

**ISOLATION AND IDENTIFICATION OF FUNGAL DISEASE OF MAIZE GRAINS
AT DIFFERENT STORAGE FACILITIES**

BY

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CERTIFICATION

This project by **MUHAMMAD HAUWA’U MAHUTA ADM. NO. 2010302005** has meet the requirement for the award of BS.c in Biology Usmanu Danfodiyo University, Sokoto

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DEDICATION

This project is dedicated to my beleoved parents, Prof. Muhammad Garba Mahuta and Hajiya Shafa'utu isah Wamale.

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First and foremost, I give thanks to Almighty Allah for His grace, protection, and guidance during the course of this project. Without His divine support, this achievement would not have been possible. I am deeply grateful to my project supervisor, Professor Sanusi Muhammad, for his outstanding mentorship, constant encouragement, and insightful guidance, which were instrumental to the success of this research. My sincere appreciation goes to the Head of the Department of biology, Prof. M.M. Yahaya for his leadership and support throughout my time in the department. I also wish to acknowledge the efforts of the Departmental Project Coordinator, Mal. S.L. yelwa, whose coordination, guidance, and support greatly contributed to the smooth execution of this project. Furthermore, I extend my heartfelt thanks to all the lecturers and staff of the Department of Biology, for the knowledge, support and dedication they have shown throughout my academic years.

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ABSTRACT

Maize (*Zea mays L.*) is a major staple crop in Nigeria but suffers significant post-harvest losses due to fungal contamination during storage. Traditional storage methods often expose maize grains to poor aeration, high humidity and pest infestation, which encourage fungal growth and increase the risk of mycotoxin contamination. These losses reduce grain quality, nutritional value, and pose serious public health risks. This study was conducted in the Mycology Laboratory of Usmanu Danfodiyo University, Sokoto. Maize samples were collected from silos, barns, warehouses and household storage facilities within the study area. Using Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA), fungal pathogens were isolated, cultured and identified through microscopic techniques. The findings revealed five fungal species: *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus oryzae*, *penicillium chrysogenum* and *Mucor racemosus*. Among these, *A. niger* was the most prevalent (53.33%), indicating high adaptability across diverse storage environments. *A. flavus* was the second most common (20%), raising significant concern due to its aflatoxin-producing potential. Other fungi such as *Rhizopus oryzae*, *Penicillium chrysogenum* and *Mucor racemosus* occurred less frequently but were associated with particular storage conditions. These results demonstrate that environmental factors, especially humidity, aeration, and hygiene, strongly influence fungal colonization of stored maize. In conclusion, *A. niger* poses the greatest threat to maize during storage, while *A. flavus* presents food safety risks linked to aflatoxins. To minimize fungal contamination, maize should be properly dried, stored under low-humidity, well-ventilated conditions and regularly inspected. Further research is recommended to explore advanced storage technologies and management strategies.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Maize (*Zea mays L.*) is a major staple food crop in Nigeria and many other parts of sub-Saharan Africa. It provides food for millions of people and serves as a key ingredient for animal feed and raw materials for food industries (Asibe *et al.*, 2023). According to the Food and Agriculture Organization (FAO, 2018), Nigeria is the largest maize producer in West Africa. However, despite high production, a significant amount of harvested maize is lost due to poor post-harvest handling and storage diseases (Mukanga *et al.*, 2010).

After harvest, maize grains are usually stored to be consumed later, sold in markets, or used for processing (Ekwomadu *et al.*, 2018). Unfortunately, these grains often suffer from post-harvest fungal infections, especially when stored under poor conditions. This field of study called post-harvest pathology focuses on how and why maize grains become infected by fungi and other microorganisms after harvesting (Afolabi *et al.*, 2007). Fungal contamination is the most common cause of spoilage in stored maize and leads to major losses in both quantity and quality (Bankole *et al.*, 2006).

In Nigeria, many farmers and traders store maize in traditional ways such as jute bags, wooden cribs, clay pots, or open barns. These storage methods usually have poor air circulation, high humidity, and exposure to pests and rainfall (Fandohan *et al.*, 2003). Such

conditions are ideal for the growth of harmful fungi such as *Aspergillus flavus*, *Fusarium verticillioides*, *Penicillium spp.*, and *Rhizopus spp.* (Udoh *et al.*, 2000). These fungi can infect maize even before harvest and continue to grow during storage if moisture content is not reduced to safe levels (ur Rehman *et al.*, 2021).

One of the most dangerous problems caused by storage fungi is the production of mycotoxins, such as aflatoxins and fumonisins (Dutton *et al.*, 2009). These are toxic chemicals that remain in food even after cooking and drying. According to the International Agency for Research on Cancer (IARC, 2002), aflatoxins are known to cause liver cancer and immune suppression. (Makun *et al.*, 2010) found high levels of fumonisins in maize samples from different parts of Nigeria, posing a serious health risk to both humans and animals (Owolade *et al.*, 2001). Mycotoxins also reduce the market value of maize and can result in the rejection of maize exports that do not meet international safety standards.

Hell *et al.*, (2000) conducted a study in Benin and Nigeria and found that maize stored in improved structures like metal silos and sealed plastic containers had lower levels of fungal infection compared to maize stored in traditional systems. Similarly, (Fapohunda *et al.*, 2013) reported that poor awareness of proper storage techniques and lack of access to modern storage facilities are major causes of fungal contamination in rural Nigeria (Beukes *et al.*, 2018).

The impact of these losses is wide-ranging. At the household level, it leads to food insecurity and health problems. At the national level, it causes economic loss and lowers the

competitiveness of Nigerian maize in regional and international markets. According to (Adebajo and Idowu 1994), over 30% of stored maize in Nigeria can be lost to fungal spoilage within a few months if not stored properly (Benjamin *et al.*, 2024).

Despite the known risks, there is still limited data on the specific types of fungal organisms affecting stored maize grains in different parts of Nigeria and under various storage systems. Many farmers and grain handlers also do not fully understand how storage conditions affect the growth of fungi and the production of mycotoxins (Mabuza *et al.*, 2018).

Therefore, this study aims to fill this gap by isolating and identifying the major fungi found in maize grains stored in different types of storage facilities across selected areas in Nigeria. The findings will help to assess which storage practices are more prone to fungal contamination and will provide useful recommendations for safer maize storage. This will ultimately help to reduce post-harvest losses, improve food safety, and support better income for farmers and traders (Kibe 2015).

1.2 Statement of the Problem

Maize is a staple food crop in Nigeria and is consumed by a large portion of the population. However, a major challenge affecting its availability, quality, and safety is the high rate of post-harvest losses due to fungal infections during storage (Alunga *et al.*, 2016). These losses are not only economic but also pose serious health risks due to the presence of

harmful mycotoxins such as aflatoxins and fumonisins. Despite efforts by agricultural extension services and food safety organizations, fungal contamination remains a widespread problem in many parts of the country.

Despite being a major staple food in Nigeria, maize grains often suffer significant post-harvest losses due to fungal contamination during storage. Many farmers and traders use traditional storage methods such as sacks, barns, or open containers, which expose the grains to moisture, poor ventilation, and warm temperatures conditions that favor the growth of harmful fungi (Van Rensburg *et al.*, 2016). These fungi not only cause spoilage but also produce dangerous mycotoxins like aflatoxins and fumonisins, which can lead to serious health issues in humans and animals, as well as economic losses.

There is limited awareness and access to improved storage practices in many rural areas, and the specific types of fungi affecting maize in different storage systems are not well documented. Without proper identification of these fungal species, effective control and prevention strategies remain difficult to implement.

This study seeks to fill this gap by isolating and identifying the fungal pathogens present in maize grains stored under various storage conditions in Nigeria (Gxasheka *et al.*, 2015). Understanding which fungi are most prevalent in specific storage environments will help in developing better storage techniques and reducing food contamination.

