ISOLATION AND MOLECULAR CHARACTERIZATION OF *ASPERGILLUS FUMIGATUS* VARIANTS FROM SOME AGRICULTURAL PRODUCTS OBTAINED FROM DIFFERENT LOCATIONS

BY

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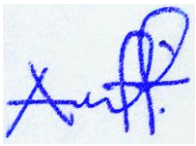
DECLARATION

I, undersigned Asante Danso Adjoa Asante, declare that, I am the author of this thesis. I do declare that the work is a result of my research work carried out in the Department of Plant and Environmental Biology, School of Biological Sciences, University of Ghana, Legon, under the supervision of Dr. Michael Wiafe-Kwagyan and Emeritus Professor George T. Odamtten from October 2023 to April 2025. References made in this work are duly acknowledged.



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DEDICATION

First of all, I dedicate this work to Almighty God for His grace, mercy and guidance. I also dedicate it to my late mother, Madam Nana Ama Kyeretwie Kwanin for her unconditional support and encouragement throughout my education. May God keep you in His bosom.

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"Rejoice always, pray continually, give thanks in all circumstances; for this is God's will for you in Christ Jesus" (**1 Thessalonians 5:16-18**)

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List of Abbreviations

BLAST – Basic Local Alignment Search Tool

CFU/g – Colony Forming Units per gram

cm – Centimeter

℃ – Degrees Celsius

DNA – Deoxyribonucleic Acid

Ecto – Epicarp, skin or peel of fruit

IA – Invasive Aspergillosis

Madina – Madina Market

mm – Millimeter

MM – Mallam Atta Market

PDA – Potato Dextrose Agar

pH – Hydrogen Ion Concentration

PCR – Polymerase Chain Reaction

µL – Microlitre

µm – Micrometre

v/v – Volume per volume

ABSTRACT

Post-harvest fungal and bacterial contamination significantly contributes to global food insecurity, particularly in regions like Ghana, where inadequate storage practices and environmental conditions exacerbate losses. This study focused on the isolation and molecular characterization of *Aspergillus fumigatus* variants from selected agricultural products, including fruits (oranges, mangoes, and tomatoes) obtained from Accra markets and cocoa beans from Breman Asikuma, with additional assessments of their pathogenicity and possible control strategies. Total fungal populations varied by fruit type and the market from which they were obtained. For instance, fruit juices (orange, mango and tomato) recorded $\log_{10}$ 3.0–3.4 CFU/g, and orange juice showed the least contamination at $\log_{10}$ 3.08 CFU/g). Fruit peels on the other hand, recorded lower contamination ($\log_{10}$ 1.0–1.4 CFU/g), highlighting the pericarp's protective role. The aeromycoflora populations also varied. For example, Mallam Atta Market showed marginally higher airborne fungal loads ($\log_{10}$ 1.43 CFU/g) compared to Madina Market. The prevalence of *Aspergillus fumigatus* in the various markets and on the different substrates sampled varied significantly ($P \leq 0.05$). The percentage incidence of *A. fumigatus* for Mallam Atta Market was 12.4% in airspora and was isolated from all fruit samples. Madina Market: 7.3% in airspora but absent in mango juice. Cocoa bean shells registered 6% *A. fumigatus* contamination, posing potential mycotoxicosis and causing detrimental health implications to consumers. The *A. fumigatus* complex isolated in this study had morphological and microstructural differences in hyphal shape, conidia, conidiophore, phialide and vesicle sizes. Conidia were deciduous, globose, oblong and ellipsoidal with dark-green suede-like colour with dense felt mycelial mass. Analysis of variance of morphological variability indicated significant differences ($p \leq 0.05$) in hyphal structures, conidia shapes (globose, ellipsoidal), and vesicle sizes among isolates, suggesting intra-species variants.

The disease progression observed in this study revealed that mango, orange, and tomato exhibited sigmoid-shaped curves, indicating a monocyclic disease pattern. However, there were significant differences ($p \leq 0.05$) in their levels of infection and susceptibility to the pathogen, as shown in the pathogenicity assays. Mango recorded the highest Area Under Disease Progress Curve (AUDPC) value of 13.69 cm, followed by tomato with 11.7 cm, and orange with 3.98 cm. These values corresponded with the observed variation in disease severity over seven days. Consequently, mango showed the highest susceptibility to infection, tomato displayed moderate susceptibility, and orange exhibited the lowest susceptibility. The monocyclic sigmoid curves observed further confirmed the presence of a single infection cycle.

Genetic analysis using conventional DNA techniques, including PCR amplification, gel electrophoresis, and ITS characterization, revealed phylogenetic divergence among the isolates. Two distinct genetic groups were identified based on ITS sequencing. Group 1 (550 bp) showed close genetic relatedness to the reference *A. fumigatus* strain (Af293_REF), whereas Group 2 (650 bp) represented a distinct evolutionary lineage with bootstrap values ranging from 0.4 to 0.98. Cocoa bean isolates exhibited the highest level of genetic divergence, suggesting possible niche adaptation. Among the three antifungal agents tested for controlling *A. fumigatus* growth *in-vitro,* Itraconazole (1:200 v/v) fully inhibited *A. fumigatus* growth due to triazole activity. The emergence of azole-resistant strains recorded in this study underscores the need for alternative antifungal agents. Pericarp integrity reduced contamination, but poor handling (e.g., damaged fruit) increased fungal ingress. The higher *A. fumigatus* prevalence in Mallam Atta Market may be linked to environmental or handling differences. Contaminations of cocoa beans by *A. fumigatus* highlight the urgent need for post-harvest monitoring to prevent huge losses and the likely accumulation of mycotoxins in the beans. This study underscores the critical role of localized fungal surveillance

and adaptive management in mitigating post-harvest losses. The genetic diversity of *A. fumigatus* strains in Ghana necessitates tailored interventions to address evolving antifungal resistance and preserve food safety.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Background Information

Fruits and vegetables are essential components of the human diet, providing a rich source of vitamins, minerals, fiber, and antioxidants, all of which are crucial for maintaining health and preventing disease. The consumption of fruits and vegetables has been consistently associated with a reduced risk of chronic illnesses, including cardiovascular diseases, cancers, and diabetes (Slavin and Lloyd, 2012). Fruits and vegetables are vital for human health due to their high nutrient density and low-calorie content. They are primary sources of essential vitamins, such as vitamin C, vitamin A, and folate, as well as minerals like potassium and magnesium (Steinmetz and Potter, 1996). These nutrients are necessary for various bodily functions, including immune system support, bone health, and the maintenance of healthy blood pressure levels.

In addition to vitamins and minerals, fruits and vegetables are rich in dietary fiber, which is important for digestive health. A diet high in fiber helps prevent constipation, lowers cholesterol levels, and reduces the risk of developing cardiovascular disease (Slavin, 2013). Moreover, the antioxidants found in many fruits and vegetables protect the body against oxidative stress, which is linked to aging and the development of chronic diseases (Boeing *et al.,* 2012). The World Health Organization (WHO) recommends consuming at least 400 g of fruits and vegetables daily to meet the nutritional needs of the body and to help reduce the risk of chronic diseases (WHO, 2020). Despite this recommendation, global consumption rates often fall short, highlighting the need for increased awareness and access to these vital food groups. There are many fruits on the Ghanaian

trading markets. But for the purpose of this thesis oranges, mangoes, tomatoes and a product of national interest, cocoa beans, were selected.

Oranges (*Citrus sinensis*)

Oranges (*Citrus sinensis*) are among the most popular and widely consumed fruits in the world, known for their sweet flavor and high vitamin C content. Vitamin C is a powerful antioxidant that plays a critical role in immune function, skin health, and the absorption of iron from plant-based foods (Carr and Maggini, 2017). A single medium-sized orange can provide over 100% of the recommended daily intake of vitamin C, making it an essential fruit for maintaining good health.

Beyond vitamin C, oranges also contain a variety of other nutrients, including fiber, folate, and potassium, which contribute to heart health by helping to lower blood pressure and cholesterol levels (Potter *et al.,* 1998). The presence of flavonoids, particularly hesperidin, in oranges further enhances their cardiovascular benefits by improving blood vessel function and reducing inflammation (Erlund, 2004).

Oranges are also significant from an economic standpoint, particularly in countries like Brazil and the United States, where they are major agricultural products. The orange juice industry, in particular, is a substantial contributor to the economy, providing employment and export revenues (FAO, 2022). Our market in Ghana is replete with oranges in their season, but these quickly get rotten due to biodeterioration. Biodeterioration is primarily caused by microbial activity, especially fungi such as *Aspergillus*, *Penicillium*, and *Fusarium* species, which invade damaged or overripe fruits under humid and poorly ventilated conditions, leading to decay and spoilage (Singh and Sharma, 2018).

Mangoes (*Mangifera indica*)

Mangoes (*Mangifera indica*), often referred to as the "king of fruits," are celebrated for their delicious taste and rich nutritional profile. They are an excellent source of vitamins A and C, both of which are essential for immune function, vision, and skin health (Shah *et al.,* 2010). Vitamin A, in particular, is crucial for maintaining healthy vision and preventing night blindness, while vitamin C aids in collagen formation and protects against oxidative stress.

Mangoes also provide dietary fiber, which supports digestive health and helps regulate blood sugar levels. The fruit is rich in polyphenols, including mangiferin, which has been shown to have antioxidant, anti-inflammatory, and anti-cancer properties (Masibo and He, 2008).

In Africa, mangoes are of particular importance, both nutritionally and economically. Countries such as Nigeria and Kenya are major producers of mangoes, with the fruit being a key source of income for smallholder farmers. The global demand for mangoes has been steadily increasing, driven by their popularity as fresh fruit and their use in processed products such as juices, dried fruit, and sauces (Neguse *et al.,* 2019). In Ghana, mangoes are seasonal delicacies enjoyed by both young and old. Much of the harvest goes to waste owing to lack of effective storage and preservation methods of conservation.

Tomatoes (*Solanum lycopersicum*)

Tomatoes (*Solanum lycopersicum*) are one of the most versatile and widely consumed vegetables worldwide. Although botanically classified as a fruit, they are commonly used as a vegetable in cooking. Tomatoes are known for their rich lycopene content, a powerful antioxidant that gives them their red color, and has been linked to a reduced risk of certain cancers, particularly prostate cancer (Giovannucci, 1999). In addition to lycopene, tomatoes are a good source of vitamins C and K, potassium, and folate, all of which contribute to overall health. Regular consumption of

tomatoes has been associated with a reduced risk of heart disease, as they help lower blood pressure and cholesterol levels while improving blood vessel function (Rao and Agarwal, 2000).

Tomatoes are also economically significant, in countries like China, India, and the United States of America being among the top producers. The global tomato industry is vast, encompassing fresh tomatoes as well as processed products like sauces, pastes, and canned tomatoes (FAOSTAT, 2022). This versatility makes tomatoes an essential crop for food security and economic stability in many regions not excepting Ghana.

