida *et al.*, 2016). In order to create vascular connections with the host, the haustorium eventually reaches the endodermis by penetrating the host root (Runo and Kuria, 2018). Here, the haustorial cells lengthen and divide. Vascular continuity signals the start of host material translocation to the parasite. *Striga* induces wilting and chlorosis in its host long before it erupts and becomes evident above ground.



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2.5.5 Economic Importance of Striga

One of the biggest and most difficult living constraints on the production of cereal, particularly maize, is the *Striga* species. It is mostly found in the Middle East of the Asian continent, as well as high-temperate climates in portions of SSA. The parasite effect greatly hinders the development and yield of food crops. Farmers find the control to be disappointing because the parasite's detrimental effects are visible even before it leaves the soil. *Striga* spp. has been found to be infested on about 50 million hectares of tillable soil used for the growth of cereals, including maize (Dafaallah, 2019).

Grain losses due to this parasite have been predicted to reach up to 75%, depending on the host infected, usual weather, cultivar variations, level of infection, and soil type (Anitha *et al.*, 2020). Anitha *et al.* (2020) reported that the enhanced cultivar's drop in grain harvest has improved to 90%. An estimated \$10 billion worth of damages are caused each year, and farmers who experience losses of up to 80% as a result of parasite invasion are eventually evicted from their properties (Dafaallah, 2019). But if more action is not done, *Striga's* onslaught on agriculture will intensify and pose a threat to agricultural production.

Striga decimate Africa's agricultural industry by 30–50% on 40% of its fertile land (Hearne, 2009). More than 50 million hectares of arable farmland in sub-Saharan Africa that are planted with cereals are reportedly home to *Striga* (Westwood *et al.*, 2010). 17.2 million hectares in West Africa are infested with *Striga*, accounting for approximately 64% of the total area of cereal farms (Gressel *et al.*, 2004). Additionally, it has been shown that the parasites' infectious range has grown (Ejeta, 2007)

In an experimental study, *striga* infection has been anticipated to result in crop yield losses ranging from 10% to 31% (Ramaiah, 1983). Gressel *et al.* (2004) reported an average yield

loss of 26% in sorghum and pearl millet in sub-Saharan Africa. In locations with high Striga infection, yield losses might range from 90 to 100% (complete crop failure) in some years. As a result, studies by Atera *et al.* (2011) state that farmers have been forced to abandon farms extensively infested with *Striga*.

Andrews and Kumar (1992) and Mannuramath *et al.* (2015) state that some cereals are the main source of magnesium and zinc for impoverished farmers, as well as carbs and other vitamins and minerals. Consequently, yield losses have a significant detrimental socioeconomic effect. More than 300 million people in Africa suffer from stroke, which results in yearly economic losses for the continent of more than \$10 billion (Obilana and Ramaiah, 1992; Ejeta, 2007; Scholes & Press, 2008). Consequently, sub-Saharan Africa has recently been found to have the highest rate of poverty and malnutrition, with an estimated one in four (1/4) people (24.8%) living in hunger (McGuire, 2015).

2.5.6 Striga Management Strategies

2.5.6.1 Chemical Control

Since these chemicals can cause the parasitic weed to sprout without a host, they represent a growing progress in the control of *Striga*. These compounds mimic the activities of strigolactone. Suicidal germination is the term for this process (Zwanenburg *et al.*, 2016). Previously, strigolactones created by multiple chemical processes were unstable in the field, making this technology unfeasible due to its high cost, adequately implemented procedure and user-friendly formulation. However, simple strigolactones can currently be obtained in the field by chemical combination. The Striga seed bank is quickly depleted, which makes this strategy attractive. While several techniques have been developed for this parasite's suicide killing, their application in field settings is restricted because of their high cost, lack of a well-established approach, and challenging formulation (Kountche *et al.*, 2019). TIS108 reduced SL levels in



rice seedlings and had no influence on plant height, according to Kawada et al. (2020), in contrast to TIS13, which dramatically reduces plant height. Additionally, it had the ability to minimize striga germination. According to the most current reports, SL analog MP16 was able to reduce striga emergence as much as 97% in greenhouse evaluations, and Nijmegen-1 was able to reduce striga emergence which was 60% and 40% higher in sorghum and pearl millet fields, respectively, than with the traditional GR-24. These SL analogs could be used to further suicidal germination technology, which would abolish the Striga seed bank in SSA (Kountche et al., 2019). The application of imidazolinone herbicides, such as pyrithiobac, imazapic, imazaquin, and imazapyr, along with imazapyr-resistant (IR) coating on maize seeds has inhibited Striga growth during all farming seasons, leading to intermittent increases in maize production yields (Kanampiu et al., 2018).

But persistent chemical application to the soil has resulted in deteriorating soil quality, water contamination, greenhouse gas emissions, and decreased soil biodiversity (Basu et al., 2021).

2.5.6.2 Cultural Strategies



For the purpose of managing *Striga* on cereal farms, several cultural management techniques have been put forth. According to David et al. (2022) these techniques help minify striga seeds slow down the germination and growth of seedlings while also enhancing the prolificacy and maturation of the soil. The strategies include the following: combining nitrogen fertilizers with trap crops (Tadesse, 2018); intercropping legumes with cereals (Mutyambai et al., 2019; Jamil et al., 2021); managing water (Goldwasser and Rodenburg, 2013); mixed cropping; crop rotation (Kuyah et al., 2021); cover cropping (Randrianjafizanaka et al., 2018); push-pull technology (Ndayisaba et al., 2020); and fertilization (Jamil et al., 2011). In push-pull technology, the desmodium root secretes chemicals that promote the growth of parasitic striga weeds while preventing them from adhering to roots of crops through radical growth inhibition.