1.3 Justification of the Study

The statement of the problem highlights the urgent need to identify the specific fungal species responsible for maize spoilage under different storage conditions. This is particularly important because different storage methods are used across rural and urban areas in Nigeria, and not all of them provide the same level of protection against fungal growth (Munkvold 2003). Unfortunately, many farmers and grain handlers are not fully aware of the connection between poor storage practices and the development of fungal infections and toxins.

While several studies have been carried out on maize fungi and mycotoxins in Nigeria, most have focused either on pre-harvest contamination or general fungal presence without comparing the effects of different storage systems. Therefore, there is a clear need for localized research that examines how storage type affects the kind of fungal organisms found in stored maize.

This study is justified because it will provide practical and scientific information on the types of fungi present in maize stored under various conditions. By identifying the most vulnerable storage methods and the fungi commonly associated with them, the research can help farmers, traders, and policymakers take better action to reduce losses, improve food safety, and increase the shelf life and quality of stored maize. The results will also contribute to existing literature and support national efforts to promote food security and public health (Lamboni *et al.*, 2009).

1.4 Aim and Objective of the Study

The main aim of this study is to isolate and identify the fungal species that affect stored maize grains in different types of storage facilities in Nigeria, and to assess how storage conditions influence fungal growth and contamination. The specific objectives were to:

1. isolate and identify the fungal species present in the stored maize grains using standard laboratory techniques.
2. compare the level and type of fungal contamination across the different storage methods.
3. determine the storage conditions (e.g., temperature, humidity, duration) that favor fungal infection.

1.5 Significance of the study

This study is important because it helps to understand the types of fungi that spoil maize grains during storage in Nigeria. Many farmers and sellers lose a lot of maize after harvest because of poor storage (Biemond *et al.*,2021). Some of these fungi also produce harmful substances called mycotoxins, which can make people and animals sick.

By finding out which fungi are present in different storage methods, this research will help people know the best way to store maize and keep it safe. The study will also give useful information to farmers, traders, and food safety officers. It can help reduce food loss, protect public health, and improve the quality of maize in the country (Price *et al.*,2024).

1.6 Scope and delimitations

This study focuses on the isolation and identification of fungal species affecting maize grains stored in various storage facilities such as sacks, barns, silos, and plastic containers within selected regions of Nigeria such as sacks, barns, Silos and plastic containers within the northern region of Nigeria (Kano, Katsina and Sokoto) (Miller 2008). It examines the relationship between storage conditions and the level of fungal contamination, aiming to recommend better storage practices. The study is limited to post-harvest maize and considers only fungal infections, excluding bacterial or pest-related damage. It is also restricted to areas, such as Anambra,Cross Rivers (e.t.c.) and relies mainly on morphological identification methods rather than advanced molecular techniques.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Maize (*Zea mays L.*) is more than just a crop it's a lifeline. As one of the world's most vital cereal grains, it feeds millions, supports livestock production, and fuels various industries. Yet, despite its importance, maize faces a silent but destructive enemy after harvest: fungal infections. These unseen invaders thrive in poorly managed storage facilities, turning healthy grains into spoiled, toxin-ridden produce. The consequences are severe reduced nutritional quality, economic losses for farmers, and health risks for consumers due to dangerous mycotoxins.

Understanding these fungal threats is crucial to safeguarding our food supply. This chapter explores the scientific literature on the fungi responsible for maize grain spoilage, how they spread under different storage conditions, and the methods used to detect and identify them. By examining these factors, we can better address the challenges of post-harvest losses and work toward more effective storage solutions.

2.2 General Consideration of Maize Grain Disease

Maize (*Zea mays L.*) is a critical staple crop worldwide, serving as a primary food source for over a billion people and a key component in livestock feed and industrial products (Shiferaw *et al.*, 2011). Despite its importance, post-harvest losses due to fungal

infections remain a major challenge, with estimates suggesting that 10–30% of maize grains are lost annually due to spoilage FAO, (2019). These losses are exacerbated in developing countries where storage infrastructure is inadequate (Neme & Mohammed, 2017).

Corn is affected by both biotic and abiotic agents. This chapter addresses both types of causal agents. It is the responsibility of the plant pathologist to assist in attempts to minimize crop losses caused by disease (Payak *et al.*, 1985). Because of its stature as a food and feed crop, its genetics, and its adaptability to most environments, corn and its diseases can serve as a model system by which certain principles of plant disease development and disease control can be understood and practiced to accomplish the pathologist's responsibility.

Abiotic diseases of corn are caused by noninfectious entities (Czarnecka *et al.*, 2022). Two main types of abiotic diseases affect corn. One type of disease is the result of internal genetic factors within the plant. The other is a result of external stimuli being exerted upon the corn plant. This type of disease can be further grouped into climatically induced diseases, management practice-induced diseases, and diseases caused by air pollutants (Juroszek & Tiedemann, 2013). This mini-review summarizes the existing knowledge and hypotheses on the potential changes to the future importance of maize diseases due to projected global climatic changes. In contrast to fungal pathogens, there is almost no information available on viral and bacterial diseases (Subedi, 2015). Most studies related to fungi refer to *Aspergillus* and *Fusarium* species, which are causal agents of maize

ear rot, and the related risk of mycotoxin contamination of maize grain, potentially harmful to animals and humans. Just a single long-term simulation study based on a modelling approach driven by a climate change scenario has been reported for a maize disease so far (Munkvold, 2003). It projects a reduced risk of *Puccinia polysora* (southern rust) occurrence in Brazil in this century.

More simulation studies, ideally those which also generate quantitative disease-yield loss data for different maize diseases and locations are certainly needed in order to include the future potential disease risk in maize to the climate change debate (Degani, 2021). This will enable the estimation of the future maize productivity based on both abiotic factors such as temperature and biotic factors such as diseases. A fundamental conclusion of this mini review is that global maize disease problems caused by a changing climate will probably not consistently worsen, because climatic changes may also improve the crop health situation in maize depending on the disease, location and time scale considered, although ear rots and associated mycotoxin contamination of maize grain are expected to increase in many countries worldwide (Gxasheka *et al.*, 2015). Reducing ear rot disease risk is already of high priority and will likely demand particular attention in the future as well.

Fusarium species cause two distinct diseases on ears of maize, *Fusarium* ear rot (or pink ear rot) and *Gibberella* ear rot (or red ear rot), both of which can result in mycotoxin contamination of maize grain. The primary causal agent for *Fusarium* ear rot is *Fusarium verticillioides*, but *F. subglutinans* and *F. proliferatum* are also important. *Gibberella* ear rot

is caused primarily by *F. graminearum*, but *F. culmorum* can also be important, especially in Europe (Berger, 2024). Aspects of the epidemiology of both diseases have been studied for decades, but only recently have efforts been made to synthesize this information into comprehensive models of disease development. Much of the work on *F. graminearum* has focused on *Fusarium* head blight of small-grain crops, but some of the results obtained are also relevant to maize (Oldenburg *et al.*, 2017). The primary mycotoxins produced by these fungi, fumonisins and deoxynivalenol, have differing roles in the disease-cycle, and these roles are not completely understood, especially in the case of fumonisins. Progress is being made toward accurate models for risk assessment of both diseases, but key challenges remain in terms of integrating models of pre- and post-infection events, quantifying the roles of insects in these diseases, and characterizing interactions among competing fungi and the environment (Renfro *et al.*, 1976).

Maize is produced primarily in temperate and warm-temperate zones of the world, but its production is increasing in many tropical regions. Some diseases are common to both of these environmental zones even though their importance may vary greatly. Other diseases are confined to a single zone (Thompson *et al.*, 2018). The important factors influencing the geographical distribution of maize diseases and some of their vectors are temperature, moisture, cultural practices, and the type and diversity of maize germplasm used. Within tropical zones maize diseases may be segregated into those in humid environments and those favoured by drier, upland climatic conditions (Mukanga *et al.*, 2010). Elevation within the tropics, as this influences temperature, is a factor in distribution of maize diseases.