Cocoa (*Theobroma cacao*)

Dry beans of cocoa (*Theobroma cacao*) are the primary raw material used in the production of chocolate and other cocoa-derived products. Native to the tropical regions of Central and South America, cocoa beans have become a globally significant agricultural commodity, with West Africa being the largest producer. The importance of dry cocoa beans extends beyond their economic value; they are rich in bioactive compounds that offer potential health benefits (Scapagnini *et al*., 2014; Melo *et al*., 2021)

Dry cocoa beans are essential in the global chocolate industry, which relies on them as the fundamental ingredient in products ranging from chocolate bars to cocoa powder. The beans are processed to extract cocoa solids and cocoa butter, both of which are key components in the manufacturing of various chocolate products (Afoakwa, 2010). Cocoa beans are not only economically important but also nutritionally valuable, providing a range of bioactive compounds, including flavonoids, theobromine, and caffeine, which contribute to their health-promoting properties (Martin and Sampeck, 2016).

The global demand for cocoa has a significant impact on the economies of producing countries, particularly in West Africa, where countries like Côte d'Ivoire and Ghana dominate the market. These two countries together account for more than 60% of the world's cocoa production (ICCO, 2020). The cocoa industry is a critical source of income for millions of smallholder farmers in these regions, supporting livelihoods and contributing to national economies through export revenues.

Nutritional and Health Benefits of Cocoa Beans

Cocoa beans are rich in flavonoids, particularly epicatechin, catechin, and procyanidins, which have been linked to various health benefits. These compounds have potent antioxidant properties, helping to neutralize free radicals and reduce oxidative stress, which is associated with the aging process and the development of chronic diseases (Katz *et al.,* 2011). Regular consumption of cocoa flavonoids has been shown to improve cardiovascular health by enhancing endothelial function, lowering blood pressure, and reducing inflammation (Hollenberg *et al.,* 2009).

In addition to flavonoids, cocoa beans contain theobromine, a stimulant similar to caffeine, but with milder effects. Theobromine has been found to have positive effects on mood, cognitive function, and respiratory health (McShea *et al.,* 2008). Cocoa also contains a small amount of caffeine, contributing to its stimulating effects on humans.

The potential health benefits of cocoa have led to its increasing popularity as a functional food ingredient. Dark chocolate, which contains higher concentrations of cocoa solids, is particularly noted for its health benefits when consumed in moderation (Katz *et al.,* 2011). This has spurred a growing interest in the nutritional value of cocoa products and their role in a healthy diet.

The economic importance of dry cocoa beans is key to development, particularly in West Africa, where cocoa farming is a major agricultural activity. Côte d'Ivoire, the world's largest producer of cocoa beans, heavily relies on cocoa exports for its economic stability. The cocoa industry in this region supports millions of people, from smallholder farmers to those involved in the processing and export sectors (ICCO, 2020). The revenue generated from cocoa exports is vital for the economies of producing countries, helping to fund public services and infrastructure development.

Cocoa production also plays a significant role in international trade, with the global chocolate industry being a multibillion-dollar market. The demand for cocoa is driven by consumer preferences for chocolate products, which continue to grow, particularly in emerging markets. As a result, the cocoa market has seen steady growth, leading to increased efforts to improve yield, quality, and sustainability in cocoa farming practices (Squicciarini and Swinnen, 2016).

1.2 Post-Harvest Infection by Fungi in Storage

Despite the economic importance of fruits, vegetables, and cocoa beans, they are susceptible to post-harvest contamination and spoilage by fungi, which can cause significant spoilage and economic losses. Post-harvest losses due to fungal contamination can reach up to 30% in some crops, affecting the profitability of farmers and the overall food supply chain (Honger *et al*., 2018). For instance, oranges are often attacked by *Penicillium* species, particularly *Penicillium digitatum* and *Penicillium italicum*, which cause green and blue mold, respectively (Eckert and Ogawa, 1988). These molds can rapidly spread during storage and transportation, leading to substantial spoilage and losses. Mangoes are vulnerable to *Colletotrichum gloeosporioides*, which causes anthracnose, a fungal disease that can significantly reduce the market value of the fruit (Arauz, 2000). Similarly, tomatoes are frequently affected by *Alternaria alternata*, leading to black mould rot, which not

only reduces their shelf life but also poses health risks due to the production of mycotoxins (Thomma, 2003).

Aspergillus species are among the most notorious post-harvest fungal pathogens, particularly in dry cocoa beans. *Aspergillus flavus* and *Aspergillus parasiticus* are known for producing aflatoxins, potent carcinogens that can contaminate a wide range of food products, including cocoa beans (Pitt and Hocking, 2009). The presence of aflatoxins in cocoa products poses significant health risks and can lead to the rejection of contaminated batches in international markets, causing substantial economic losses. In addition to aflatoxin-producing species, *Aspergillus fumigatus* is of particular concern due to its pathogenicity in both humans and plants. In stored cocoa beans, *A. fumigatus* can proliferate under conditions of high humidity and poor ventilation, leading to the spoilage of the beans and reduced quality of the final product (Pitt and Hocking, 2009).

The impact of *Aspergillus* species is not limited to cocoa beans; they also affect other crops. For example, *Aspergillus niger* is responsible for black mould in onions and can cause significant losses in storage (Snowden, 1990). This species is also known to produce ochratoxin A. This mycotoxin can contaminate cereals, coffee, and grapes, leading to food safety concerns and economic losses due to the need for stringent testing and regulation.

Fruits, vegetables, and dry cocoa beans are economically important agricultural commodities that contribute significantly to global food security and economic growth, particularly in developing countries. However, post-harvest fungi, including *Aspergillus* species, of which *A. fumigatus* is no exception, pose a major threat to the sustainability and profitability of agricultural and food-processing industries such as fruit production, vegetable storage, and cocoa processing. Addressing this challenge requires a multifaceted approach involving improved storage and handling practices,

effective fungal management strategies, and continued research into the prevention and mitigation of fungal contamination.

1.3 The Genus *Aspergillus*

Aspergillus is used for a genus of fungi that reproduces by only asexual means. The morphology of the conidiophore, the structure that bears the asexual spore is the most important taxonomic character used in *Aspergillus* taxonomy (Bennet, 2010). *Aspergillus* species are among the most successful and widespread fungi with important roles in natural ecosystems and human economy. There is a continuous fascination with their technological potential in producing useful extracellular enzymes and organic acid and other important secondary metabolites in bio-technology (Moore-Landecker, 1996).

One of the oldest named genera of fungi *Aspergillus* received its name from by the Italian Priest and Biologist Pier Antonio Micheli in 1729. Micheli was reminded of a device by the Roman Catholic clergy to sprinkle holy water during liturgy called asperges which resemble the microscopic spore-bearing structure- conidiophore of *Aspergillus* (Ainsworth,1976 as cited by Benett, 2010). The *asperges* was described as follows in Encyclopedia Britannica: Asperges (thou wilt sprinkle, from the Latin verb *aspergere*), the ceremony of the people with holy water before High Mass in the Catholic Church, with which the priest begins the ceremony. The brush used in sprinkling is an Aspergilli (singular, Aspergillum). By the time Thom and Church published the first monograph on the genus in 1926. *Aspergillus* has become one of the best-known and most studied fungal groups. Their prevalence in the natural environment, their ease of cultivation on laboratory media and economic importance of several of its species insured that many mycologists and industrial microbiologists were attracted to studying them in the laboratory.

1.4 Substrate Preferences

Aspergillus grows profusely as saprophytes on decaying vegetation, where they are found in large numbers on mouldy hay, grass, organic compost piles, leaf litter, dry cocoa beans, fruits and vegetable matter, mangoes (Shah et al., 2010; Neguse et al., 2019), and tomatoes (FAOSTAT, 2022). They can also be found on dung, human tissues and antique parchment. There is even a report of an unidentified *Aspergillus* species solubilizing low rank coal (Torzilli and Isbister, 1994).

Aspergillus is a large genus that belongs to the Phylum Ascomycota containing more than 100 recognized species such as *Aspergillus amstelodami, Aspergillus sojae, Aspergillus awamori, Aspergillus rogulosus, Aspergillus saito, Aspergillus clavatus, Aspergillus glaucus, Aspergillus ornatus, Aspergillus cervinus, Aspergillus restrictus, Aspergillus fumigatus, Aspergillus ochraceus, Aspergillus niger, Aspergillus candidus, Aspergillus flavus, Aspergillus wentii, Aspergillus cremeus, Aspergillus sparsus, Aspergillus oryzae, Aspergillus versicolor, Aspergillus nidulans, Aspergillus ustus, Aspergillus flavipes*, and *Aspergillus terreus* (Samson *et al*., 2014;Kale *et al.,* 2003). Several mycologists including Clark (1966), Thom and Raper (1945), Raper and Fennell (1965), Samson *et al.* (2014), Kale *et al.,* (2003) put the genus into 17 distinct teleomorphic group namely, *A. fumigatus group, A. ochraceus (A. alutaceus)* group, *A. clavatus* group, *A. glaucus* group, *A. ornatus* group, *A. restrictus* group, *A. niger* group. *A. candidus* group, *A. flavus* group, *A. sparcus* group, *A. versicolor, A. nidulans, A. ustus, A. flavipes, A. terreus, A. cremeus, A. wentil (*Aneja and Mehrotra, 2011; Gams *et al*., 1986)

1.5 Nutritional, Biotechnological, and Health Benefits of the genus *Aspergillus*

The versatile physiology of the genus *Aspergillus* enables members (depending on the substrate and environmental conditions) to produce many primary and secondary metabolites useful for industries, medicines for human health, for agricultural purposes (such as crop protection), and as

toxins. Table 1. summarizes some of the compounds formed by the genus *Aspergillus*. In a recent study, Danso (2021) surveyed the preponderance of the genus *Aspergillus* decomposing and decaying vegetables (garden eggs, green pepper, cherry pepper, onions, and tomatoes on sale in the local markets of Greater Accra Region (Kaneshie, Dome, Madina, and Night Markets, located on the University of Ghana campus).

Table 1. Summary of some useful and harmful compounds formed by *Aspergillus species*

COMPOUND	FUNGI PRODUCING	FUNDAMENTAL GROUP
Mycotoxins		
Aflatoxins	*Aspergillus flavus, Aspergillus parasiticus*	Mycotoxin
Griseofulvin	*Aspergillus clavatus*	Mycotoxin/ Antibiotic
Fusidic acid	*Aspergillus fumigatus*	
Xanthoallin	*Aspergillus flavus, A. parasiticus*	Antibiotic
Helvolic acid	*Aspergillus fumigatus*	Antibiotic
Ochratoxin	*Aspergillus alutaceus (=Aspergillus ochraceus)*	Mycotoxin
Sterigmatocystin	*A. nidulans, A. versicolor*	Mycotoxin
Organic Acids		
Citric acid	*Aspergillus niger*	
Gluconic acid	*Aspergillus niger*	Organic acids and derivatives
Gluconate salts	*Aspergillus niger*	
Mannitol	*A. candida*	Organic acid
3-hydroxy-phthalic acid	*A. flavus and A. niger*	Plasticizers, dyes etc.
Enzymes		
α-amylase	*Aspergillus oryzae, A. niger*	For brewing, food processing, textile and paper industry etc.
Protease	*Aspergillus oryzae*	Pharmaceuticals, biotechnology, detergents.
Amylase amylignoglucoside	*A. foetidus, A. oryzae*	Industrial and biotechnological use, health benefits
Naninginase	*A.niger*	
Oriental Food Fermentation		
Koji	*A. niger, A. soyae*	Rice, grains
Soya sauce	*A. niger, A. soyae*	Cereals
Miso	*A. Oryzae*	As substrates
Hamanatto	*A. Oryzae*	For fermentation of foods

Sources: Smith and Berry (1975); Griffin (1981); Carlile, Watkinson and Gooday (2005); Webster and Weber (2007)

Aspergillus fumigatus was the most prevalent *Aspergillus* species found on the vegetables sampled from Kaneshie, Dome, Madina, and Legon night Markets. The spores of *A. fumigatus* were highest on the onions, followed by garden eggs, tomatoes, green pepper, and cherry pepper in decreasing

order. She found little morphological and physiological variation in the *Aspergillus fumigatus* strain isolated from the listed vegetables (Danso, 2021).