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The *striga* seed bank is thus exhausted (Ndayisaba *et al.*, 2020). Early planting before rains in semi-desert settings also lessens the chance of Striga plague, which sometimes appears a few months after cultivation (Mrema *et al.*, 2017). Smallholder farmers find it difficult to adopt these tactics due to their lack of capital, labor, time, and technical expertise. The application, rate, and timing of fertilizers are still challenges for developing-nation maize growers.

Using hands or equipment to remove *Striga* weeds is the least expensive cultural control technique. This technique is best used prior to *Striga* weed blossoming in order to prevent soilbased propagation of the plant. However, it is difficult, time demanding, and less effective at reducing crop damage. (Mahuku *et al.*, 2017). To lessen the impact of *Striga* on rice and maize, cover crops and the use of pesticides with different levels of *Striga* resistance have recently been developed (Teka, 2014). Understanding the benefits and drawbacks of the currently available management techniques is necessary for the application of integrated management strategies to lessen the impact of *Striga* and boost maize output.

2.5.6.3 Integrated Management Strategies

It is ineffective to control *Striga* using just one mechanism. But combining two or more approaches has produced interesting results (Sibhatu, 2016; Jamil *et al.*, 2021). For example, a considerable reduction in *Striga* was observed when F. oxysporum was combined with host resistance (Mrema *et al.*, 2020; Shayanowako *et al.*, 2020). The coating of the hybrid maize seed resistant to imazapyr reduced *striga* resistance (Menkir *et al.*, 2020). Additionally, germination boosters in addition to rotating crops between grain and legumes can significantly reduce the number of parasite seeds in the soil (Kountche *et al.*, 2019). An experiment by Abdullahi *et al.* (2022) found that combining resistant varieties, P fertilizer, and Brandyrhizobium was able to decrease *Striga gesnerioides* and boost cowpea grain output. According to Kanampiu *et al.* (2018), the usage of herbicide- and *Striga*-resistant maize combined with legumes inhibited the germination of *Striga* seeds and decreased the amount of

striga seedbank in the soil. Furthermore, it has been suggested that the best method for preventing striga seed banks death is to combine genetically resistant varieties with selfdestructive development (Kountche et al., 2018).

2.5.6. 4 Biological Control

It has been suggested that fungi and insects can be used to control Striga. The majority of the seeds, but not all of them, are consumed by the insects when they feed on the seedpods. Accordingly, minimal yield promotion is required to sustain the weed population through seed bank replenishment (Smith and Webb, 1996; Watson et al., 2007). Though not all fungi have been thoroughly field tested, their pathogenicity on striga has been studied. Fusarium species are the most common type of fungus on sick striga plants (Watson, 2013). A well-managed environment chamber study of 81 fungal isolates from three countries (Mali, Niger, and Burkina Faso) revealed that the emergence of S. hermonthica was effectively inhibited by the Mali Fusarium oxysporum isolate (isolate M12-4A), which was cultivated on sorghum straw and integrated into pots (Ciotola et al., 1995).



Sorghum dry matter increased fourfold as a result (Ciotola et al., 1995). The M12-4A isolate was then tested in the field in Mali using ground or chopped sorghum straw inoculum. At 82 days after sowing, emergent Striga was reduced by 60% and sorghum biomass increased by double as compared to the control (Watson et al., 2007; Ciotola et al., 2010; Watson, 2013). After more investigation on Isolate M12-4A, it was discovered that S. hermonthica emergence was totally prevented by adding powdered fungal spore (chlamydospore) to the soil with sorghum seeds or by sowing sorghum seeds that were also coated with the chlamydospore (Ciotola et al., 2010). Between 78 and 92% less S. hermonthica emerged after receiving chlamydospore powder treatments (Ciotola et al., 2010; Yonli et al., 2010). Studies from Nigeria and Burkina Faso in additional isolates of F. oxysporum (PSM197, 4-3-B) inhibited

the establishment of S. hermonthica plants in pots and field trials, and they also stopped Striga seeds from germinating, according to Marley et al. (2010) (Marley and Shebayan, 2005; Yonli et al., 2010). The host range of F. oxysporum is restricted. It was discovered that the following crops are resistant to isolate M12-4A7: sorghum, pearl millet, maize, rice, fonio, cotton, peanuts, cowpea, and okra. These and other crops are also resistant to isolates from Ghana, Sudan, and Nigeria (Elzein, 2004; Venne et al., 2009). All F. oxysporum isolates from S. hermonthica are only pathogenic to S. hermonthica and potentially S. asiatica, according to Lobulu et al. (2021). Elzein and Kroschel (2004) reported that isolate M12-4A did not produce mycotoxins under any of the investigated conditions, meaning that it does not present a known health concern to humans or livestock. Mass producing and delivering the biocontrol agent to the intended target are essential phases in biocontrol programs. For the mass manufacturing of F. oxysporum inoculum, three different models have been proposed: on-farm, cottage industry, and small entrepreneur industry (Marley et al., 2004). Fusarium can be grown on a range of inexpensive, raw agricultural products, including the stubble of sorghum. Numerous methods for producing the fungus in large quantities on sterilized sorghum straw have been developed (Watson et al., 2000; Ciotola et al., 2010). Just 80g of the chlamydospore powder are needed per hectare when used as a seed coat. Yonli et al. (2010) presented a cottage industry-style inoculum production technique that employs a liquid fermentation process and inexpensive locally available substrates (such as gum arabic and sorghum straw) to increase the utilization of the F. oxysporum isolate M12-4A. Four Mali villages participated in the liquid mass manufacturing of M12-4A in seed coating and cooking pots in 2000, according to Bastiani (2001). Despite the great success of seed coating, no viable inoculum was produced and all of the manufacturing vessels became infected. A thorough assessment of other demanding production systems is necessary. In addition to the previously mentioned powder formulation, a variety of granular formulations, such as sodium-alginate and wheat flour-kaolin "Pesta"

granules, have been studied (Anderson, 2004). Inoculum has been directly inserted into the seeding holes.