Late wilt, banded leaf and sheath blight, and Rhizoctonia, Botryodiplodia and grey ear rots occur only in the tropical zone and the warm, temperate areas, while the Sclerospora-incited downy mildew diseases, southern rust, Pythium and bacterial stalk rots, Curvularia leaf spot, and ear rot caused by Diplodia macrospora are much more prevalent there, occurring only occasionally beyond latitudes 34°N and 34°S. Conversely, bacterial wilt, eye spot, and yellow leaf blight have been found to occur only in the temperate zone (ur Rehman *et al.*, 2021). Ergot has been found only in a few locations at high elevation (250–270 m) in Central Mexico. Northern leaf blight, common rust, and stalk and ear rots caused by Diplodia maydis and Gibberella zeae prevail in the temperate zones and at high elevations and in winter seasons within tropical latitudes.

Field fungi invade the seeds before harvest while the crop is still in the field. Field fungi may affect the appearance and quality of seed or grain (Qiu *et al.*, 2020). Usually damage caused by field fungi occurs before harvest, can be detected by routine inspection and does not continue to increase in storage if grain is stored at the proper moisture content and temperature. Most field fungi are more prevalent when rainfall is above normal during grain fill and harvest. Invasion by field fungi may be more severe if the crop has been damaged by insects, birds or hail (Masiello *et al.*, 2019). With corn, ears well covered by husks and maturing in a downwards position usually have less rot than ears with open husks -or ears maturing in an upright position. Agricultural crops are vulnerable to infections by a wide spectrum of plant pathogens (Jeffers, 2004). In today's marketplace, the increasing complexity and wide distribution chain represent enormous challenges for food production.

The increased fungal infection and cross-contamination hazards are associated with the globalization of cereal trade (Waage *et al.*, 2006). Cereals are one of the most important sources of food (FAO, 2002), which have contributed to human nutrition for millennia. However, cereals are exposed to numerous biotic and abiotic stress factors, from cultivation and throughout their life cycle to processing (Gulbis *et al.*, (2016). The grain losses recorded during storage period on worldwide scale according to FAO estimations are between 5-10% of total production. In developing countries, due to reduced possibilities of implementing appropriate technologies, the reported damages during storage period may increase up to 30%. In 2015, the maize grains which are to be stored on an indefinite period of time is affected by the presence of various pests specific to warehouse ecosystem (Pfeiffer *et al.*, 2021). The paper work presents a study regarding the occurrence and development of specific warehouse micromycetes during the first months of maize storage. Immediately after being deposited, it has been identified the specific microflora for this period, respectively species of *Alternaria*, *Trichoderma*, *Cladosporium*, *Aureobasidium*, *Cephalosporium*, *Aspergillus* and *Fusarium* general (Soonthornpoet *et al.*, 2001). Crop safety and security can be achieved by maintaining climatic factors in stored spaces, thus limiting the populations' level of contaminating microorganisms.

Field fungi invade the seeds before harvest while the crop is still in the field. Field fungi may affect the appearance and quality of seed or grain (Tilahun *et al.*, 2002). Usually damage caused by field fungi occurs before harvest, can be detected by routine inspection and does not continue to increase in storage if grain is stored at the proper moisture content

and temperature. Most field fungi are more prevalent when rainfall is above normal during grain fill and harvest (Bressan, 2003). Invasion by field fungi may be more severe if the crop has been damaged by insects, birds or hail. With corn, ears well covered by husks and maturing in a downwards position usually have less rot than ears with open husks or ears maturing in an upright position.

Maize ear rots reduce grain yield and quality with implication on food security and health. Some of the pathogenic fungi produce mycotoxins in maize grain posing a health risk to humans and livestock (Matos *et al.*, 2004). Unfortunately, the levels of ear rot and mycotoxin infection in grain produced by subsistence farmers in sub-Saharan countries are not known. A survey was thus conducted to determine the prevalence of the ear rot problem and levels of mycotoxins in maize grain (Fisher *et al.*, 1992). A total of 114 farmsteads were randomly sampled from 11 districts in Lusaka and southern provinces in Zambia during 2006. Ten randomly picked cobs were examined per farmstead and the ear rot disease incidence and severity were estimated on site. This was followed by the standard seed health testing procedures for fungal isolation in the laboratory (Singh *et al.*, 2012). Results indicated that the dominant ear rots were caused by *Fusarium* and *Stenocarpella*. Incidence of *Fusarium verticillioides* ranged from 2 to 21%, whereas that of *Stenocarpella maydis* reached 37% on ear rot diseased maize grain. In addition, 2–7% *F. verticillioides*, and 3–18% *Aspergillus flavus*, respectively, were recovered from seemingly healthy maize grain. The mean rank of fungal species, from highest to lowest, was *F. verticillioides*, *S. maydis*, *A. flavus*, *Fusarium graminearum*, *Aspergillus niger*, *Penicillium spp.*, *Botrydiplodia spp.*,

and *Cladosporium spp* (Bertus *et al.*, 1927). The direct competitive ELISA-test indicated higher levels of fumonisins than aflatoxins in pre-harvest maize grain samples. The concentration of fumonisins from six districts, and aflatoxin from two districts, was 10-fold higher than 2ppm and far higher than 2ppb maximum daily intake recommended by the FAO/WHO (Niaz & Dawar, 2009). The study therefore suggested that subsistence farmers and consumers in this part of Zambia, and maybe also in similar environments in sub-Saharan Africa, might be exposed to dangerous levels of mycotoxins due to the high levels of ear rot infections in maize grain.

Fungal genera commonly affect maize grains, including *Aspergillus*, *Penicillium*, and *Fusarium*. These fungi can cause significant damage during both pre-harvest and storage, leading to yield losses and potential mycotoxin contamination (Haque *et al.*, 2022).

2.3 Fungal Disease of Maize Grains

Fungal genera commonly affect maize grains, including *Aspergillus*, *Penicillium*, and *Fusarium* (Pal *et al.*, 2022). These fungi can cause significant damage during both pre-harvest and storage, leading to yield losses and potential mycotoxin contamination.

2.3.1 Fusarium

Fusarium is one of the major fungal genera associated with maize in Africa. This genus comprises several toxigenic species including *F. verticillioides* and *F. proliferatum*, which are the most prolific producers of fumonisins (Degani *et al.*, 2020). The fumonisins

are a group of economically important mycotoxins and very common contaminants of maize-based foods and feeds throughout the world. They have been found to be associated with several animal diseases such as leukoencephalomalacia in horses and pulmonary oedema in pigs. Effects of fumonisins on humans are not yet well understood. However, their occurrence in maize has been associated with high incidences of oesophageal and liver cancer (Do Amaral *et al.*, 2005). Infection of maize by *Fusarium* species and contamination with fumonisins are generally influenced by many factors including environmental conditions (climate, temperature and humidity), insect infestation and pre- and postharvest handling. Attempts to control *F. verticillioides* and to detoxify or reduce fumonisin levels in maize have been undertaken. However, more research studies are urgently needed in order to understand more about this toxin. Fumonisins are less documented because they are recently discovered mycotoxins compared to aflatoxins (Sermons *et al.*, 2018). To date in Africa, apart from South Africa, very little information is available on *Fusarium* infection and fumonisin contamination in maize. It is a matter of great concern that on this continent, millions of people are consuming contaminated maize and maize-based foods daily without being aware of the danger.

Infections of maize with phytopathogenic and toxinogenic *Fusarium* spp. may occur throughout the cultivation period. This can cause different types of diseases in vegetative and generative organs of the plant (Pfordt *et al.*, 2020). Along with these infections, mycotoxins are often produced and accumulated in affected tissues, which could pose a significant risk on human and animal health when entering the food and feed chain. Most

important fungal species infecting European maize belong to the *Fusarium* sections Discolour and Liseola, the first being more prevalent in cooler and humid climate regions than the second predominating in warmer and dryer areas (Tilahun *et al.*, 2012). Coexistence of several *Fusarium* spp. pathogens in growing maize under field conditions is the usual case and may lead to multi-contamination with mycotoxins like trichothecenes, zearalenone and fumonisins. The pathways how the fungi gain access to the target organs of the plant are extensively described in relation to specific symptoms of typical rot diseases regarding ears, kernels, rudimentary ears, roots, stem, leaves, seed and seedlings (Jakhar *et al.*, 2017).