Aspergillus fumigatus is a saprophytic fungus known for its critical role in the recycling of environmental carbon and nitrogen. It is predominantly found in soil, where it thrives by decomposing organic matter (Latgé, 1999); its spores (conidia) are frequently present in the air. It stands out as the leading cause of invasive aspergillosis (IA), responsible for approximately 90% of human infections (Latgé, 1999; Diba *et al.,* 2007).

1.6 Aspergillosis and human health

Two species of *Aspergillus* cause most infections associated with *Aspergillus*. *A. fumigatus* (69% of all reports) and *A. flavus* (17% of all reports) where they are present (Summerbell, 2003). Both species produce similar diseases. Like many other fungal human pathogens, these species primarily cause infections of the respiratory tract and lungs, although wound infections can also occur occasionally. The immune-competent, non-spreading fungal ball "Aspergilloma" may be formed in the lung in cavities formed, for example, by previous tuberculosis. In immunocompromised patients, invasive aspergillosis may arise, that is the infection that spreads throughout the lung and even to other organs. Aspergillosis is a major cause of death among cancer patients and is strongly on the increase among AIDS sufferers. One of the reasons why *A. fumigatus* is a more frequent cause of the infection than *A. flavus* is possibly because its conidia is smaller *than A. flavus*. The conidia (3µm diameter or less) of *A. fumigatus* can penetrate more deeply into the lung, they are also more buoyant in the air (Croft *et al.,* 2016) and have routinely measured concentrations above 1 conidia m^{-3} air even in protected hospital environments. This means that every human normally inhales several hundreds of conidia of *A. fumigatus* every day (Webster and Weber, 2007). Another disease caused by *A. fumigatus* is allergic bronchopulmonary aspergillosis, in which infections

occur in patients already suffering from chronic irritation of the lung, e.g. due to asthma or cystic fibrosis. The disease can lead to fatal destruction of the lung tissue. This health danger confronts to refuse/garbage sanitary collectors' daily by the inhalation of *A. fumigatus* conidia. The women who are also exposed to all refuse damps and garbage cans without ancillary nasal protection and nose masks are also susceptible. Interestingly, Rudramurthy *et al.* (2019) stated that *A. flavus* was the second most prevalent etiology agent of invasive aspergillosis (IA) followed by *A. fumigatus*. Signs and symptoms of aspergillosis may include asthma, pneumonia, sinusitis, or rapidly compromising systematic illness (Revanker, 2021). Despite these reports, it is generally reported that invasive aspergillosis is a disease caused by *A. fumigatus* and related *Aspergillus* species. Presumably, in Africa, *A. fumigatus* causes wider range of human diseases while across Asia, the Middle East, *A. flavus* has a better physiological capacity to thrive in hot and arid climates (Rudramurthy *et al.,* 2019).

There is hardly any information in Ghana on the environmental preponderance of *A. fumigatus* and its effects on human workers in Ghana, namely sanitary workers and markets women who spend many hours of the day exposed to piles of garbage within the vicinity of their stalls. The pathogenicity of *A. fumigatus* is partially attributed to its inherent ability to thrive and proliferate in the human body, despite immunity system defense. It is reported that conidia of *A. fumigatus* can evade the early responses of the immune system and when inhaled, can germinate and produce the hyphae which penetrate and proliferate the lung tissue (Webster and Weber, 2007). The virulence of *A. fumigatus* is also linked to the production of various enzymes and toxins (Table 1) that enable it to breach host defenses and establish infection.

1.7 Ecological Adaptation of *Aspergillus fumigatus*

Soil is the primary resident reservoir of *A. fumigatus*, serving as a natural habitat where it persists and proliferates. Presumably, organic matter in the soil, not excepting decomposing plant debris and animal waste matter provides adequate nutrients for its growth. Walsh and Groll (2001) reported that agricultural soils, enriched with manure or compost, are ideal for developing *A. fumigatus* due to high humidity and nutrient availability. The humidity of 70% and above facilitates fungal growth, sporulation, and dispersal of conidia (Latgé, 1999).

Aspergillus fumigatus is a typical thermotolerant species with an upper growth limit of 52°C (Dix and Webster, 1995), although it can survive up to 80°C for 60 minutes (Jesenská, *et al.,* 1993). It is one of the abundant fungi found in compost heaps and other environments in which the decay of the vegetation generates heat. Workers at compost sites are therefore subjected to massive spore inoculum, although the incidence of aspergillosis does not seem to be generally higher among them. This indicates the opportunistic nature of aspergillosis in man. Truly, the spores of thermophilic actinomycetes seem to cause most of the problems associated with compost workers' lungs (Van den Bogart *et al.,* 1993). Fluctuation in temperature can influence fungal sporulation rates and conidial viability, thereby modulating the dispersal potential of infections of *A. fumigatus* (Latgé, 1999).

In healthcare facilities, the presence of specific substrates, including medical devices, surgical instruments, and hospital surfaces, poses potential risks of nosocomial *A. fumigatus* infections (Abdolrasouli *et al.,* 2015). This therefore necessitates the need for rigorous inspection of medical facilities to mitigate the risk of a healthcare-associated aspergillosis outbreak.

1.8 Morphological and molecular characteristics of *Aspergillus fumigatus*

Aspergillus fumigatus species identification often relies on the macroscopic characteristics and recently on genetic multi-locus sequencing typing (MLST). The ongoing modern molecular techniques have revolutionized our understanding of fungal phylogeny. Traditional classification based on morphological characteristics has been complemented and, in many instances, revised by molecular methods such as DNA sequencing. These techniques have revealed cryptic species and characteristics within what was once considered a single species and have led to the reclassification of several *Aspergillus* species (Samson *et al.,* 2014).

The primary macroscopic features used in species identification include the growth rate of hyphae (mm ¯day/sec), colony colour, and thermotolerance of the fungi. *Aspergillus* colonies typically exhibit a downy to powdery texture, which varies depending on the environmental conditions. The surface colour of the colonies varies depending on the species, ranging from green to blue-green/bluish-grey, yellow, to black and white. *Aspergillus niger* colonies are typically black, *A. flavus* colonies are greenish yellow; *A. terreus* are often sand-brown or tan while *A. candida* has white spores. In addition to the surface characteristics, the reverse (underside) of the plate of colonies also provides important identification characteristics. In most *Aspergillus* species, the reverse is either uncoloured or pale yellow, although this can vary among isolates (Samson *et al.,* 1995).

Aspergillus fumigatus is a filamentous fungus with distinct morphological characteristics, and these features (its conidiophore, conidia, colony appearance, colour, etc) play a major role in its physiological and pathogenic characteristics. Conidiophores are columnar, and their heads consist of flask-shaped vesicles, a single layer of phialides (known as uniseriate phialides), and long chains of conidia (Sugui *et al.,* 2014).

The colonies of *A. fumigatus* typically grow rapidly on various culture media, reaching a diameter of 30 to 40 mm within a few days at optimal temperatures of 37°C to 42°C (Hohl and Feldmesser, 2007; Sugui *et al.,* 2014). As stated earlier it can survive up to 80°C for 60min (Jersenské *et al.,* 1993). The ability to grow at high temperatures not only supports its role as a pathogen but also allows the species to occupy varying ecological niches where other fungi might not survive (compost piles, decaying vegetation, vegetables, fruits, etc.). According to Sugui *et al.* (2014), *A. fumigatus* prefers a pH range between 2.1 and 8.8.

The colonies are initially white, but as they mature, they develop a characteristic greenish-blue to gray-green color due to the formation of conidia (See Plate 1). The texture of the colony surface is usually velvety or cottony, reflecting the high density of conidiophores and conidia. The reverse side of the colony in a Petri dish often appears uncolored or pale yellow, which can help distinguish *A. fumigatus* from other species with similar surface coloration (Samson *et al.,* 1995).

The conidiophores of *A. fumigatus* are another key morphological feature. These structures are typically short, smooth, and unbranched, measuring 300 to 400 micrometers in length (Kwon-Chung and Bennett, 1992) (See Plate 2). The conidiophores arise from specialized foot cells and terminate in a vesicle, which is columnar and typically measures 20 to 30 micrometers in diameter. The vesicle supports a layer of phialides (flask-shaped cells) that are arranged in a uniseriate pattern, (i.e., they directly cover the upper two-thirds of the vesicle without an intervening layer of metulae (secondary sterigmata) (See Plate 2). (Raper and Fennell, 1965). Table 2. Summarizes the morphological characteristics of a typical colony of *A. fumigatus*.

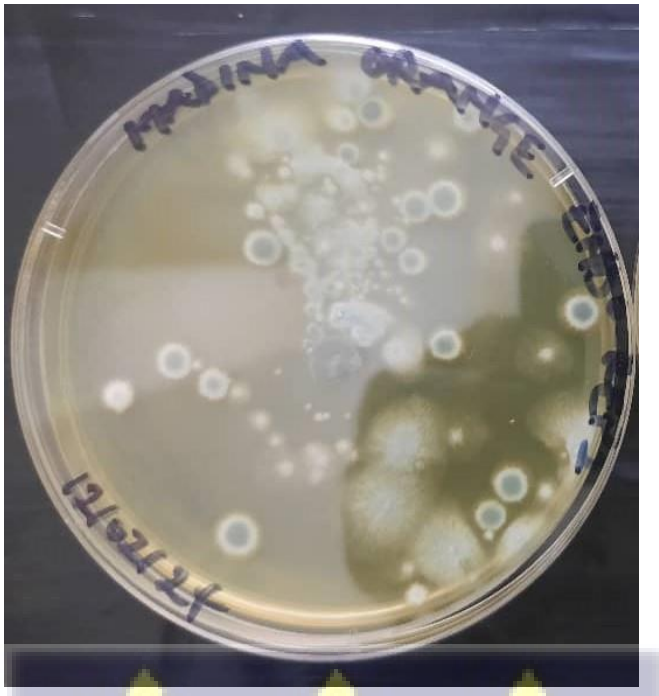


Plate 1. Pure culture of colonies of *Aspergillus fumigatus* on potato dextrose agar plate incubated at 30 ±2 ℃