2.6 Fusarium

For many years, Fusarium species have been significant plant pathogens that cause vascular wilts on a variety of horticulture crops and diseases like crown rot, head blight, and scab on cereal grains (Okungbowa and Shittu, 2012). Due to the potential hazard that their mycotoxins pose to both human and animal health, Fusarium species have been the subject of much research over the past 20 years (Munkvold, 2017). Secondary metabolites known as mycotoxins are created by fungi and have been linked to a number of animal illnesses as wells as certain health issues in humans (Zain, 2011).

Fusarium species have grown in importance as infections of immunocompromised humans in more recent times (Hof, 2020). They are common in both temperate and tropical areas, as well as in arctic, alpine, and desert locations with severe climates (Mandeel et al., 2005). While many Fusarium species are reasonably frequent in forest soils, they are plentiful in fertile cultivated and rangeland soils. Because of their prevalence in soil and their frequent interaction as either saprophytes or parasites with plant roots, Fusarium species are frequently thought of as soilborne fungi (Okungbowa and Shittu, 2012).

Nonetheless, a large number possess both active and passive mechanisms for atmospheric dissemination, and they frequently colonize aerial plant segments, where they have the potential to cause illnesses with significant economic implications (Magyar et al., 2016). Even though the distribution of some species is more dependent on climatic conditions, some species complexes, like the Fusarium oxysporum species complex (FOSC), the Fusarium solani



species complex (FSSC), and the *Fusarium incarnatum-Fusarium equiseti* species complex (FIESC), are thought to be ubiquitous (Summerell *et al.* 2010).

2.6.1 Fusarium Oxysporum

Filamentous plant-pathogenic fungus, Fusarium oxysporum is ranked fifth among the top 10 plant diseases of scientific and commercial significance. In a variety of host plants, it results in necrosis, wilting, and root rot (Dean et al., 2012). Leslie and Summerell (2006) have identified strains of this soilborne asexual fungus that are known to be both nonpathogenic and pathogenic, meaning they can damage humans, animals, and plants. Plant-pathogenic strains are classified as formae speciales based on their host specificity (Bora et al., 2018). Depending on which cultivars they can infect, some of these strains can be further categorized into races or pathotypes. F. oxysporum is classified into more than 100 formae speciales according to its host species specialization. Among the numerous formae speciales of F. oxysporum are the tomato pathogen F. oxysporum f. sp. lycopersici (Fol), the banana pathogen F. oxysporum f. sp. cubense (Foc), the common bean pathogen F. oxysporum f. sp. phaseoli (Fop), and the melon pathogen F. oxysporum f. sp. melonis (Fom).

After *F. oxysporum* enters a plant by its roots, it colonizes the vascular system and causes disruption to the xylem vessels that carry water, which causes the plant to wilt and eventually die (Yadeta, 2013). Mycotoxins, effect or proteins, and plant cell wall-degrading enzymes (CWDEs) are only a few of the virulence factors that *F. oxysporum* uses to subvert target host cells during the infection process (Ma *et al.*, 2013).

CWDEs, such as polygalacturonases, pectate lyases, xylanases, and cutinases, may contribute to pathogenesis by degrading waxes, cuticles, and cell walls to induce tissue invasion and pathogen dispersal (Ma et al., 2013). Furthermore, Fusaric acid, beauvericin (Li et al., 2013), and fumonisins (Rheeder et al., 2002) are among the mycotoxins that *F. oxysporum* may create, and they all contribute to the pathogenicity of hosts. Fusaric acid and beauvericin have been connected to Foc, which makes banana plantlets wilt, break down, and eventually die in vitro, according to Li et al. (2013). In Foc tropical race 4 (TR4), fusaric acid plays a significant role as a favorable virulence factor, particularly in the early phases of disease development (Liu et al., 2020). The mycotoxin beauvericin, which disrupts mitochondrial functions and causes DNA fragmentation and death, is produced by a variety of Fusarium species and Beauveria bassiana (Mallebrera et al., 2018). Beauvericin lowers the ascorbic acid content of tomato cells in Fol, which causes the ascorbate system to collapse and protoplast death (Paciolla et al., 2004).



CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Samples were taken from Manga in the Binduri District, Upper East region of Ghana.

The study was conducted in the Spanish laboratory complex at the University for Development Studies (UDS) Nyankpala campus, located in the Northern Region of Ghana.

3.2 Sample Collection and Storage

Disease *S. gesnerioides and hermonthica* were randomly sampled from cowpea, millet, sorghum and maize fields in Manga. The samples were packed into paper envelopes labelled and sent to the Spanish laboratory. The samples were air dried and stored at room temperature for seven days.

3.3 Isolation, Identification and Characterization of Fungi Isolates

The leaves of *S. gesnerioides and hermonthica* were taken for the fungal isolation. Samples were washed with sterile distilled water to do away with soil debris. Stems sections were cut into 0.5-1.5 cm pieces, and surface sterilized along with the leaves in 3.5% sodium hypochlorite (NaClO) solution for 3 min, rinsed three times in distilled water and dried on sterile filter paper to remove excess water. The dried leaves and pieces of stems were cultured on a growth medium containing 10 g glucose, 2 g peptone, 0.5 g K2HPO4, 0.5 g MgSO4.7H2O, 20 g agar, 10 mL triton-X 100 and 0.5 mg chloramphenicol in 1 L distilled water (autoclaved at 105 °C for 15 minutes) poured into 90 mm Petri dish. The Petri dishes with leaves and stems were wrapped in cling film and left at room temperature for 7 days for fungi growth. All fungal colonies were transferred to potato dextrose agar (PDA, Oxoid) amended with 0.5 mg chloramphenicol and incubated at room temperature for 14 days.