Both *Gibberella* and *Fusarium* ear rots are of major importance in affecting the toxinogenic quality of grain or ear-based products as well as forage maize used for human or animal nutrition. Although rudimentary ears may contain high amounts of *Fusarium* toxins, the contribution to the contamination of forage maize is minor due to their small proportion on the whole plant dry matter yield. The impact of foliar diseases on forage maize contamination is regarded to be low, as *Fusarium* infections are restricted to some parts on the leaf sheaths and husks (Anjorin *et al.*, 2019). Mycotoxins produced in rotted basal part of the stem may contribute to forage maize contamination, but usually remain in the stubbles after harvest. As the probability of a more severe disease progression is increasing with a prolonged cultivation period, maize should be harvested at the appropriate maturity stage to keep *Fusarium* toxin contamination as low as possible (Zou *et al.*, 2022). Ongoing surveillance and research is needed to recognise changes in the spectrum of dominating

Fusarium pathogens involved in mycotoxin contamination of maize to ensure safety in the food and feed chain.



Figure 1 Symptoms of fusarium ear rot on maize grain

Description: image showing symptoms of fusarium ear rot on Maize grain. Source: Research Gate

Fusarium species, particularly *F. verticillioides* and *F. graminearum*, are significant pathogens of maize, causing ear rots, stalk rots, and mycotoxin production (Debnath *et al.*, 2012). Fusarium can infect maize before harvest and continue to develop during storage. Fusarium is a large genus of soil-borne fungi with both saprophytic and pathogenic species. They are widespread globally and can infect maize during pre-harvest, harvest, and post-harvest stages (Mrope, *et al.*, 2024). Fusarium was first described in the early 19th century, and by the early 20th century, its role in plant diseases, including maize, was recognized.

1950s–1970s: Increased awareness of Fusarium spp. due to livestock poisoning from contaminated maize.

1988: Fumonisin (toxic secondary metabolites from *F. verticillioides*) were first identified and linked to equine leukoencephalomalacia (ELEM) and human esophageal cancer.

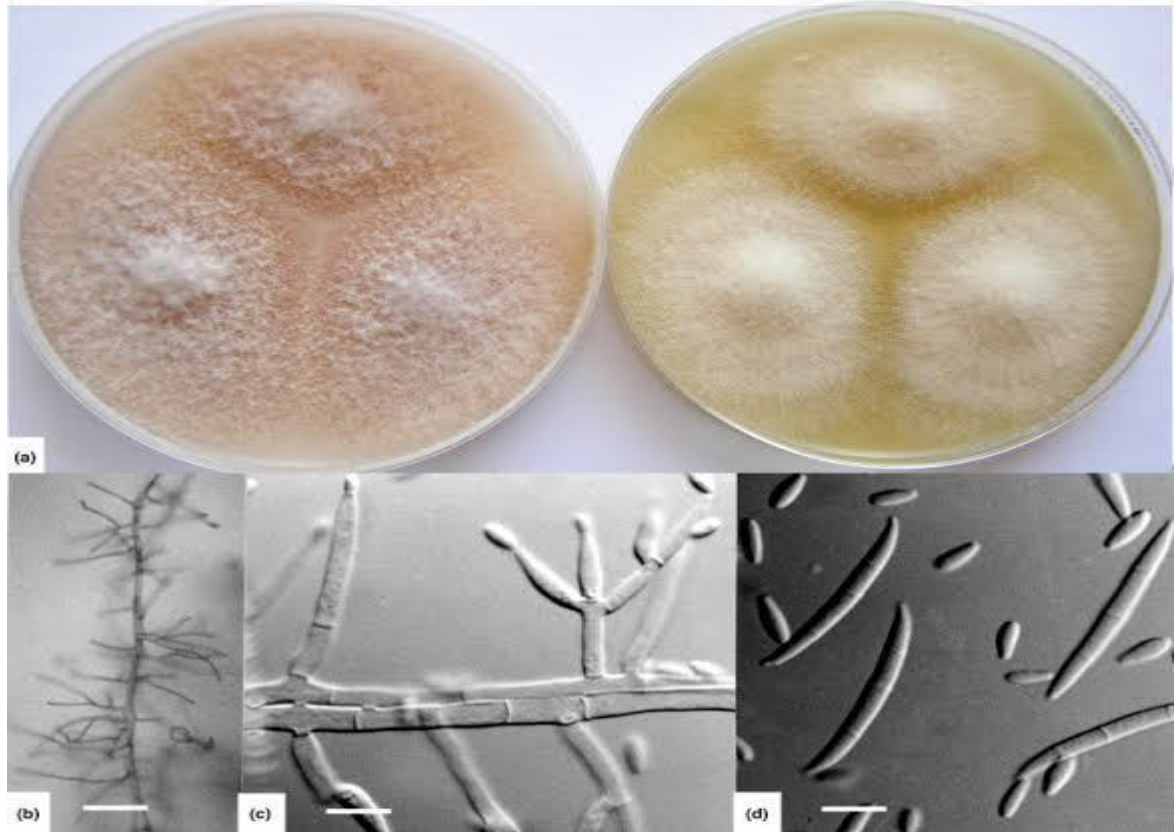


Figure 2 Image of fusarium verticillioides on growth Agar

Description: image showing the fusarium verticillioides growth on Agar (Lag, Log, Stationary and death phase. Source: Research Gate

2.3.2 *Aspergillus SPP*

Maize (*Zea mays L.*) is a staple crop worldwide but is highly susceptible to fungal contamination, especially by *Aspergillus* species during post-harvest storage. This study explores the pathogenic effects of *Aspergillus spp.*, particularly *Aspergillus flavus* and *Aspergillus niger*, on maize grains. These fungi not only deteriorate grain quality but also produce toxic secondary metabolites like aflatoxins, posing severe health risks to humans

and livestock (Moratelli *et al.*, 2015). Maize is one of the most important cereals consumed globally. However, its susceptibility to fungal infestation during harvest, drying, and storage poses a major threat to food safety and security (Borah *et al.*, 2016). Among various fungal pathogens, *Aspergillus* species are predominant, especially in warm and humid climates. Common *Aspergillus* Species Affecting Maize: *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus niger*, *Aspergillus ochraceus*.

2.3.3 Pathogenicity and Infection Mechanism

Aspergillus fungi infect maize through: Wounds caused by insects or mechanical damage, improper drying (moisture >13–15%), high relative humidity (>70%), poor ventilation and storage hygiene, once infected, the fungus colonizes the kernel, leading to grain discoloration, shriveling, and rot.

2.4 Effects on Fungal Disease on Maize Grains Maize Grains

2.4.1 Physical Effects

Discoloration (black, green, or yellow patches), Kernel shrinkage and brittleness, reduced grain weight and germination capacity. Maize grain have several physical effects on human health, both positive and negative. As a rich source of carbohydrate maize provide energy necessarily for daily physical activities and supports body strength and endurance. It fiber contents promotes healthy consumption. Yellow maize, in particular contains carotenoids such lutein and zeaxanthin which contribute to improved eye and skin health.

Furthermore, contaminated maize stored in poor conditions may harbor fungal toxins like aflatoxins, leading to serious health problems such as vomiting, abdominal swelling, stunted growth in children and liver damage.

2.4.2 Chemical Effects

Maize grains also have various chemical effects on the body. Especially due to their biochemical composition and the chemical changes they undergo during digestion processing or storage. Production of aflatoxins (especially by *A. flavus*), which are: carcinogenic, mutagenic and hepatotoxic, it also contains bacteria compound such as phenolic acid, flavonoids and carotenoids which act as antioxidant and help neutralize harmful free radicals in the body. However, if maize is stored under poor conditions. It may become chemically contaminated by mycotoxins like aflatoxins or fumonism which are toxic secondary metabolites produced by fungi such as *Aspergillus* and *fusarium*. These mycotoxins interfere with DNA replication, liver enzymes function and can lead to carcinogenic effects.