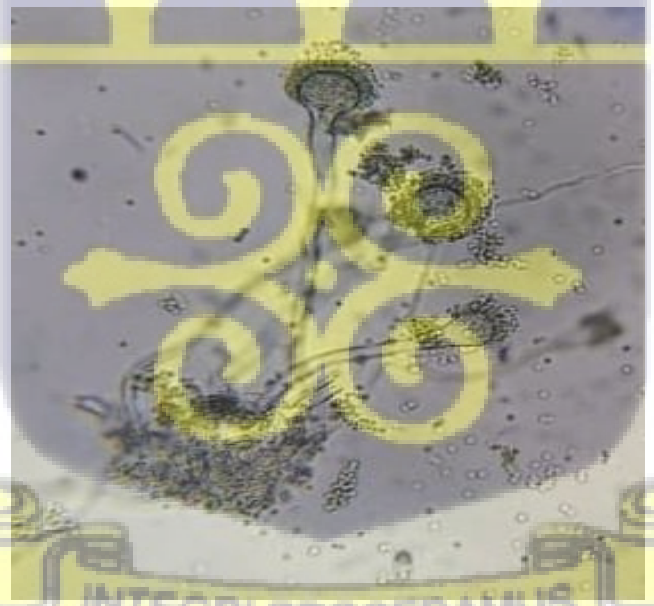


Plate 2. Photomicrograph of *A. fumigatus* showing the gross morphology and characteristic un-branched conidiophores and vesicle bearing uniseriate phialides and conidia (×400)

Table 2. Summary of cultural and morphological characteristics of *Aspergillus fumigatus*

Character	Description and Dimensions
Colony diameter	21-75 mm
Colony colour	greyish turquoise or dark turquoise to dark green to dull green
Conidiation	abundant, rarely less abundant
Reverse colour	creamy, yellow to orange
Colony texture	Velutinous
Conidial head	Columnar
Conidiophore	300-400 µm
Stipe dimension	50–350 × 3.5–10 µm
Vesicle diameter	10–26 µm
Vesicle shape	pyriform to subclavate, sometimes subglobose, but rarely globose
Conidia length	2–6 µm in diameter
Conidia shape	globose to ellipsoidal
Conidia surface texture	smooth to finely rough

SOURCE Samson, Hong, Peterson *et al.* (2007)

Generally, the unique morphological characteristic of *A. fumigatus* can be stated as follows:

- The conidiophores are typically short, smooth, and unbranched, 300-400 µm in length (Kwon-Chung and Bennett, 1992).
- The conidiophores arise from specialized foot cells and terminate in a vesicle which is columnar and measures 20-30 µm in diameter.

- The vesicle supports a layer of phialides (flask-shaped cells arranged in a uniseriate pattern; i.e. they are directly over the upper two-thirds of the vesicle without intervening layer of metulae (secondary sterigmata)). Plate 2. Illustrates these features.
- The conidiophores spores exhibit a bluish-grey to pale green colour attributed to the accumulation of 1,8 dihydroxy naphthalene-melanin (Hohl and Feldmesser, 2007; Sugui *et al.,* 2014). See Plate 1.
- The conidia are typically small (2.5-6.0) µm in diameter; globose to sub-globose in shape.
- The surface of the conidia is finely roughened or echinulate, a feature that can be observed under scanning electron microscopy only.
- The spores (conidia) are hydrophobic due to the presence of rodlet layers on their outer surface. These rodlets, which are proteinaceous protrusions, play a crucial role in the effective dispersal ability of spores in the environment (Hohl and Feldmesser, 2007).
- The conidial surface of *A. fumigatus* is rich in Sialic Acid Residues; the high density of these sialic acids may be linked to the fungus's ability to cause disease in humans, as they are involved in the adhesion of conidia to host tissues or in evading the host immune system (Hohl and Feldmesser, 2007).
- There are some distinctive features of the conidia of *A. fumigatus* which facilitate their survival and spread in various environments and contribute significantly to the pathogenicity of the fungus. For example:

  The colour, size and hydrophobicity, and surface composition help in the dispersal efficiency and allow the colonization and ability to infect immunocompromised individuals. The melanin in the conidia also offers protection against environmental stresses, e.g. UV

radiation and oxidative damage, thus enhancing the survival of the spores under adverse conditions.

1.9 Modern Molecular Techniques and DNA Sequencing in the Reclassification of *Aspergillus fumigatus* in Ghana

The study of *Aspergillus* taxonomy has been greatly advanced by modern molecular techniques, which have revolutionized our understanding of fungal phylogeny. Traditional classification methods based on morphological characteristics have been complemented and, in many cases, revised through molecular approaches such as DNA sequencing. These techniques have revealed cryptic species that were previously considered a single taxon and have led to the reclassification of several *Aspergillus* species, including *A. fumigatus* (Samson et al., 2014).

Multi-locus sequence typing (MLST) and whole-genome sequencing (WGS) have provided deeper insights into the genetic diversity within the genus, enabling researchers to delineate species boundaries more precisely and identify new species with distinct pathogenic or industrial properties (Houbraken *et al.,* 2016). There has been a survey on Ghanaian vegetable garden eggs, green peppers, cherry peppers, onions (Danso, 2021), and soil mycoflora for the presence of *A. fumigatus* (Asante, 2021) from five major Markets and different locations at the University of Ghana campus, Legon respectively, in the Greater Accra region. *A. fumigatus* was predominant on the samples. However, there were morphological and physiological variations among the cultures.

The isolation and morphological characterization of *A. fumigatus* from different markets was continued in this project with the view to expand the mycoflora profile of our Ghanaian vegetables and fruits from our local markets at different locations and their implications on human health. There was another objective; to use molecular techniques to delimit *A. fumigatus* encountered into different strains (if possible) with the view to elucidating the potential of these strains in causing

diseases such as aspergillosis producing potent food toxins and pathological deterioration of food-stuff.

1.10 Prevention of fungal Growth

Fungi are important agents of biodeterioration, causing spoilage of food, timber, and a wide range of other products, both in storage and in use. They are a major cause of disease in plants, causing heavy crop loss. They also cause diseases of man and animals. Among the fungi, *Aspergillus* species grow as saprophytes on decaying vegetation and are found in large numbers (Shah *et al.,* 2010, Neguse *et al.,* 2019) growing on stored tomatoes, vegetable matters, mangoes, oranges, etc. (FAO-STAT, 2022). *A. fumigatus* is also ubiquitous and thermotolerant with upper growth at 52°C (Dix and Webster, 1995) and can survive up to 80°C for 60min (Jesenská *et al.,* 1993). *A fumigatus* is an opportunistic fungus responsible for aspergillosis in man (Van den Bogart *et al.,* 1993). A wide range of antifungal agents are used to combat biodeterioration and prevent or treat fungal diseases of plants. In these contexts, they are referred to as fungicides. Others are used for treating diseases in animals and humans, and are termed as antifungal drugs or in produced employing microbial fermentation are called antifungal antibiotics (Carlile, Watkins and Gooday, 2005).

Fungicides of low biochemical activity have to be used as contact fungicides which for as long as they persist on the plant surface, protect the plant from fungal attack. Some other agents of high specificity are also used as contact fungicides, but still, others can be used as systemic fungicides are taken up and translocated throughout the plant, suppressing established fungal disease as well as preventing fresh infection. Griseofulvin and the unrelated Benzimidazoles inhibit fungal mitosis and act by preventing tubulin polymerization and microtubule elongation in the nucleus (McDaniel and Robertson (1998). Griseofulvin, given orally, is used in treating infections by dermatophytes, *Trichophyton*, and *Microsporum*.

The triazole drugs: fluconazole, itraconazole, and voriconazole have a broad spectrum of antifungal activity and itraconazole has been used for the treatment of a wide range of fungal infections orally (Carlile, Watkinson, and Gooday, 2005)

1.11 The General Objective of this Thesis

To isolate and characterize *Aspergillus fumigatus* from different geographical locations and different substrates, oranges, mangoes, tomatoes, and dry cocoa beans in Ghana.

Specific Objectives

- **Isolate and Characterize**: Identify *Aspergillus fumigatus* from various substrates using both their conventional taxonomic features and modern molecular techniques.
- **Molecular Identification**: Use DNA sequencing for precise identification and phylogenetic analysis of the fungus.
- **Antifungal Testing**: Assess the susceptibility of isolated strains to antifungal agents commonly used in agricultural pathogen control systems.

EXPERIMENTAL PRECAUTIONS

1. All glassware used was thoroughly washed and dried before autoclaving for use in all experimental work.
2. All prepared media were sterilized by autoclaving at 121 °C and 1.1 kg/cm^3 pressure for 15 minutes.
3. New and sterile syringes were used for the serial dilution.
4. Inoculating pins and rods used in the experiment were kept in 100% absolute alcohol and after that, they were sterilized by flaming them over a spirit lamp until they appeared red hot and allowed to cool before use.
5. Conical flasks used in the experiment were all covered with non-absorbent cotton wool plugs.
6. The laminar flow hood where most of the experiments were conducted was switched on 15 minutes before use.
7. 70% Alcohol was used to swab all benches as well as the laminar flow hood before any work was carried out there.
8. The antibiotic chloramphenicol was added to all media prepared to inhibit bacteria growth.
9. The fruit samples were kept in Ziploc bags and sealed to prevent contamination.
10. The fruit samples were collected in the morning between 7 AM to 9 AM to prevent cross-contamination from vendors.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 MATERIALS

Fruits (orange, mango, tomato and dry cocoa beans) samples (were purchased from two different markets in Accra; Mallam Atta Market and Madina Market), Ziploc bags (180 mm × 260 mm), Petri dishes (obtained from Korle Bu), syringes, McCartney tubes, Petone water, distilled water, PDA (potato dextrose agar), gloves, alcohol, Sodium peroxide, parafilm, beaker, conical flask, measuring cylinder, cork borer, inoculating pins, Microscope and staining reagents (e.g., Lacto-phenol plain), Bunsen burner, Incubator, Autoclave, Lamina flow, forceps (all obtained from Department of Plant and Environmental Biology).

The sampling collection locations are presented in Table 3.

Table 3. Global Positioning System (GPS) of the Sampling Sites

Sampe Area	**Coordinates**
Mallam Atta Market	5°34’44’’N Latitude -0°12’27’’W Longitude
Madina Market	5°40’48’’N Latitude -0°10’13’’ W Longitude
Central Region (Breman Asikuma)	CB-0005-9310 BL482 COCOASI RD.

2.2 METHODS

2.21 Sample Collection of Fruits

The fruits were collected from two major markets in Accra: Mallam Atta Market and Madina Market. These markets were selected due to their high volume of fruit sales and diverse fruit varieties available from producing forms scattered in the country. Sample collection was done early in the morning, between 7:00 AM and 9:00 AM, to obtain samples before significant handling by vendors and customers. For each fruit type, three to five samples were purchased from different vendors to account for variability in storage, handling, and exposure to environmental factors. The samples were collected early in the morning to avoid cross-contamination. Samples were handled with clean latex surgical gloves to avoid contamination and were placed in sterile, labeled polyethylene bags. Detailed records were kept for each sample, including the type of fruit, the vendor's location within the market, and the time of collection. The open plate exposure method for airspora was carried out at the sites where the fruits were purchased.

Cocoa beans

Dry cocoa beans were collected from the Central Region (Breman Asikuma cocoa depot) using the conventional cocoa sampling technique.

Composition and Preparation of Culture Media

Composition:

- **Potato infusion:** 200 g of peeled and cut potato tubers, boiled in 1 liter of distilled water for 20 minutes, and filtered through cheesecloth.
- **Glucose:** 15 g
- **Agar:** 12 g

- **Chloramphenicol:** 25.0 µg

The medium was prepared using potato infusion (200 g), glucose (15 g), agar (12 g), and chloramphenicol (25.0 µg). Potatoes were peeled and cut into pieces, and 200 g of the pieces were boiled in 1 liter of distilled water for 20 minutes. The resulting potato infusion was filtered through cheesecloth. Glucose (15 g) and agar (12 g) were then added to the infusion, and the mixture was boiled until fully dissolved. The prepared mixture was poured into McCartney bottles and sterilized in an autoclave at 121°C under a pressure of 1.1 kg/cm^2 for 20 minutes. Finally, chloramphenicol (25.0 µg) was added aseptically to the medium to prevent bacterial growth.