3.4 Molecular Characterization of *Fusarium* Isolates



3.4.1Deoxyribonucleic acid (DNA) Extraction

The genomic DNA of Fusarium isolates was extracted using the modified CTAB extraction method described by Lee et al., (1988). Briefly, mycelium was scraped off the surface of a 14day-old culture and ground with 500 µl of CTAB buffer (2% CTAB, 100 mM tris-HCl; pH 8.0, 20 mM EDTA, 1.4 M NaCl, 2% PVP, and 0.2% beta mercapto-ethanol) in a 1.5 microcentrifuge tube using Kontes' pestle. The tubes were incubated for 1 hrs at 65 °C in a heating mantle. After incubation, 500 µl of chloroform: isoamyl alcohol (24:1) was added, vortex for 1 min, and centrifuge at 2500 g (Centro-4-BL, J.P. Selecta, Spain) for 20 min. The upper aqueous fraction was reextracted with 500 µl of chloroform: isoamyl alcohol. After centrifugation, the supernatant was then transferred into a 2.0 mL microcentrifuge tube containing 300 µl of 100% ice-cold isopropyl alcohol. The supernatant was kept in a freezer overnight (approximately 14 hours) and DNA was pelleted at 2500 g for 3 min. The pellet was washed with 500 µl of ice-cold 70% ethanol and centrifuged at 2500 g (Centro-4-BL, J.P. Selecta, Spain) for 3 min. After removal of the supernatant, the DNA pellet was air-dried for 1 h and resuspended in 50 µl of molecular water. After extraction, the DNA concentration was measured using a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

3.4.2 Molecular Identification

Fusarium isolates were identified using translation elongation factor 1-alpha (EF1: ATGGGTAAGGAAGACAAGAC; EF2: GGAAGTACCAGTGATCATGTT). The PCR amplification reactions were conducted in 25 μl reaction volumes, consisting of 2 μl of DNA, 1 μl of 10 μmol each of forward and reverse primers, and 12.5 μl of premix PCR standard buffer [Tri-HCl pH 8.5, (NH4)2SO4, 1.5 mM MgCl2, 0.2% Tween® 20, 0.4 mM of each dNTP, 0.2 units/μl Taq polymerase (VWR International, Belgium). The thermal cycling conditions for the PCR amplification were set as follows: an initial denaturation at 95°C for 30 s, extension min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing 57°C for 30 s, extension

at 72°C for 1 min, and a final extension step at 72°C for 10 min. The resulting PCR amplicons were separated and visualized on a 2% agarose gel that had been stained with 250 μ l of 60× ethidium bromide.

3.4.3 DNA sequencing and analysis

PCR amplicons were sequenced at Inqaba Biotechnology (Pty) Limited, Pretoria, South Africa. Quality control of illumine reads was performed with GENtle software v.1.9.4 by cleaning and trimming sequences. Nucleotides were aligned in Clustal Omega (Madeira et al., 2022). Nucleotide sequences were compared to other sequences available in the National Centre for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed on 24 August 2022). TCS haplotypes network was constructed in PopArt 1.7 (Leigh and Bryant, 2015). DnaSP software was used to determine the nucleotide and haplotype diversities of the sequences (Librado and Rozas, 2009). The maximum likelihood phylogenetic tree based on the TEF 1-α sequences obtained in this study and sequences retrieved from GenBank was drawn using Molecular Evolutionary Genetics Analysis (MEGA X) software v.11 (Kumar *et al.*, 2016).



The cycling conditions used in PCR

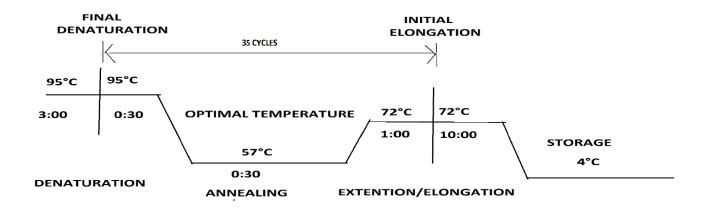


Figure 1.0 PCR cycling condition, above is the cycling condition(s) used in the PCR amplification.

3.4.4 Gel Electrophoresis

Products from the amplification were separated through electrophoresis in 1.2% (w/v) agarose gel containing ethidium bromide for 30 minutes at 80 V. The 1.2% agarose gel was prepared as follows: 1.0g of agarose granules was dissolved in 50ml of 1X Tris Borate Ethylenediaminetetraacetic acid (TBE) buffer. For complete dissolution, the mixture was put in a microwave for 1 min 20 s after which 250µl of ethidium bromide was added. The mixture was swirl gently to make it uniform. Gel was casted into a prepared gel tray to solidify for about 40 minutes.

Visualization of the PCR amplicons was performed using the UV transilluminator (Cleaver Scientific Ltd., UK) and the images captured with an in-built digital camera.

A total of 21 samples were run through the gel, seven of the samples recorded no amplification.



3.5 Pathogenicity Test

3.5.1 Preparation of Fusarium spp. Inoculum

All the 14 cultures spores were harvested individually. To collect spores from *Fusarium* isolates, 10 mL of sterile distilled water was flooded into each of the ten-day-old cultures. The spores were scraped from the agar's surface using a sterile L-shaped rod. To get rid of any mycelia, the suspensions were subsequently passed through a four-layer sterile gauze swab (MicroTech). Using a Neubauer enhanced hemocytometer (Germany), spore concentrations were measured and adjusted to roughly 1×10^5 spore mL-1.