2.4.3 Nutritional Degradation

Loss or reduction of essential nutrient during harvesting processing storage or cooking. This degradation can significantly affect the grains quality and its ability to meet human nutritional needs. During long term storage, especially under warm and humid

condition maize is prone to fungal infestation, which not only lead to spoilage but also cause the breakdown of vital nutrients such as proteins, vitamins (especially B-complex) and fat.

Economic Effects

The production of maize is vulnerable to economic losses due to factors of contamination and climate change which can reduce yield quality and quantity, leading to increased food prices and financial strain on farmers. Rejection in local and international markets, reduced yield and market value, increased health expenditure due to toxin exposure.

The infestation of maize by *Aspergillus* species, particularly *A. flavus*, poses serious agricultural and public health challenges due to aflatoxin contamination. Effective management requires an integrated approach, combining good agricultural practices, proper storage, and regular monitoring. Public awareness and strict regulatory standards are crucial for ensuring food safety. Over dependence on maize farming in some regions can expose economics to risks from market fluctuations and crop failures. Effect of maize in economic are extensive shaping food systems, job creation, trade and national development.

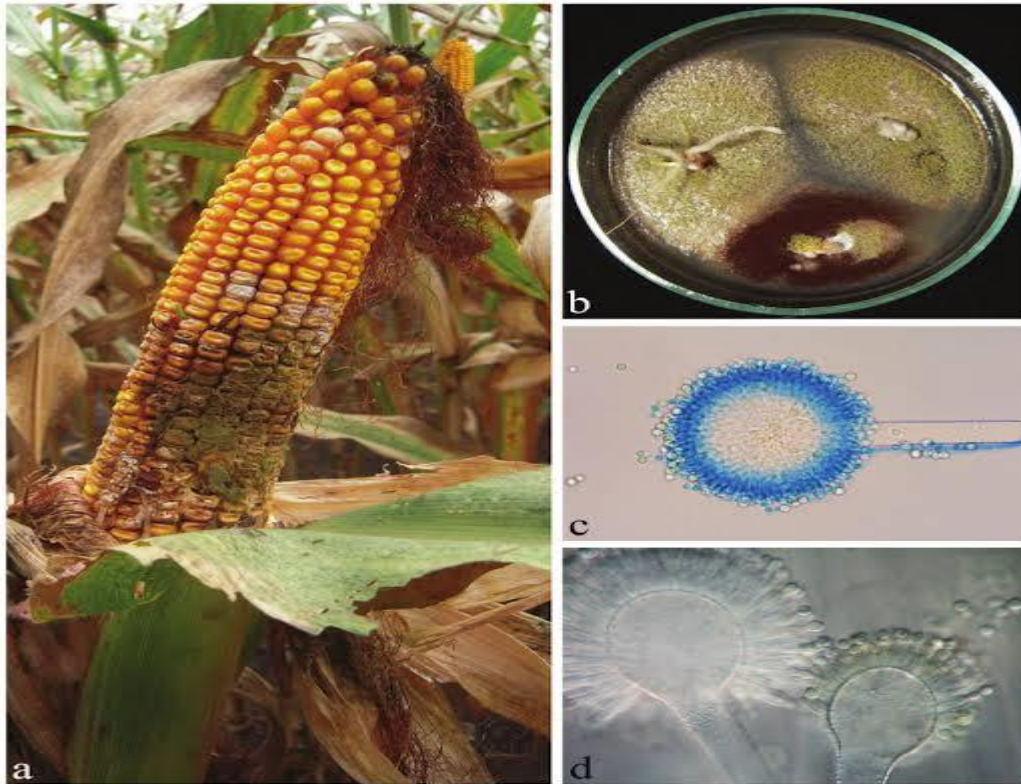


Figure 3 Occurrence of *Aspergillus flavus* on a maize crop and it's growth on media Agar.

Source: Research Gate

2.5 Storage Facilities

Maize (*Zea mays L.*) is a widely cultivated cereal with significant global importance. It is the third most important crop after rice and wheat, ranking first in production area and yield per hectare. Maize is a staple food crucial to alleviating poverty and addressing food crises. It also has various industrial applications, including the production of biofuels and the extraction of starch, oil, beverages, and food additives. Maize is a vital staple food crop,

ranking second in many African area coverage and first in total production among cereals. The Jimma zone in the Oromia region is a particularly dominant maize production area. The introduction of high-yielding hybrid varieties by the Ethiopian Institute of Agricultural Research has contributed significantly to the increase in maize production and productivity. However, maize storage presents challenges, leading to quantitative and qualitative losses caused by improper storage, insect pests, rodents, mites, fungi, and abiotic stresses. Negligence and substandard grain storage methods pose a significant obstacle to improving food security in Ethiopia. Without proper control measures, post-harvest grain losses due to insects can range from 20 to 80% within a few months after harvest.

Therefore, it is crucial to minimize stored grain losses caused by storage insect pests to meet the needs of a growing global population. Traditional storage methods such as polypropylene bags, jute sacks and conventional gombisa facilities are commonly used in Ethiopia to store maize for extended periods, typically longer than eight months. However, these traditional storage methods often result in significant losses due to rodents, insect pests, diseases, and adverse environmental conditions. On the contrary, improved storage methods have been reported to play a vital role in reducing maize losses caused by storage-related issues in Ethiopia. One highly effective method reported by many scholars is the use of hermetic storage bags. For example, a recent study by Kuyu demonstrated that hermetic storage bags resulted in less than 3.8% seed damage and preserved the nutritional composition of various grain types. On the contrary, non-hermetic bags caused up to 61.2% grain damage at the end of the storage period. These bags create an oxygen-depleted

environment by increasing carbon dioxide levels, thus protecting the grain from aerobic microorganisms and insects.

Furthermore, hermetic storage bags reduce or prevent moisture migration from the ambient environment to grains and from grains to the outside environment. Modified gombisa, an improved technology that incorporates photovoltaic panels for ventilation and drying, has shown promising results in controlling mycotoxin-producing fungi. Although hermetic storage technologies and modified gombisa have been developed and introduced in Ethiopia, small holder farmers have yet to widely adopt these technologies and continue to rely on conventional storage practices. A recent assessment conducted by (Fufa *et al.*, (2021) in significant areas of maize production in the country revealed that 98% of the farmers surveyed still use traditional storage methods (gombisa, gumbi, polythene sacks with different sizes (50 and 100 kg), woven sacks and some hang over the tree. Therefore, this study compares various storage facilities and evaluates their effectiveness in reducing quantitative and qualitative losses over eight months of storage. The findings of this research will provide valuable information to validate the performance of modified gombisa and hermetic storage technologies under Ethiopian conditions. It will also serve as a recommendation to smallholder farmers and agricultural policy makers in Ethiopia.

Storage has been the major problem of Nigerian agriculture for a long time and that has resulted in considerable waste of agricultural output. Due to inadequate storage facilities, a lot of farm produce have been condemned to wastage and our economy has

suffered dearly for it. Nigeria is losing about 2.4 billion tonnes of food yearly to poor harvest and storage facilities (Olumeko, 1999). With the world population projected to reach 9.1 billion people in the year 2050, there is a need to improve storage techniques in developing countries especially in order to preserve agricultural produce for an ever-growing population. Maize, being one of the most important cereals used majorly in human consumption is susceptible to pest infestation by weevils, reducing their nutritional value, weight and most importantly, the market price. Hence, there is a need for improved storage to protect maize and its products from damage or wastage whilst ensuring household food supply. The purpose of any grain storage facility is to prevent grain loss from; weather, moisture, rodents, birds, insects.