2.22 Quantitative Estimation of Mycoflora Population in the Expressed Juice of the Fruits

The mycoflora population in the fruit juice samples was evaluated using the Decimal Serial Dilution technique. A 1 mL aliquot of freshly squeezed fruit juice was combined with 9 mL of peptone water in a McCartney bottle and mixed on an orbital shaker (Gallenkamp, England) operating at 140 rpm for 10 minutes. Decimal serial dilutions (10^{-1} -10^{-3}) were prepared from the mixture. For each dilution level, 1 mL of aliquots were plated onto 20 mL of Potato Dextrose Agar (PDA) in duplicate. The plates were incubated at 30 ± 2 °C for 7 days. After the incubation period, the colonies of mycoflora that developed were counted and expressed as $\log_{10}$ CFU/g of the sample. Fig 1. Shows the results obtained.

2.23 Quantitative Estimation of Mycoflora Population on Fruit Using Direct Plating Method

The mycoflora population in the fruit samples was assessed using the Direct Plating method. To prepare the samples, small portions of each fruit were surface-sterilized by rinsing with sterile water and then blot-dried with sterile paper towels. Approximately 1 g of each fruit sample was then aseptically placed directly onto 20 ml of Potato Dextrose Agar (PDA) in Petri dishes. The plates were incubated at 30 ± 2 ℃ for 7 days. After incubation, the colonies of mycoflora that

developed on the fruit samples were counted and recorded as $\log_{10}$ CFU/g of the sample. Fig 2. Summarizes the results obtained.

2.24 Resident Dry Cocoa Beans Mycoflora

The same process was repeated for dry cocoa beans and aliquots plate in PDA in duplicate. Fig 2.

2.25 Estimation of Airspora Populations at the Sampling Sites

The plate exposure method was employed. Petri dishes in triplicate containing sterile PDA were opened for 5min and then covered for incubation at 30 ± 2 ℃ for up to 7 days. The population was recorded as $\log_{10}$ CFU/g of sample. Fig 4. summarizes the results obtained

2.26 Identification of *A. fumigatus*

Colonies that resembled a dense felt of dark green conidiophores intermixed with aerial hyphae-bearing conidiophores were isolated on a fresh PDA plate. Further morphological characteristics such as a typically blue-green surface with a suede-like surface consisting of a dense felt of conidiophores. Conidia heads were typically columnar. Conidiophores were short-walled, green, particularly in the upper part and phialides were directly borne on the vesicle with globose to sub-globose conidia were used to identify the colony as *A. fumigatus* (Samson and Van Reenen Hoekstra, 1988); Table 2. after Samson *et al.,* 2007.

2.27 Isolation and Sub-culturing of *Aspergillus fumigatus* from Mixed Mycoflora

After incubating the fruit samples or dry cocoa beans directly on PDA for 7 days at 30 ± 2 ℃, the plates were observed for mycoflora growth. Colonies exhibiting the characteristic features of *Aspergillus fumigatus* were identified. The resulting colonies were carefully excised using a sterile cork borer and syringe. The excised inoculum was inoculated on a fresh PDA in Petri plate. The

plates were incubated under the same conditions to allow for the growth of pure cultures of *A. fumigatus*.

2.28 Spore count, spore diameter, and Conidial Head Morphology

A microscopic slide was prepared from each plate by putting a drop of plain Lactophenol on a clean slide. Mycelia plug was placed and teased using a sterile inoculating pin and covered with a cover slip. It was observed under a compound light microscope for the presence of conidia and spores.

Spore count, spore diameter, vesicle diameter, and conidiophore diameter were measured respectively with the aid of an analyzing software called ImageJ (V.1.8.0). Results obtained were summarized and presented in Fig 6-9

2.3 Pathogenicity Test

The pathogenicity test was carried out using the direct wound inoculation method. Fruit samples without blemishes were first obtained and thoroughly washed with distilled water. The fruits were then placed in a 5-liter transparent square plastic bowl with a cover. To create a sterile environment, sterilized paper towels were laid at the bottom of the plastic bowl, and the cleaned fruits were arranged on top of the moistened paper towels

A cork borer of size 5mm was used to excise plugs of the pathogen culture, which were then directly inoculated onto each fruit. There were three replicates to ensure consistency in the inoculation process. The bowls with inoculated fruits were covered and incubated at room temperature (28 ± 2 ℃) for 7 days. Daily lesion size measurements were recorded daily to monitor the progression and severity of infection. The data obtained are presented in Plate 5. Figs 11 and 12

2.31 Antifungal Agents Efficacy Test

In this experiment, three antifungal drugs-Griseofulvin, Cefuroxime, and Itraconazole were prepared at three concentration levels (1:25, 1:100, and 1:200) v/v. These antibiotic solutions were mixed with potato dextrose agar (PDA) media, poured into Petri dishes, and triplicates, and allowed to solidify before inoculating the pathogen onto the prepared media. The plates were then incubated for 7 days at 30 ± 2 °C to observe the antifungal effects of the growth/ inhibition of the culture on the plates.

2.4 Methodology for DNA Extraction of *Aspergillus fumigatus*

Materials and Reagents

Notes:

- All buffers were ensured to be at room temperature before use, and it was confirmed that ethanol had been added to Buffers LP3 and GW2 as indicated on their labels.
- Repeated freezing and thawing of samples were avoided to prevent DNA fragmentation.
- DNA purity and quantity were evaluated using spectrophotometry (e.g., A260/A280 ratio) or agarose gel electrophoresis.

This methodology effectively combined specific steps tailored for *A. fumigatus* with general protocols outlined in the NuClean Plant Genomic DNA Kit manual.

2.5 Data Analysis

2.51 The Heatmap Analysis of Spore Counts

The heatmap is a two-dimensional data visualization technique that uses colour to represent the magnitude of the values in a data set. Heatmaps are used in many areas, including analytics, football, and biology. In this study context of spore counts, darker colours usually indicate higher

frequency or severity, while lighter colours indicate fewer intensities or weaker correlations. Positive correlations, show when one variable increase and the other dies are often represented by warm colours. Any kind of heatmap goes from cold to warm. In all types of heatmaps, brighter colours are used to represent larger values (https://mouseflow.com > blog 2023). A heatmap is therefore a graphical representation of data where values are depicted by colour to display the complex information in a way you can quickly comprehend. You can visualize the most popular (hot) and unpopular (cold) elements of your data using colours on a scale from red to blue (Anon, 2024)

2.52 The Box Plot

This is a descriptive statistical method of demonstrating graphically the locality spread and skewness groups of numerical data throughout quartiles. The box plot can be plotted either horizontally or vertically which is lucidly described by en.m.wikipedia.org; Spear, 2024; Marmolejo-Ramos and Tian, 2010).

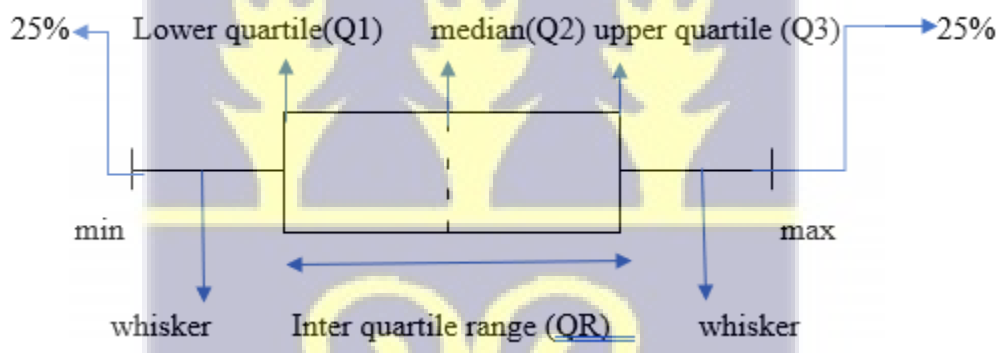
The boxplot is a means you can graphically display a bit of information about your data. Among other things, the median, the interquartile range (IQR), and the outliers can be real in a Boxplot. A boxplot is created to compare and contrast two or more groups of age or length of different fungi.

On DATAtab one can create a boxplot outlier by clicking on the statistics calculated to copy your data onto the Table selecting the tab Description or charts and clicking on the variables you want to create (DATAtab Team, 2024)

A boxplot is a special type of diagram that shows the quartiles in a box and the line extending from the lowest to the highest value. In a boxplot, we draw from the first quartile to the third quartile. A vertical line goes through the box at the median. A box plot or a whisker plot is a graphical

representation of the distribution of a dataset. It displays the key summary statistics of median, quartile, and potential outliers in a concise visual manner. A boxplot is given a five-number summary of a set of data.

- Minimum – min value in the dataset excluding outliers.
- First quartile (Q1)- 25 % of the data lies below the first quartile (Lower Quartile)
- Median quartile (Q2)- it is the midpoint of the dataset. Half of the values lie below it and half above it.
- Third quartile (Q3)- 75 % of the data lies below the third (Upper quartile).
- Maximum- it is the maximum value in the dataset excluding outliers.

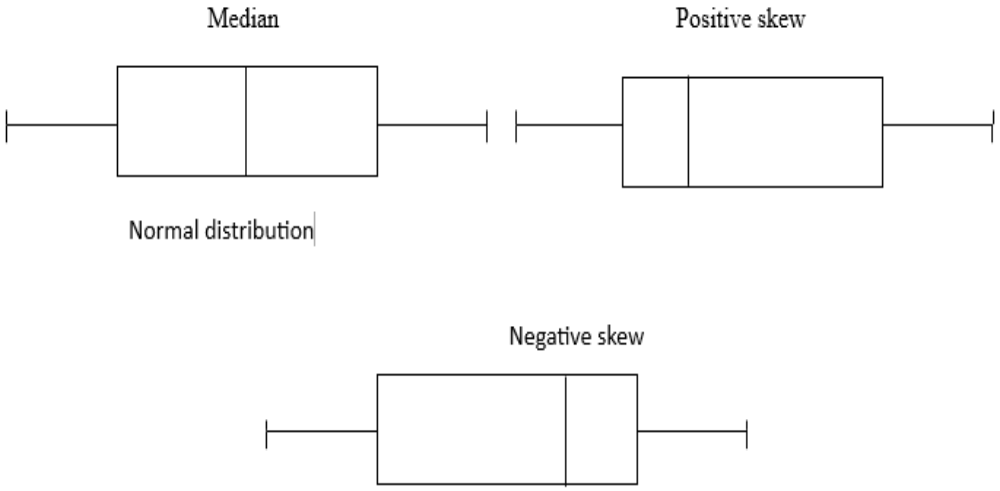


QR = Q3 – Q1

Lower Limit = Q1 + 1,5* |QR

Upper limit = Q3 + 1,5* |QR

The values below and above these limits are considered outliers and the minimum and the max values are calculated from the point which lie under the lower and upper limits.