Hemocytometer was to determine the spores concentration which were then stored into Eppendorf tubes and stored at -20 C

3.5.2 Leaves inoculation and disease assessment

Striga leaves were monitored and harvested on the 21st day of emergence on farms around Nyankpala- Tolon district in the Northern region and transported to the Spanish laboratory. Using Opoku *et al.* (2011)'s protocol, a pathogenicity test was conducted to determine the *Fusarium* isolates' effectiveness against Striga hermonthica. The antagonistic potential of the *Fusarium* isolates was tested against S. hermonthica, the freshly harvested leaves were cleaned with sterile distilled water and a wound was created in the middle of the adaxial surface. The wounded leaves were placed carefully in Petri dishes containing 0.5% water agar amended with kinetin with the adaxial surface facing upward. Subsequently, the wounded area was inoculated with 5 ul of the spore suspension and incubated at room temperature for 14 days. Four leaves were inoculated per isolate and the same number of leaves were inoculated with sterile distilled water only, as control treatments.

3.5.3 Statistical analysis

Version 11.1 of the GenStat statistical software (VSN International Ltd) was used to conduct the statistical analysis. One-way analyses of variance were used to assess the pathogenicity test data (ANOVA). For ANOVAs with significant (P < 0.05) differences between means, the Fisher's unprotected LSD test was employed.



CHAPTER FOUR

4.0 RESULTS

4.1 Morphological characterization of Fusarium species

Fusarium species growth on PDA started as white mycelial growth and changed into different shades of pink 7 days after culturing. The identified isolates were similar in mycelial growth with their colour. For the first week, all fungal colonies showed vigorous growth morphologically which affirms the fungal isolate ability to utilize the nutrient composition provided by PDA which confirms the findings of Wallis (2021). Cultures of Fusarium isolates on the PDA started as white mycelial growth and after seven days mycelial colour started changing to shades of pink, however F. oxysporum isolates remained the same with creamy white cotton colour. This could be attributed to aging of the fungal mycelial and the processes of metabolism.





Figure 4.1: Shows the growth pattern of the Fusarium on PDA



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4.2 Molecular Characterization of Fusarium

4.2.1 Identification by Amplification of the TEF Primer (760bp)

A total of 21 samples were used in the molecular analysis. The TEF 1-alpha gene (760bp) successfully amplified 14 DNA samples. These were C6,C7,C9,C10,C11,C12 (from cowpea field); Mi16,Mi17,Mi18,Mi19 (from millet field); M11,13 (from maize field); So6, So8(from sorghum field). Since these primers set employed was *Fusarium* specific, the amplified samples were therefore confirmed at this stage to be *Fusarium* spp.



Figure 4.2: Gel electrophoresis for the *Fusarium* isolates

4.2.2 Identification of *Fusarium* Isolates using the TEF1-alpha gene sequences

In a BLAST search in the Genbank database, all 14 samples sequenced were identified as *Fusarium sp.* with 77-100% identity to previous accessions (Table 4.1). Five *Fusarium sp* were identified, namely: *F. incarnatum, F. duofalcatisporum, F. verticillioides, F. oxysporum, F. fredkugeri*. Out of the 14 sequenced samples, 12 samples representing 95% showed homology above 90% with previous GenBank accessions. Only two representing 5%, that is Mi17(millet 17) and C12(cowpea12) showed 89.01% and 76.58% respectively with GenBank accessions (Table 4.1).

Table 4.1: Percentage identity and accession numbers of identified species of *Fusarium* species from NCBI-BLAST base on haplotype groups

Sample	%		Accession		
code	Identity	GenBank Reference	Number	Country	Host
M11	98.44	Fusarium incarnatum	OP487159	Ethiopia	sorghum
		Fusarium			
M13	98.41	duofalcatisporum	OP487130	Ethiopia	sorghum
Mi16	96.52	Fusarium verticillioides	LC102197	Thailand	rice
		Fusarium			
Mi17	89.01	duofalcatisporum	GQ505651	USA	
Mi18	99.7	Fusarium oxysporum	MN539104	Mali	rice





		Fusarium			
Mi19	99.28	duofalcatisporum	GQ505651	USA	
		Fusarium			
So6	100	duofalcatisporum	OP487130	Ethiopia	sorghum
		Fusarium			
So8	99.67	duofalcatisporum	GQ505651	USA	
C10	100	Fusarium oxysporum	MN539104	Mali	rice
		Fusarium			
C11	99.53	duofalcatisporum	GQ505651	USA	
C12	76.58	Fusarium oxysporum	MN539104	Mali	rice
					pinus
C6	99.84	Fusarium fredkugeri	OP273041	USA	taeda
		Fusarium			
C7	100	duofalcatisporum	GQ505651	USA	
C9	90.45	Fusarium oxysporum	MN539104	Mali	rice

4.2.3 Phylogenetic Tree Analysis

A phylogenetic tree shows the evolutionary relationships between various biological species by comparing and contrasting their physical and/or genetic characteristics. The species represented in the tree are:

F. duofalcatisporum (So8, C11, M13, Mi19, So6, C7, Mi17)

F. incarnatum (M11)

F. verticillioides (Mi16)

F. oxysporum (C12, C9, Mi18, C10)

F. fredkugeri (C6)

A number of *F. duofalcatisporum* isolates (So8, C11, M13, and Mi19) show evidence of a recent common ancestor by forming a closely related group.

The tight evolutionary relationship between the *F. oxysporum* isolates (C12, C9, Mi18, and C10) is further evidenced by their clustering.