Conditions for storage

Maize can be stored for a long period of time (a year or more) if the moisture content is below the safe moisture level (below 14% or 12% and less). The moisture level should not rise during the storage process by either re-wetting by rain or imbibing moisture from the surrounding air. The maize must be protected from insects, rodents and birds. The longer the grain needs to be stored, the lower the required moisture content will need to be.

Methods of Storage

Prior to storage, immediately after harvesting, maize should be dried in the field as much as possible to ensure that it reaches safe moisture level and can then be stored

properly. Once the products have reached the safe moisture content, they can be stored permanently. There are various ways to store maize products depending on the duration of storage and relative humidity of the outside air during the period of storage. An efficient method of storage post the harvest season can empower the socio-economic scenario in India. These storage structures are eco-friendly, cheap, and provide high shelf life for the commodities to be stored. Though the chemical methods do save the commodities but then they also leave behind toxic residues. The wisdom of farmers to store through traditional methods is more secure and protects the commodities from infestation for a longer period. To meet the demand in the off-season grains, legumes and cereals must be stored throughout the year. Adequate storage and processing facilities will bridge the gap between demand and supply. It has been observed that different localities of India have storage methods that are peculiar to them. This also depends upon if the storehouses are outdoor, indoor, big or small, permanent or temporary, and last but not least it also depends upon if the storage is for community or individual. The storage structures could be semi-open, open, or closed. Certain methods are traditional and are unique to a particular community. These are entirely based upon the wisdom of the locals who has the consciousness of the environment of their native place.

The Crib:

This is entirely made up of bamboo, wood, metal wires, and roofed with thatch straws in a way that air can perpendicularly pass through them. It is a rectangular-shaped

structure and elevated above ground by 0.5m to 1m. The legs are fitted with a rat-proof device to prevent them from harming the produce. Its shape allows the drying process of grains with ease as the natural ventilation continues.



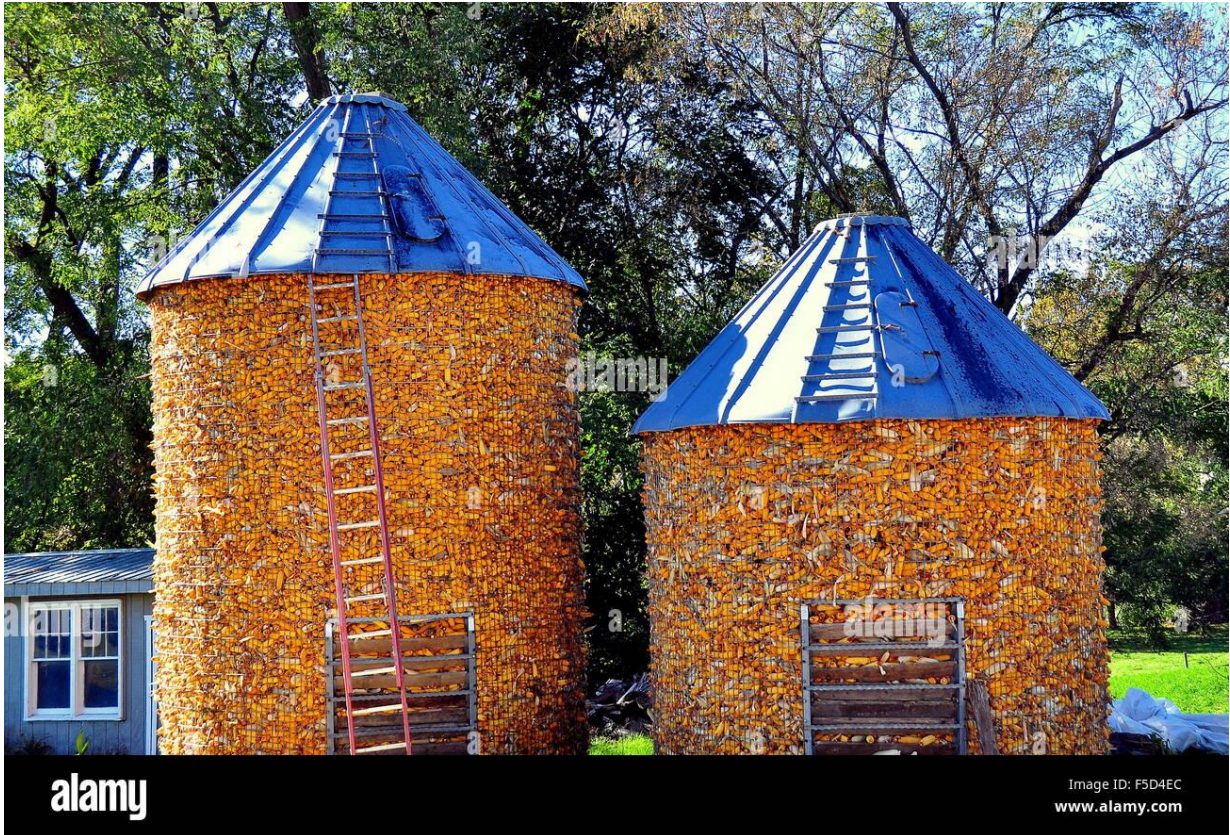
Description: Corn Cribs hi-res stock photography. Source: Harvest money

Storage with table salt:

For short-term storage Farmers sometimes use table salt. The abrasive nature of the salt prevents the insects from entering the storage and destroying the produce.

Camphor:

Another damage preventive method used by farmers for short-term storage is camphor. Its repellent, fumigant, and antifeedant pungent smell prevents insects from attacking the grains.



Description: Traditional method of food preservation and storage in Nigeria and India.

Source: sciencedirect.com

Constructed in agro-ecological zones where the water table is low, this is an age-old system of storage. It can store up to 200 tons of grains like millet, cowpea, sorghum, etc. It's

bell-shaped or cylindrical. Insulated with corn husk or straw mat. 1-3 meters deep and in diameter, it's covered with wooden planks and polythene/iron sheets.



Description: underground Silos made of dry mud and covered with adobe lids, the compound of a walled. **Source:** Alamy

Silo:

Silo is a tall cylindrical structure mostly used to store threshold food grains like paddy. It is pest resistant and is made of various materials like metal, concrete, plastic, and

mud. In a Silo, you can store grains for 6 months to some years. It is placed on an embankment made of coarse gravel and insulated from moisture.



Description: Grain storage Silos. Source: TOFNUM

Storage with cow dung:

Some farmers believe that cow dung has pesticide property and can protect seeds from insects. After being dried these seeds are embedded in the plate-like structures of cow dung and then sun-dried for 2-3 days.

Food grains are the main source of nutrition for a larger population hence their spoilage should be stopped. The above-mentioned traditional methods of storing can be further improvised with modern technologies. This will help us meet the requirement of food grains and stop starvation worldwide. To reduce the losses post-harvesting following measures like drying the grain before storing, using the storage structures that are sufficiently aired and are moisture-proof is a great practice followed by the farmers. The fundamental need for grain storage is to protect them from rodents, insects, microorganisms and a well-built storage facility can easily do that.

2.5.1 Types of Storage Facilities of Maize Grain

1. The traditional practices of grain storage:

- i. **Solarization:** solarization is the process in which the grains are put under the sun with a temperature of 20 degrees and above. This process kills the insects before the grain goes for storage. The period of solarization varies with each grain and is best to the knowledge of the farmer. The best way to determine if they have dried enough for storage is by chewing them and farmers are well versed with it.
- ii. **Open fireplace:** Traditionally the majority of the farmers have been storing the grains near the place used for cooking so that the heat and the smoke can keep the pests away. When storing a larger quantity barns are built with slow-burning fire to keep the grains dry.

- iii. **Diatomitized Earth:** It's a cloud of dust formalized out of fossilized diatoms and consists of amorphous silicon. It acts as a natural insecticide to protect the grains from insects.