2.53 Area Under the Disease Progress Curve (AUDPC)

The Area Under the Disease Progress Curve (AUDPC) is a quantitative measure used to integrate disease severity data collected at multiple time points into a single value, reflecting both the intensity and duration of disease progression. The calculation follows the trapezoidal rule, as described by Madden and Campbell (1990):

$$\text{AUDPC}= \sum_{\{i=1\}}^{\{n-1\}} \left[\frac{\{(Y_i+Y_{\{i+1\}})\}}{2}\right] \times \left(T_{\{i+1\}} - T_i\right)$$

Madden, L. V., Campbell, G. (1990)

Where:

- *Yi* = Disease severity at time Ti.
- *Ti* = Time of disease severity recording.
- *n* = Total number of observations.

This formula represents the trapezoidal integration of disease progress over time, providing a reliable way to compare disease dynamics across different treatments, cultivars, or management strategies.

2.54 Phylogenetic Analysis

The Internal Transcribed Spacer (ITS) sequences obtained from *Aspergillus fumigatus* isolates were edited and aligned using ClustalW in MEGA X software. The Neighbor-Joining (NJ) method was used to construct the phylogenetic tree, and evolutionary distances were calculated using the Kimura 2-parameter model. The reliability of the branching patterns was assessed through bootstrap analysis with 1,000 replicates.

A reference sequence of *A. fumigatus* (Af293_REF) was retrieved from GenBank for comparison to confirm species identity and determine genetic relationships. The resulting phylogenetic tree was visualized and annotated using the Interactive Tree of Life (iTOL) platform, revealing distinct clustering patterns that indicated genetic variation among isolates from different sampling locations.

2.6 DNA Extraction, PCR Amplification, And Gel Electrophoresis

2.61 DNA Extraction - NuClean Plant Genomic DNA Kit (Jiangsu CoWin Biotech Co., Ltd.)

Materials Used

- Centrifugal adsorption columns with collection tubes
- Buffers
- RNase A (10 mg/mL)
- 100% ethanol

- Liquid nitrogen
- Agarose, TAE buffer, and DNA loading dye (for electrophoresis)
- DNA ladder (550-650 bp, kappa universal DNA ladder for comparison)
- Fresh or dried *Aspergillus fumigatus* mycelium (100 mg fresh or 20 mg dry weight)
- Centrifuge (12,000 rpm) (Model: Labwe)
- PCR thermocycler (Model: Bio-Rad T100)
- Primers: ITS1 (TCCGTAGGTGAACCTTGCGG) and ITS4 (TCCTCCGCTTATTGA-TATGC) (White *et al*, 1990)
- PCR reagents: Taq DNA polymerase, dNTPs, $MgCl_2$, and PCR buffer
- Electrophoresis system with UV transilluminator (Model: blueGel™)

DNA Extraction

Genomic DNA was extracted from *Aspergillus fumigatus* mycelium cultivated on cellophane over potato dextrose agar (PDA) media. The mycelium was frozen in liquid nitrogen and ground into a fine powder to ensure complete cell disruption. This powder was resuspended in 400 µL of Buffer LP1 (lysis buffer) and 6 µL of RNase A, followed by vortexing for 1 minute. The mixture was incubated at room temperature (28±2 °C) for 10 minutes to facilitate cell lysis.

Next, 130 µL of Buffer LP2 (precipitation buffer) was added, and the mixture was vortexed before centrifugation at 12,000 rpm for 5 minutes. The supernatant was carefully transferred to a new tube, and 1.5 times its volume of Buffer LP3 (binding buffer) was added. The resulting solution was loaded into a spin column positioned in a collection tube, with multiple rounds of loading if

necessary. After centrifugation at 12,000 rpm for 1 minute, the flow-through was discarded. The column was washed twice with 500 µL of Buffer GW2 (Genomic Wash 2) ensuring proper ethanol addition as instructed. A final centrifugation step removed any residual ethanol, and the column was left to air dry at room temperature.

To elute the DNA, 50-100 µL of sterile water was added to the column membrane and incubated for 1-2 minutes before centrifugation at 12,000 rpm. The eluted DNA was collected in a clean microcentrifuge tube for subsequent analysis.

2.62 PCR Amplification

The extracted DNA was used as a template for PCR amplification of the Internal Transcribed Spacer (ITS) region. The PCR reaction mix consisted of 2 µL of template DNA, 10 µL of PCR buffer, 1 µL of dNTP mix, 1.5 µL of $MgCl_2$, 0.5 µL of Taq DNA polymerase, and 1 µL each of ITS1 and ITS4 primers (10 µM concentration), with the volume adjusted to 50 µL using nuclease-free water.

The PCR cycling conditions included an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. A final extension step was performed at 72°C for 7 minutes. The amplified products were stored at 4°C until further analysis.

2.63 Gel Electrophoresis

The PCR products were analyzed using gel electrophoresis. A 1.5% agarose gel was prepared in TAE (Tris-Acetate-EDTA) buffer and incorporated with a DNA stain (such as ethidium bromide) for visualization under UV light. Five microliters of the PCR product were mixed with 2 µL of DNA loading dye and loaded into the gel wells alongside a DNA ladder (550 bp-650 bp) for size

comparison. The gel was electrophoresed at 100 V for 30-40 minutes and visualized using a UV transilluminator.

2.64 DNA SEQUENCING

Materials Used

- PCR-amplified ITS region (purified PCR product)
- Primers: ITS1 (TCCGTAGGTGAACCTTGCGG) and ITS4 (TCCTCCGCTTATTGA-TATGC) (White *et al*, 1990)
- BigDye™ Terminator v3.1 Cycle Sequencing Kit (or equivalent)
- Sequencing buffer
- Nuclease-free water
- Ethanol (70 % and 100 %)
- Sodium acetate (3 M, pH 5.2)
- Distilled water
- Spin columns or Sephadex™ G-50 columns (for purification)
- Thermal cycler (Model: Bio-Rad T100)
- Capillary electrophoresis system (e.g., Applied Biosystems 3130 Genetic Analyzer)

The PCR-amplified ITS region was purified to remove residual primers and unincorporated nucleotides. Purification was performed either via a spin column or ethanol precipitation. The purified product was then used for the sequencing reaction, which included the PCR product, either the ITS1 or ITS4 primer, BigDye™ Terminator mix, sequencing buffer, and nuclease-free water.

The sequencing reaction was conducted under the following thermal cycling conditions: an initial denaturation at 96 °C for 1 minute, followed by 25 cycles of denaturation at 96 °C for 10 seconds, annealing at 50 °C for 5 seconds, and extension at 60 °C for 4 minutes.

Following the sequencing reaction, the products were purified again using ethanol precipitation. The final sequencing products were loaded onto a capillary electrophoresis system (Applied Biosystems 3130 Genetic Analyzer) for separation and detection. The raw sequence data were analyzed using software tools to evaluate quality and perform base calling. The resulting sequences were compared against known sequences in databases such as GenBank using BLAST to accurately identify *Aspergillus fumigatus* based on its ITS region. All procedures, except DNA extraction, were performed at Functional Biosciences Inc., USA.

CHAPTER THREE

3.0 RESULTS

Experiment 1 (a-d)

a) Quantitative estimation of total fungal flora population in the fruit juice extract (endoderm extract) of orange, mango, and tomato samples purchased from Mallam Atta and Madina Markets.

There was a miscellany of fungal flora isolated on PDA including *Aspergillus fumigatus*, other genera encountered included *Penicillium, Neurospora, Mucorales, Rhodotorula, Fusarium* etc.

The total fungal population isolated from the juice of the orange, mango, and tomatoes varied from one market to another ($\log_{10}$ 3.0 ± 0.1 CFU/g - $\log_{10}$ 3.35 ± 0.1 CFU/g) depending on the type of fruit (Fig. 1). In the case of orange juice, the fungal population varied slightly at the Mallam Atta and Madina Markets ($\log_{10}$ 3.01 ± 0.1 CFU/g - $\log_{10}$ 3.08 ± 0.1 CFU/g) (Fig. 1).

Mango juice from Madina Market contained a higher population of fungi ($\log_{10}$ 3.35 ± 0.15 CFU/g) than what was obtained in samples from Mallam Atta Market ($\log_{10}$ 3.07 ± 0.3 CFU/g) sample (Fig.1)

The population of fungi resident in tomato juice from Mallam Atta was higher ($\log_{10}$ 3.2 ± 0.5 CFU/g) than the same fruit samples purchased from Madina Market ($\log_{10}$ 3.07 ± 0.5 CFU/g). Mango fruits were laden with fungal spores in the juice sample ($\log_{10}$ 3.35 ± 0.1 CFU/g) while orange was the least contaminated with ($\log_{10}$ 3.08 ± 0.1 CFU/g) (Fig. 1).

b) Quantitative estimation of the total fungal floral population on the exterior ectoderm of the test fruits (orange, mango, and tomato) purchased from Mallam Atta, Madina (Greater Accra), and cocoa beans from Breman Asikuma (Central Region).

The mycoflora appearing in the Petri plates (PDA) inoculated with samples of the ectoderm (skin) of the samples of oranges, mangoes, tomatoes, and cocoa beans showed variation in populations isolated (Fig. 2).

Generally, the external surfaces of the samples harboured lower fungal population ($\log_{10}$ 1.0 CFU/g – $\log_{10}$ 1.43 CFU/g) as compared to extracted juice sample ($\log_{10}$ 3.0 CFU/g - $\log_{10}$ 3.50 CFU/g) (Fig. 2). Clearly cocoa beans harboured the least fungal population ($\log_{10}$ 1.0 CFU/g) on the testa (Fig. 2). Both markets had oranges, mangoes, and tomatoes on sale with similar fungal populations on the ectoderm (skin) of the samples ranging from ($\log_{10}$ 1.20 CFU/g - ($\log_{10}$ 1.43 CFU/g) (Fig. 2).

c) Aeromycoflora of the Markets

Fig. 3 summarizes the results of the plate exposure method of sampling the aeromycoflora from the two markets. The Airspora population was slightly higher at Mallam Atta Market ($\log_{10}$ 1.51 CFU/g) sample than what existed in Madina Market ($\log_{10}$ 1.43 CFU/g) sample. However, the difference was only marginal ($\log_{10}$ 0.0 8CFU/g) (Fig. 3).