Divergence places: Evolutionary divergence from a common ancestor is represented by the places where branches break. For example:

The separation seen between *F. incarnatum*-M11 and the isolates of *F. duofalcatisporum* implies that, although diverging into different species, they had a common ancestor.

Outgroups: Species that split off early in the tree, such *F. fredkugeri*-C6 and *F. verticillioides*-Mi16, are seen as being more out of grouping than those with more recent common ancestors.



The strains of *F. duofalcatisporum* exhibit a closer evolutionary link with each other than with other species.

Within the tree, strains of *F. oxysporum* exhibit a higher degree of mutual relatedness, resulting in the formation of a unique clade.

The placement of *F. fredkugeri* and *F. verticillioides* on different branches illustrates their different evolutionary routes from those of the other species mentioned.

The evolutionary links between the many *Fusarium* species are depicted in the phylogenetic tree. The grouping of closely related species suggests that they have a common evolutionary history. The tree illustrates the differences between these species and their shared predecessors.



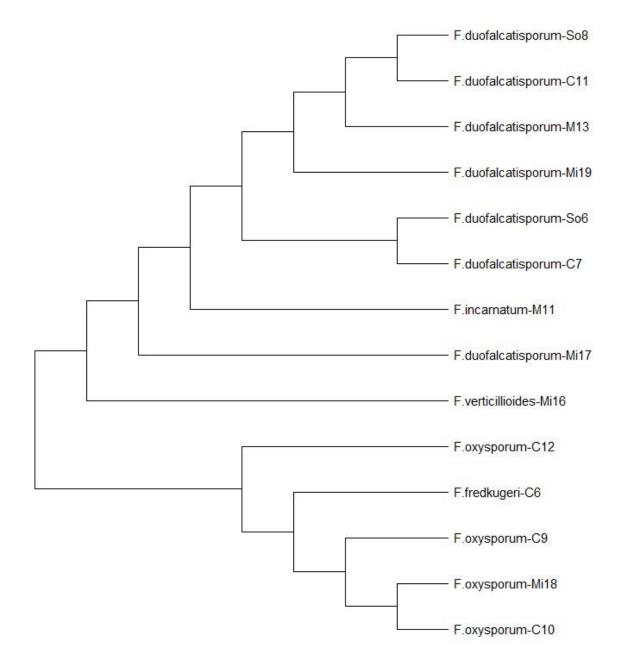


Figure 4.3: Phylogenetic tree show the relationship among Fusarium isolates

No.

4.3 Pathogenicity Test

Each *Fusarium* species was tested on four leaves (4 reps) and lesion growth was measured for two periods that is 1 week after inoculation and two weeks after inoculation. The development of lesion on the leaves are related to how pathogenic the *fusarium* species are. The larger the lesion growth, the more the pathogenicity.

Table 4.2: Lesion diameter induced by *Fusarium* spp. Isolates 14 days after inoculation on *Striga hermonthica* leaves

Isolate code	Species	Mean lesion diameter (mm)
C10	F. fredkugeri	4.44 ± 1.26 ^a
M11	F. incarnatum	3.81 ± 0.98 ^{ab}
C6	F. duofalcatisporum	3.75 ± 0.28 ^{ab}
So8	F. oxysporum	3.75 ± 0.50 ab
So6	F. duofalcatisporum	3.313 ± 1.02 abc
M13	F. duofalcatisporum	2.94 ± 1.29 bc
Mi18	F. oxysporum	2.81 ± 0.55 bcd
C11	F. duofalcatisporum	2.75 ± 0.35 bcd
C7	F. duofalcatisporum	2.75 ± 1.42 bcd
Mi17	F. duofalcatisporum	2.75 ± 0.50 bcd
C12	Fusarium spp*	2.75 ± 0.35 bcde
	C10 M11 C6 So8 So6 M13 Mi18 C11 C7 Mi17	C10 F. fredkugeri M11 F. incarnatum C6 F. duofalcatisporum S08 F. oxysporum S06 F. duofalcatisporum M13 F. duofalcatisporum Mi18 F. oxysporum C11 F. duofalcatisporum C7 F. duofalcatisporum Mi17 F. duofalcatisporum

S. hermonthica	Mi19	F. duofalcatisporum	$1.94 \pm 0.37 ^{\text{cdef}}$
S. gesnerioides	С9	F. oxysporum	1.44 ± 1.04 df

The value represents means \pm standard deviation of triplicate determination. Values within a column with unlike superscript letters are significantly different at a 95% confidence interval (p < .05). *Could not be identified to the species level due to low similarity with sequences in GenBank and FUSARIOID-ID

Lesions caused by the five Fusarium species on Striga hermonthica varied from 1.00 to 3.75 mm and 1.44 to 4.44 mm for week 1 and week 2 respectively (Table 1). An analysis of variance indicated significant interactions between Fusarium isolates and the Striga leaves. Isolate F. oxysporum-C9 had the lowest lesion effect while F. fredkugeri-C10 exhibited the highest lesion on the Striga hermonthica leaves

Lesion caused by *F. fredkugeri* generally had a very dark edge around the wounded part with some yellowish spot and some traces of water.

Lesion caused by *F. oxysporum* had a light green spot with traces of water surrounded by a dark edge. For the first week, no dark edge was observed around the wound, it started in the second week.

Lesion caused by *F. duofalcatisporum* had some leaves light green spot and some were yellowish with traces of water surrounded by a dark edge.

F. verticillioides and F. incarnatum lesion development had similar characteristics with F. fredkugeri with slower growth as compared with F. fredkugeri.







Figure 4.4: Wounded detached leaves 7 days post-inoculation.