Description: Traditional methods of food grain storage. Source: sciencedirect.com

2. **Modern practices of grain storage**

In modern storage walls of the warehouse are built between the supporting pillars. If the supporting posts are thicker than the walls, it is important that the extra thickness is on the outside of the building so that the internal surfaces of the walls are smooth and free from projections. This facilitates cleaning of the store, and avoids interference with other

operations as well. The walls may be made of breezeblocks, or stabilised earth bricks 15 to 20 cm thick, and should be rendered smooth on both sides. They should be painted white, on the inside to facilitate the detection of insect pests, and on the outside to help keep the warehouse as cool as possible. Alternatively, the walls may be made of a lightweight material such as fibro-cement, galvanised metal sheet, or aluminium sheeting. However, walls of this kind are easily damaged, have poor insulating properties, and are sometimes prone to erosion. A vapour-proof barrier should be incorporated into the base of the walls, to prevent damp rising and causing damage to the warehouse structure and its contents. Also, a concrete strip about 1 metre wide should be laid around the outside of the warehouse, to prevent rain from eroding the base of the walls below the damp course.

- i. **Roof:** Internal pillars supporting roof frames should be avoided because, as previously stated, they can interfere with pest control and other stock management procedures. Instead, roof frames should be designed so that they transfer the weight of the roof to the supporting columns (in framed buildings), or to the walls if the warehouse is small. Modern engineering techniques allow very wide 'free-span' roofs (i.e. roofs without internal supporting pillars) to be constructed. However, such roofs are very expensive and rarely used in warehouse construction. A steel portal frame should be used if the span is to be greater than 15 metres. Warehouses less than this width may have reinforced concrete roof frames. Roof frames made of wood or bamboo are only suitable for warehouses not more than 4 or 5 metres wide. The wood used must be well dried and treated with a preservative. Roof cladding

may be of galvanised steel or aluminium sheeting, or asbestos cement; the latter being more fragile but having better insulating properties. Tiles are not recommended, especially for large warehouses. The roof should overhang the gables by 0.7 to 1.0 metres, and the eaves by at least 1 metre. This ensures that rainwater is shed well clear of the walls; and obviates the need for guttering and drainpipes, which may become blocked or assist rodents entering the warehouse. The overhang also helps to keep walls cool and protects ventilation openings from rain.

- ii. **Ventilation:** Ventilation openings are necessary for allowing the renewal of air and reducing the temperature in the warehouse, they also allow some light to enter it. If such openings are located too low down they can be the source of numerous problems: entry of water, rodents, thieves, etc. These problems are avoided when ventilators are placed under the eaves. They should be fitted on the outside with anti-bird grills (20 mm mesh) and on the inside (10 cm behind the grills) with 1 mm mesh screens (removable for cleaning) which will deter most insects.



Description: An image showing a drying of corn for preservation. Source: Mecmar

- iii. **Doors:** The number of doors will vary according to the size of the warehouse. If possible there should be at least two doors, so as to be able to rotate stocks on a 'first in, first out' basis. However, this may not be possible or practical in a very small warehouse. Double sliding doors are recommended. Preferably made of steel, or at least reinforced along their lower edges with metal plate as protection against rodents, they should be sufficiently large (at least 2.5 x 2.5 m) and close fitting. If swing doors are fitted they should open outwards in order not to reduce the storage capacity of the warehouse. It is recommended that the doors be protected from rain by an extension of the roof or a separate cover.

- iv. **Illumination:** Adequate light in a warehouse is an important factor as far as the safety of workers inside it is concerned. However, there can be problems in providing sufficient natural light, while satisfying other technical aspects at the same time.



Description: Grains warehouse storage illustration and vectors. Source: dreamstome.com

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted in the mycology laboratory of the Department of Biology at Usmanu Danfodiyo University, Sokoto, Nigeria. The university is located in Sokoto State, a region within the Sudan Savannah zone of northwestern Nigeria. The laboratory work was carried out for comprehensive isolation, identification, and characterization of fungal pathogens.

Maize grains were collected from various storage facilities, including farm silos, market storage areas, and household storage. The study area represents a typical maize-growing region with diverse storage practices and environmental conditions that influence post-harvest fungal development.

3.2 Sample Collection

Maize grain samples were systematically collected from different storage facilities, including silos, warehouses, local barns, and traditional household storage units across selected locations. A purposive sampling technique was employed to ensure that both visibly infected and non-infected grains were included, representing a wide range of possible fungal contamination.

Samples were collected aseptically using sterilized gloves and placed in clean, labeled polythene bags to prevent external contamination. Each sample was coded based on its source, storage type, and collection date. The samples were then transported to the

laboratory under controlled conditions to maintain their original state for further analysis, culturing, and fungal pathogen identification. This method ensured the reliability and accuracy of the results obtained during mycological investigations.

3.3 Media preparation

In preparing media for the cultivation of *Fusarium verticillioides* and *Aspergillus flavus*, the media that were typically used are Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA), to support the growth of the pathogen while inhibiting the growth of normal flora.

3.3.1 Preparation of Potato Dextrose Agar (PDA)

This was prepared according to the manufacturer's instructions. 39g of nutrient agar powder was weighed and dissolved in 1000ml of distilled water in conical flask and was corked with a cotton wool and wrapped with aluminium foil. It was then shaken thoroughly and thereafter heated to further dissolve it completely. It was then sterilized at 121°C for 15 minutes in an autoclave. After autoclaving, the mixture was allowed, to cool to pouring temperature of 40°C and was then poured into sterile plate to solidifying.

3.3.2 Preparation of Sabouraud Dextrose Agar (SDA)

This was prepared according to the manufacturer's instructions. 65g of nutrient was weighed and dissolved in 1000ml of distilled water in conical flask and was corked with a cotton wool and wrapped with aluminium foil. It was then shaken thoroughly and thereafter heated to further dissolve it completely. It was then sterilized at 121°C for 15 minutes in an

autoclave. After autoclaving, the mixture was allowed, to cool to pouring temperature of 40°C and was then poured into sterile plate to solidifying.

3.4 Methodology

3.4.1 Isolation of fungal pathogens

The isolation of fungal pathogens from maize grains was carried out under sterile laboratory conditions to prevent contamination and ensure result accuracy. Maize samples from different storage facilities were first surface sterilized using a 1% sodium hypochlorite solution for 2–3 minutes, followed by rinsing in sterile distilled water and blotting dry with sterile filter paper. Using standard culturing techniques, 5 to 10 grains from each sample were plated on Potato Dextrose Agar (PDA) medium supplemented with chloramphenicol to inhibit bacterial growth.

The plates were incubated at 25–28°C and observed daily for fungal growth. Emerging fungal colonies were sub-cultured onto fresh PDA plates to obtain pure cultures. Each isolate was preserved and labeled based on its source and morphological characteristics for further identification. This isolation procedure allowed for effective separation and observation of diverse fungal species associated with maize grains from different storage environments.

3.4.2 Microscopy

The identification of fungal pathogens isolated from maize grains was conducted using macroscopic and microscopic techniques. Pure fungal cultures obtained from sub-culturing

on Potato Dextrose Agar (PDA) were first examined microscopic characteristics, such as color, texture, margin, pigmentation, and growth pattern. These features provided preliminary clues about the fungal genera.

3.4.3 Gramstaining

A small drop of sample on clean glass slide was placed and was spread thinly to form a uniform smear. A small drop of sterile water or saline was added to the slide, using a sterile loop, a tiny amount of the fungal colony was picked and emulsified thoroughly in the water drop, making a thin suspension. The smear was allowed to air dry completely at room temperature, the glass was then passed through flame three (3) times to heat fixed. The smear was then flooded with crystal violet for 30 seconds and rinsed with distilled water. Lugol's iodine was added to the smear for 30 seconds and rinsed again with distilled water. It was then decolourized acetone and rinsed immediately with distilled water. The smear was counterstain with safranin for 30 seconds and rinsed with distilled water. The smear was then allowed to air dry after which oil immersion was added and viewed under a microscope using x100 objective lens.