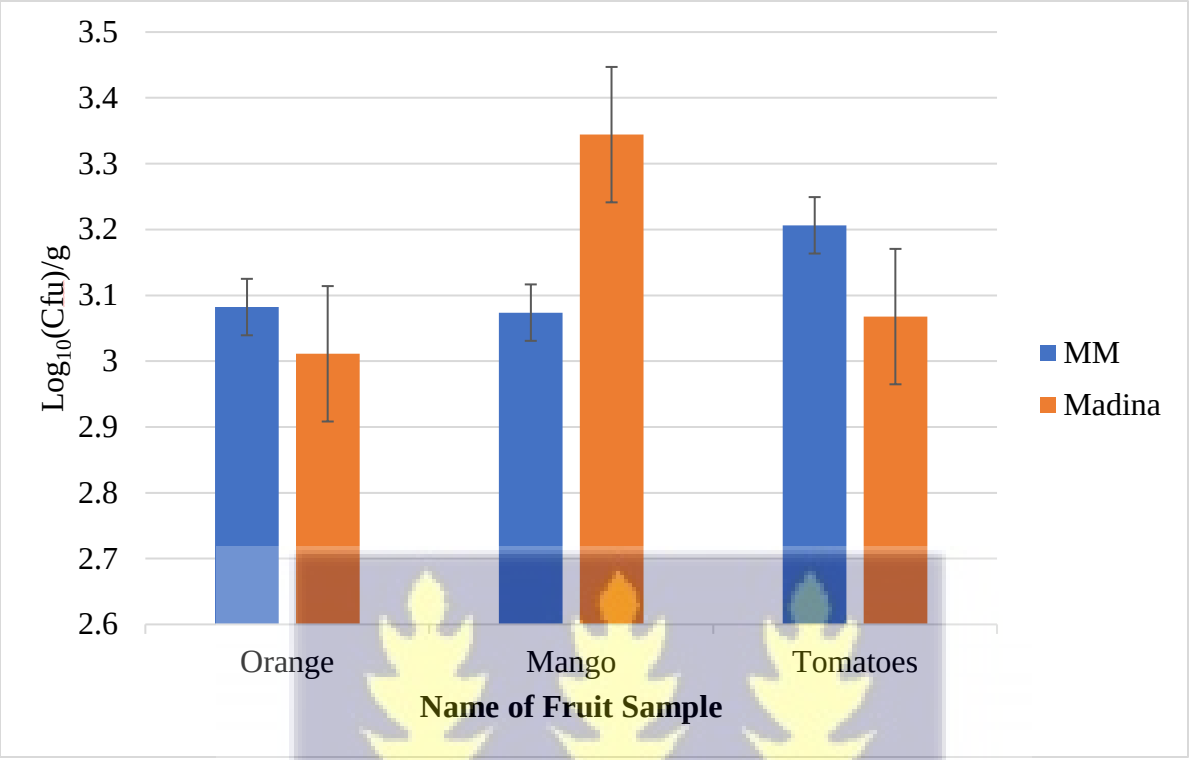


Fig.1: A profile of the fungal population isolated from the indicated juice of the fruits from Mallam Atta and Madina Market.

Key

MM- Mallam Atta Market

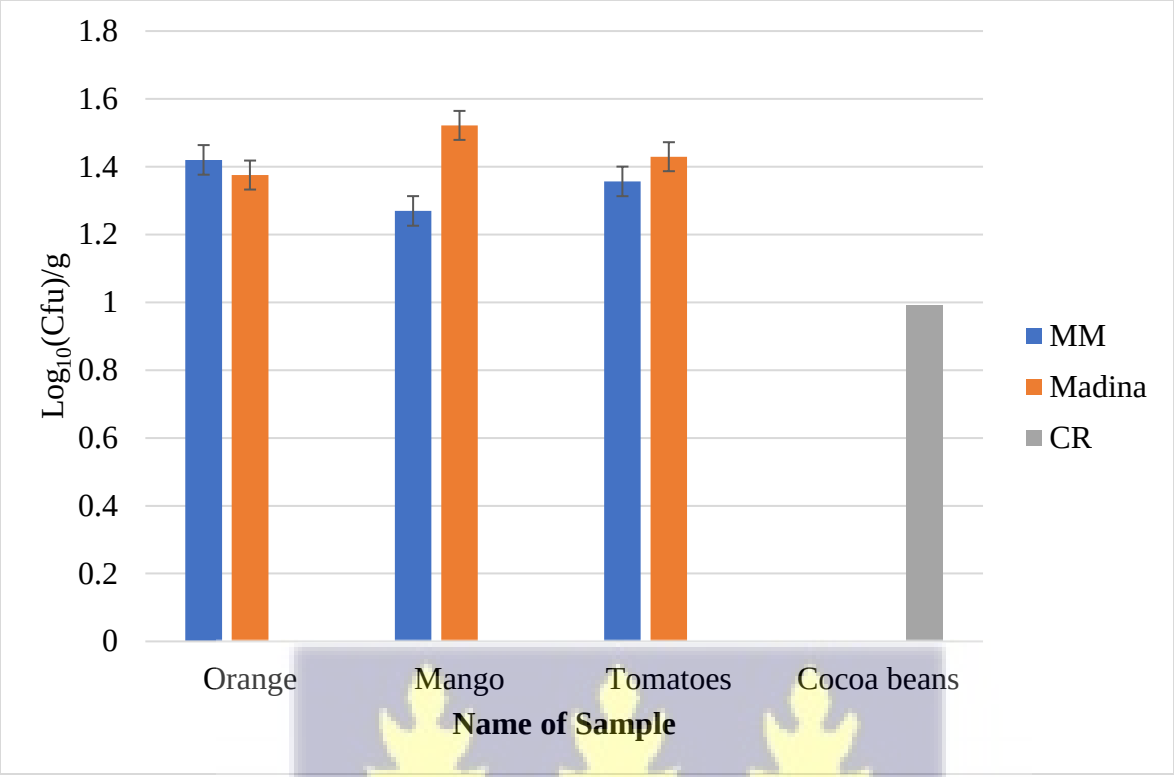


Fig 2: A profile of the fungal population isolated from the indicated skin/peel of the fruits from Mallam Atta, Madina Market, and dry cocoa beans from Central Region, Asikuma.

Key

MM- Mallam Atta Market

CR- Central Region

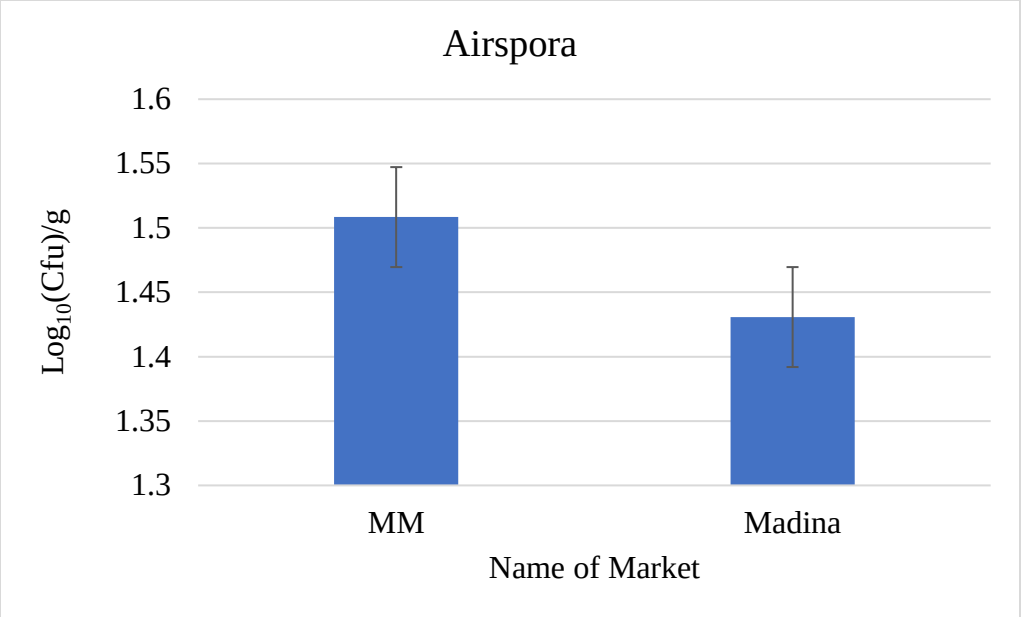


Fig. 3. Comparative population of Aeromycoflora isolated from Mallam Atta (MM) and Madina Markets.

d) Percentage contribution of *Aspergillus fumigatus* to the total mycoflora populations in the fruit extracts and ectoderm or skin of the plated samples of the test fruits from Mallam Atta, Madina Markets, and Breman Asikuma in dry cocoa beans testa

In this experiment, the percentage contributions of the *Aspergillus fumigatus* in the juice extracts and covering peel or skin as well as testa of cocoa beans were estimated in samples from Mallam Atta, Madina Markets, and Breman Asikuma in the Central Region. Fig. 4 summarizes the results obtained. *A. fumigatus* was isolated from the juice and peel (skin) of the oranges from Mallam Atta, and Madina Markets (Fig. 4); *A. fumigatus* could also be isolated from the juice of mangoes and Ectoderm (skin) of mangoes obtained from the Mallam Atta Markets. The peel of the mango fruit only from Madina Market harboured *A. fumigatus*. Interestingly, the mango juice of samples from Madina market did not record any contamination by *A. fumigatus* (Fig.4). The testa of cocoa

beans from Breman Asikuma carried only *A. fumigatus* (Fig. 4). Curiously, only tomatoes obtained from Mallam Atta Market were contaminated by *A. fumigatus* both in juice and peel/skin samples (Fig. 4). The tomatoes obtained Madina Market was devoid of *A. fumigatus* spores. There was another significant observation. The percentage incidence of *A. fumigatus* in the samples from Mallam Atta, Madina Markets was not the same for the juice extracts and ectoderm (peel) plated on the PDA medium.

Generally, *A. fumigatus* was isolated from the juice of oranges, tomatoes, and mangoes in the order shown in the table below:

Mallam Atta Market:	Orange juice < (22.5 %)	Mango juice < (5.2 %)	Tomato juice (2 %)
Mallam Atta Market:	Mango peel(ecto) < (8.2 %)	Orange peel (ecto) < (3.8 %)	Tomato peel (ecto) (2.8 %)
Madina Market:	orange juice < (6.5 %)	mango juice = (Nil; 0%)	tomato juice (Nil; 0%)
Madina Market	Orange peel(ecto) < (10.7 %)	Mango peel (ecto) < (4.9 %)	tomato peel (ecto) (Nil; 0%)

*Ecto: - refers to the outer peel/skin

Only pure cultures of *A. fumigatus* were isolated from the peel/testa of cocoa beans obtained from Breman Asikuma in the Central Region.

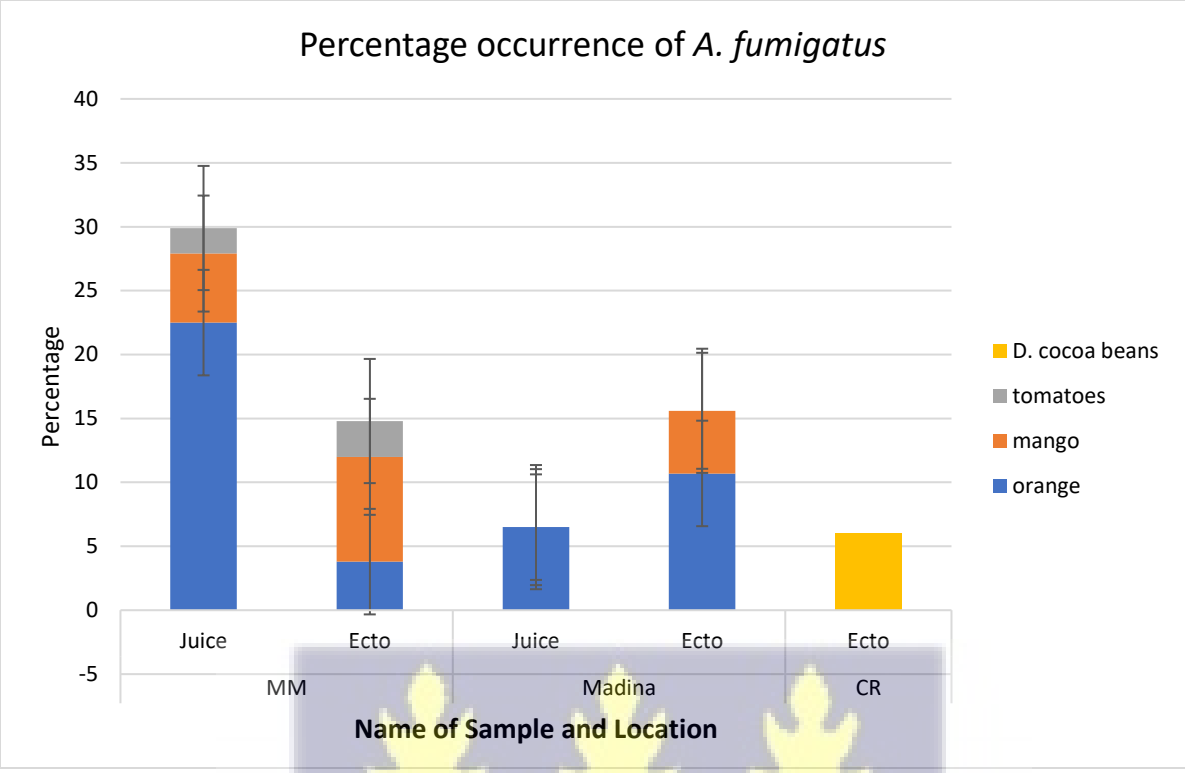


Fig 4: Percentage occurrence of *A. fumigatus* on the indicated selected fruits and vegetables from Mallam Atta and Madina markets and dry cocoa beans testa Breman Asikuma.