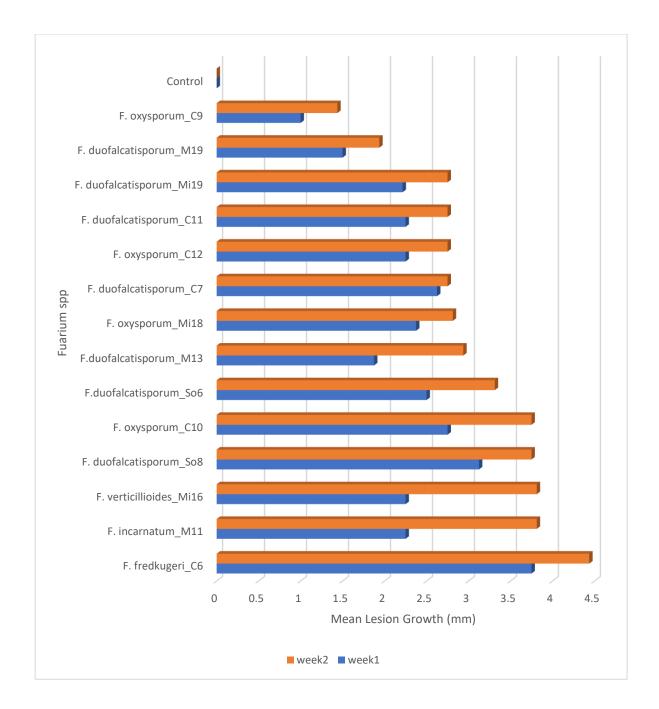


Figure 4.5: mean lesion growth of Fusarium isolates on Striga leaves

CHAPTER FIVE

5.0 DISCUSSION

This study was towards exploring *Fusarium* species as potential biological control agent against *Striga hermonthica*. A number of researches have reported on the pathogenic potential of *Fusarium oxysporum*. However, for this study *Fusarium* isolates which were suspected to be only F. oxysporum upon PCR and subsequent sequencing showed that isolates were of *F. duofalcatisporum*, *F. fredkugeri*, *F. verticillioides* and *F. incarnatum*.

5.1 Morphological and Growth Dynamics of the Fusarium isolates

The identified isolates were similar in mycelial growth with their colour. For the first week, all fungal colonies showed vigorous growth morphologically which affirms the fungal isolate ability to utilize the nutrient composition provided by PDA which confirms the findings of Wallis (2021). Cultures of fusarium isolates on the PDA started as white mycelial growth and after seven days mycelial colour started changing to shades of pink, however *Fusarium* isolates remained the same with creamy white cotton colour. This could be attributed to aging of the fungal mycelial and the processes of metabolism. Cambaza *et al.*, (2018) reported that it is very possible to verify growth by observation of colour changes of the fungi which would also probably provide better understanding of fungal growth and metabolism than just measuring the size. On the average growth of mycelia were very slow at the initial stages but rapidly increased on the fourth, fifth and seventh day of culture, which is probably a consequence of the introduction of fungi to the new environment and subsequent adaptation. This intends to confirm with Srivastava *et al.*, (2011) who reported slow growth rates for fungi mycelia at the initial stages which tends to increase at the latter stages.



Fandohan *et al.*, (2004) and Su *et al.*, (2012) stated that PDA was a good substrate that efficiently support the growth of fungi. Very high significant growths among them on PDA reveals that it probably provides the needed nutrients for the growth of the fungi as reported by Ren and Yao (2013) in a study to determine PDA to be best media for *Fusarium* growth.

5.2 Molecular Identification of the *Fusarium* Isolates

This current study confirms the efficiency of the use of TEF 1-a gene as a precise molecular marker in identifying *Fusarium* isolates. The molecular findings confirm the existence of *F. oxysporum*, *F. solani* and *F. duofalcatisporum* on *S. hermonthica* in Ghana as reported by Elzein *et al.*, (2008) and their ability to infect the weed parasite. It has important to carry out the molecular identification to scientifically prove that, we are actually working with the desired fungal. Molecular identification is carried out to eliminate all doubts in the scientific research as we cannot rely on only the morphological appearance the TEF-1 primer used confirmed the isolates were *Fusarium* species since the primers were able to amplified them and the bands produced from the PCR were within the 760 base pair.

5.3 Pathogenicity of Fusarium spp against Striga

Frequent incidences of *Striga* species have been reported in many countries in Africa. In northern Ghana particularly, *Striga hermonthica* is a major destructive parasitic weed that affects food security and safety (Akanbelum *et al.*, 2023). In an attempt to address the call for a cost-effective biocontrol agent against *Striga* species in food production, we isolated five *Fusarium* species (*F. duofalcatisporum*, *F. incarnatum*, *F. fredkugeri*, *F. verticillioides and F. oxysporum*) from diseased *S. gesnerioides and S. hermonthica*, characterized them and determined their pathogenicity against *Striga hermonthica* using in-vitro assay. Although the *Fusarium* genus contains many important field and storage pathogens that often contaminate grains with mycotoxins and reduce grain quality, a few *Fusarium* species have demonstrated



their ability to eliminate or reduce the influence of parasitic weeds on grain yield and quality (Kagot *et al.*, 2014).

It is worth mentioning that the species Fusarium duofalcatisporum which has been reported to be restricted to certain regions of North and South-eastern Africa (Lombard et al., 2022), was the dominant Fusarium endophyte associated with the Striga weed. F.duofalcatisporum is also reported to be associated with cotton and Phaseolus vulgaris seed in Sudan by Lima et al., (2021). The pathogenicity of F.duofalcatisporum may be resulting from their ability secret metabolites that contains phytotoxic effects on their hosts. Nganje et al., (2004), reported same that Fusarium species capable of producing mycotoxin are major plant pathogens found in cereals.