CHAPTER FOUR

4.0 Results

This chapter presents and interprets the results obtained from the isolation and identification of fungal pathogens associated with maize grains stored in different storage facilities. The analysis is based on observations from three sample categories, labeled A, B, and C, each representing maize stored under different conditions. The fungal isolates were identified through morphological characteristics, and the results are organized to show the distribution, frequency, and percentage occurrence of each fungal species found.

The data in Table 4.1 shows the appearance of fungal isolates across the three different maize grain samples. Among the five fungi identified, *Aspergillus niger* was the most prevalent, occurring in all three samples with counts of 5, 4, and 7 respectively. This consistent presence indicates that *A. niger* is highly adaptable and capable of surviving under various storage conditions. *Rhizopus oryzae* was found only in Sample A, suggesting that its presence may be influenced by specific environmental factors present in that particular storage method. Similarly, *Aspergillus flavus*, a member of the Zygomycota group, was detected in Samples B and C, but not in A, while *Penicillium chrysogenum* and *Mucor racemosus* were found only in Sample C and Sample A respectively. These variations suggest that different storage environments have a significant impact on the type and frequency of fungal contamination observed in stored maize.

A summary of the total number of occurrences of each fungal pathogen is presented in Table 4.2. *Aspergillus niger* was the most frequently encountered fungus, with a total of 16 occurrences across all samples. This dominance implies that it is a major fungal contaminant in stored maize, and likely poses the greatest threat to grain quality during storage. *Aspergillus flavus* had a total of 6 occurrences, while *Rhizopus oryzae* followed closely with 4. Both *Penicillium chrysogenum* and *Mucor racemosus* had the lowest frequency, with only 2 occurrences each. The frequency distribution supports the observation that *Aspergillus niger* is the most aggressive or most easily adaptable fungus under the conditions studied.

Further analysis in Table 4.3 presents the percentage occurrence of each fungal isolate relative to the total number of isolates recorded. *Aspergillus niger* accounted for 53.33% of all fungal occurrences, clearly establishing it as the dominant species in the stored maize samples. *Aspergillus flavus* followed with a percentage occurrence of 20.00%, which is noteworthy due to its ability to produce aflatoxins that are hazardous to human and animal health. *Rhizopus oryzae* made up 13.33% of the isolates, while both *Penicillium chrysogenum* and *Mucor racemosus* each accounted for 6.67%. These percentages align with the earlier tables and reinforce the conclusion that *Aspergillus niger* poses the most significant risk among the fungal species identified.

Overall, the findings highlight the diversity and varying levels of fungal contamination in maize stored under different conditions. The predominance of *Aspergillus niger* suggests that this species is highly resistant and can thrive across a range of storage environments.

The presence of *Aspergillus flavus* also raises concerns regarding food safety due to its potential to produce harmful mycotoxins. Differences in fungal distribution among the samples indicate that storage conditions—such as temperature, humidity, aeration, and hygiene—play a critical role in determining the type and extent of fungal infestation. These results provide valuable insight into the impact of storage practices on the microbiological safety of maize grains and underline the importance of adopting improved storage techniques to reduce fungal contamination and preserve grain quality.

4.1 Samples Appearance

S/N	ISOLATES	A	B	C
1	<i>Aspergillus Niger</i>	5	4	7
2	<i>Rhizopus Oryzae</i>	4	0	0
3	<i>Aspergillus flavus</i> (zygomycota)	0	4	2
4	<i>Penicillium chrysogenum</i>	0	0	2
5	<i>Mucor racemosus</i>	2	0	0

4.2 Frequency of Occurrence

S/N	PATHOGENS	NO. OF OCCURENCE
1	<i>Aspergillus Niger</i>	16
2	<i>Rhizopus Orzae</i>	4
3	<i>Aspergillus flavus</i> (zygomycota)	6
4	<i>Penicillium chrysogenum</i>	2
5	<i>Mucor racemosus</i>	2

4.3 Percentage of Occurrence

S/N	ISOLATE	PERCENTAGE (%)
1	<i>Aspergillus Niger</i>	53.33
2	<i>Rhizopus Oryae</i>	13.33
3	<i>Aspergillus Flavus (zygomycota)</i>	20.00
4	<i>Penicillium chrysogenum</i>	6.67
5	<i>Mucor racemosus</i>	6.67

Sum Total = 53.33 + 13.33 + 20.00 + 6.67 + 6.67 = 100%

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The primary objective of this study was to isolate and identify fungal species contaminating stored maize grains under different storage conditions in Nigeria and to evaluate how these conditions influence fungal growth. The results revealed five major fungal species: *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus oryzae*, *Penicillium chrysogenum*, and *Mucor racemosus*, with *A. niger* as the most dominant isolate across all storage samples.

The dominance of *Aspergillus niger* (53.33% occurrence) observed in this study concurs with recent findings that emphasize its resilience and adaptability in diverse storage environments. Adegbeye et al. (2023) reported *A. niger* as a prevalent fungal contaminant in stored maize, capable of tolerating a wide range of temperature and moisture conditions, thus making it a persistent threat to grain quality during storage. This aligns with the current findings, indicating that *A. niger* can survive across varying storage conditions.

Aspergillus flavus was the second most prevalent species, appearing predominantly in samples B and C (20% occurrence). This finding is significant due to *A. flavus*'s known ability to produce aflatoxins, which are potent mycotoxins with serious health implications (Chala et al., 2021). Research has demonstrated that *A. flavus* thrives in warm (25–30°C) and humid conditions, typical of many storage environments in Nigeria, thereby increasing the risk of aflatoxin contamination in stored maize. This highlights the importance of

maintaining controlled temperature and humidity during storage to minimize fungal growth and toxin production.

The presence of *Rhizopus oryzae* and *Mucor racemosus* exclusively in Sample A suggests that these fungi may prefer specific environmental conditions, potentially linked to higher moisture content or poor aeration. (Kumar *et al.*, 2022) demonstrated that *Rhizopus* species rapidly colonize substrates under high humidity and temperature, conditions that can lead to significant grain spoilage if storage is not adequately managed.

Penicillium chrysogenum was detected only in Sample C, which corresponds with observations by (Oladipo *et al.*, 2023), who reported that *Penicillium* species are more prevalent in poorly ventilated and humid storage settings. This underscores the influence of storage hygiene and aeration in mitigating fungal contamination.

Overall, the pattern of fungal distribution in this study supports existing literature that environmental factors such as temperature, humidity, and aeration critically affect fungal colonization in stored grains (Mohammed *et al.*, 2022). Proper drying, maintaining low moisture levels, and adequate ventilation are essential strategies to reduce fungal infestation and prevent mycotoxin production.

5.2 Conclusion

This study successfully isolated and identified the predominant fungal species contaminating stored maize grains under different storage conditions in Nigeria. The results demonstrated

that *Aspergillus niger* is the most dominant fungal pathogen across various storage facilities, reflecting its adaptability and resilience to diverse environmental conditions. The presence of *Aspergillus flavus*, particularly in certain storage types, raises significant food safety concerns due to its potential for aflatoxin production.

Variations in fungal species distribution among the different storage samples indicate that environmental factors such as temperature, humidity, and aeration significantly influence fungal contamination levels. Poor storage conditions that allow increased moisture content and inadequate ventilation contribute to a higher incidence of fungal growth and mycotoxin risks.

Overall, the findings highlight the critical role of storage management in controlling fungal contamination and ensuring the safety and quality of stored maize grains. Improved storage practices that regulate moisture, temperature, and aeration are essential to minimize fungal infestation and reduce the risk of mycotoxin contamination.

5.3 Recommendations

Based on the results, the following recommendations are made:

1. Store maize only after it is properly dried to reduce moisture content.
2. Keep storage temperature and humidity low to prevent fungal growth.
3. Clean storage facilities regularly to remove fungal spores.

4. Ensure good ventilation to improve aeration and reduce dampness.
5. Inspect stored maize regularly for signs of fungal contamination.
6. Conduct further research on storage conditions that affect fungi.
7. Explore new storage methods and treatments to reduce fungi and toxins.

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