Fusarium isolates including F. oxysporum, F. verticillioides, F. incarnatum, F equiseti and F. chlamydosporium were successfully isolated from disease Striga hermonthica from Kenya (Kagot et al., 2014. Similarly, F. duofalcatisporum and F. incarnatum were largely found to be associated with Striga hermonthica seeds sampled from Ethiopia (Lombard et al., 2022). A common pathogen that affects a variety of plant hosts, Fusarium incarnatum causes several illnesses. For instance, Fusarium incarnatum is responsible for various plant diseases, including spear rot of oil palm (Suwandi et al., 2012), black spot disease of Chinese jujube (Gai et al., 2017), stalk and ear rot of maize (Guo et al., 2016), and fruit rot of bell pepper (Ramdial et al., 2016). Fumonisins are the principal secondary metabolite produced by Fusarium verticillioides, with traces of beauvericin, fusaric acid, fusarin C, gibberiliformin, and moniliformin also being produced.

It is well established that *Fusarium*, mainly *F. oxysporum*, isolated from *S. hermonthica* selectively attack *Striga* spp. through the amino acids, L-leucine and L-tyrosin which are toxic to Striga and at the same time promote maize growth and development (Jamil *et al.*, 2021;



Nzioki et al., 2016). F. oxysporum was proved to possess most promising pathogenic potentials against the parasite which is in relation with the report of Kroschel et al., (1996) and Elzein et al., (2008) which revealed F. oxysporum as the most virulent species during the underground development of S. hermonthica. F. oxysporum are prolific producers of mycotoxins containing fusaric acid which is noted for its phytotoxicity and is also one of the first phytotoxins in tomato reported to cause its wilting according to Perincherry et al., (2019).

Despite this significant development, the validation and further development of this biocontrol control approach still require intensive research to identify new species with potential *Striga* pathogenicity activity. In the current study, all the recovered *Fusarium* isolates from the diseases *S. gesnerioides and S. hermonthica* were found to be pathogenic to wounded *Striga* detached leaves. The observed lesions' necrotic area varies among the *Fusarium* species, and was high in *F. fredkrugeri*. *Fusarium fredkrugeri* was first descripted from soil samples collected in South Africa, and is also known from *Striga hermonthica* in Madagascar (Sandoval-Denis *et al.*, 2018). However, the pathogenicity or the beneficial or deleterious effect of *F. fredkregeri* against the sampled plants were not reported. In the present study, *Fusarium fredkrugeri* was found to have necrotic effect on Striga leaves. Previously, Fusarium isolates including *F. oxysporum*, *F. verticillioides*, *F. incarnatum*, *F. equiseti and F. chlamydosporium* from Kenya successfully prevented Striga emergence and resulted in increased maize yield (Kagot *et al.*, 2018).



CHAPTER SIX

6.0 CONCLUSION AND RECOMMEDATION

6.1 Conclusion

It can be concluded from this study that all the *Fusarium* species isolate from the study are all pathogenic to *Striga*. *F. fredkugeri*, *F. duofalcatisporum* and *F. oxysporum* were highly pathogenic to the *Striga* leaves in the first week. Subsequently, in the second week *F. incarnatum* and *Fusarium verticillioides* were highly pathogenic in the second week as they occupied the second position after *F. fredkugeri*.

Fusarium oxysporum was highly pathogenic to the striga leaves in both week one and week two. It can be concluded the fusarium oxysporum is pathogenic to Striga and therefore makes it the best to be use as biocontrol agent against Striga.

Fusarium fredkugeri although a new species was the most pathogenic to the Striga leaves in the first and second weeks, this makes it a good candidate for biocontrol against Striga.

6.2 Recommendation

The five Fusarium species that were isolated; Fusarium fredkugeri, Fusarium oxysporum, F. duofalcatisporum, F. incarnatum and Fusarium verticillioides were all high pathogenic to Striga and can be considered as biocontrol agent against Striga. Fusarium oxysporum was highly pathogenic to the Striga leaves for the first and second week and should be recommended as biocontrol agent against Striga.

The other *Fusarium* species isolated from this study have the potential prospects to be as biocontrol against *Striga*. Further studies should be conducted on them to get their prospects as biocontrol agents against *Striga*.

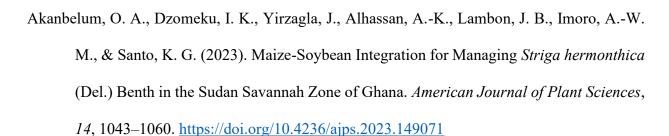
Further studies should be conducted on host specificity of *fusarium* species and how to enhance the virulence

Fusarium fredkugeri is a new species of Fusarium that has recently be discovered and from this study it was highly pathogenic to the Striga leaves for both week one and week two, I therefore recommend more research to be conducted on the Fusarium fredkugeri.



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APPENDICES

Appendix 1: Harvesting of striga on farms at Manga





Appendix II: Airdrying of striga leaves after washing with water and parazone.



Appendix III: Placing of striga parts on a selective media



Striga leaves on a selective media

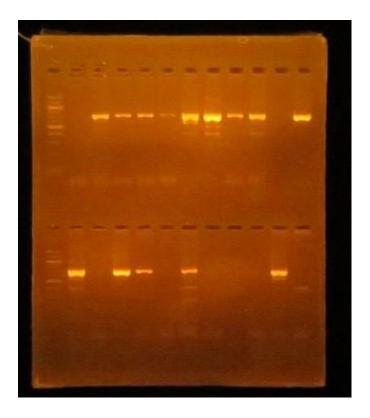


striga stems on a selective media



Appendix IV: Gel electrophoresis of fusarium isolates





Appendix v: leave assay to test the pathogenicity of fusarium isolates





Appendix VI: Microscopic picture of fusarium isolates