Experiment 2

Contribution of *A. fumigatus* to the aeromycoflora isolated from Mallam Atta and Madina Markets

Isolation of the aeromycoflora using the Open Plate Exposure Method shows marked differences in the occurrence of *A. fumigatus* in the Airspora of Mallam Atta and Madina Markets.

A. fumigatus constituted about 12.4 % of the total mycoflora in the environment of Mallam Atta Market (Fig. 5). This shows almost double what was obtained from the Madina Market (7.3 %).

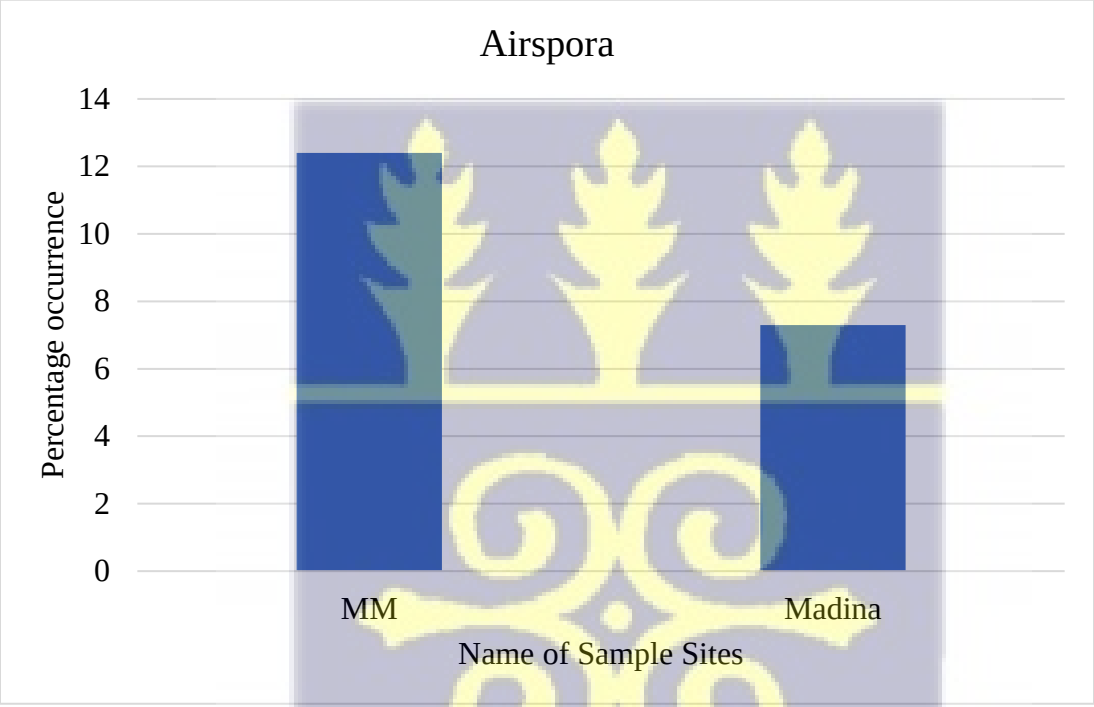


Fig 5: Comparison of Percentage Occurrence of *Aspergillus fumigatus* in Airspora from Mallam Atta Market and Madina Market.

Experiment 3

Comparative gross morphology, anatomy of the conidial head, vesicle, dimensions of the conidiophore, and the mycelium of *A. fumigatus* isolated from fruit juices, fruit peels/epicarp, and cocoa beans.

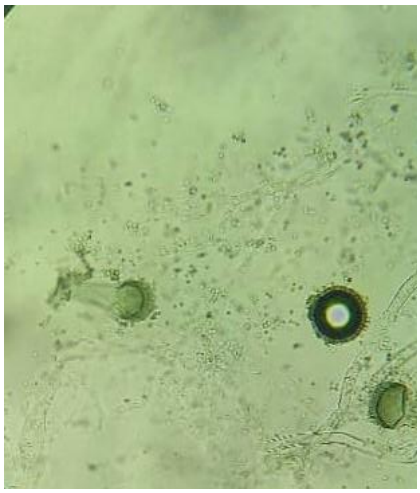
Plates 3 and 4 show the anatomical differences between the strains of *A. fumigatus* isolated from different markets in Greater Accra and from Breman Asikuma in the Central Region, resident in the orange, mango, tomato, and cocoa beans as well as those isolated from aeromycoflora.

Analysis of variance of the data (Table 5) shows that the differences observed were statistically significant at $P \leq 0.05$ (Table. 5). The microscopic and macroscopic characteristics of the *Aspergillus fumigatus* isolated from the different fruits, in widely separated geographic locations in Greater Accra and Central Region are presented in Tables 4 and 5.

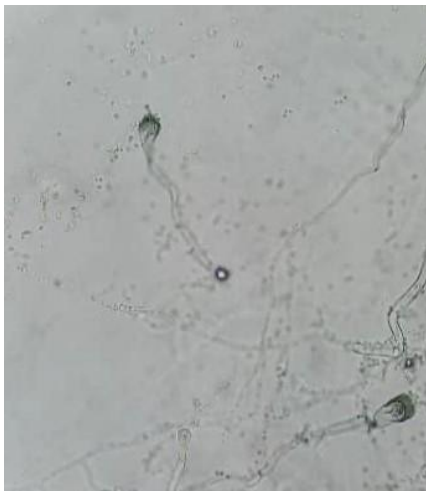
The conidiophore surfaces were smooth-walled while the colonies in the petri dishes were single, globose, and clustered and were generally bluish grey with white patches. These taken together identify the fungus as *Aspergillus fumigatus*.

Table 4. Table of Microscopic and Macroscopic Characteristics of *Aspergillus fumigatus* isolated from fruits and Airspora from different geographic locations

Microscopic Character	**Description**
Conidiophore surface	Smooth
Conidiophore wall surface	Smooth walled
Macroscopic Character	**Description**
Colony on plates	Single colonies
Shape	Globose in small column
Colour	Greyish blue with white apex

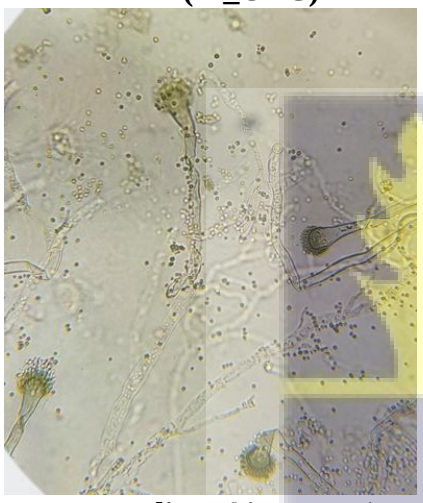


1. Madina orange ecto (M_OEC)

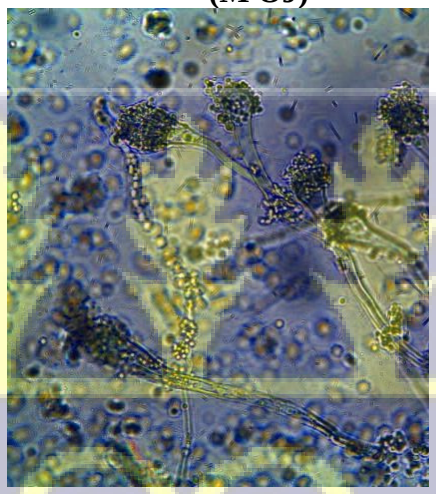


2. Madina orange juice (M OJ)

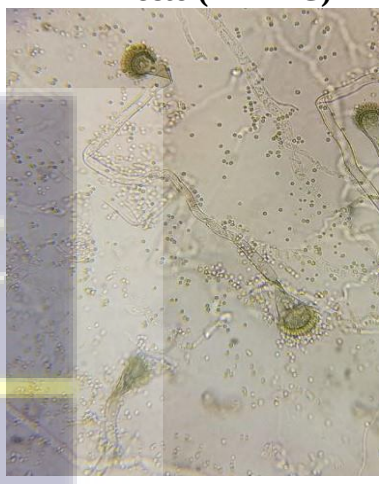
3. Madina mango ecto (M MEC)



4. Madina Airspora (M AIR)

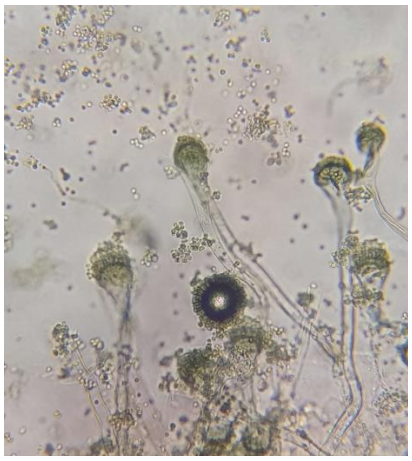


5. Mallam Atta Airspora (MM AIR)

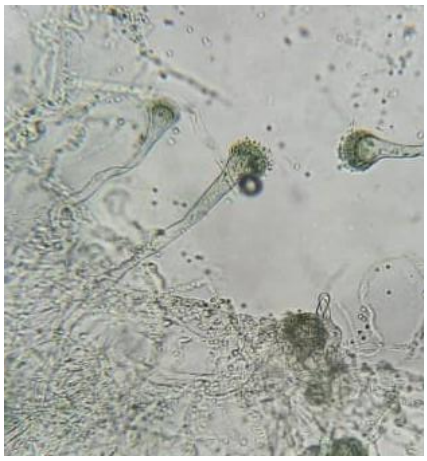


6. Mallam Atta Orange ecto (MM_OEC)

Plate 3. Photomicrographs of culture of *Aspergillus fumigatus* showing the varying morphology of conidiophores, conidial vesicles and arrangement of their phialides obtained from the indicated substrates purchased from different geographical markets locations (×400).

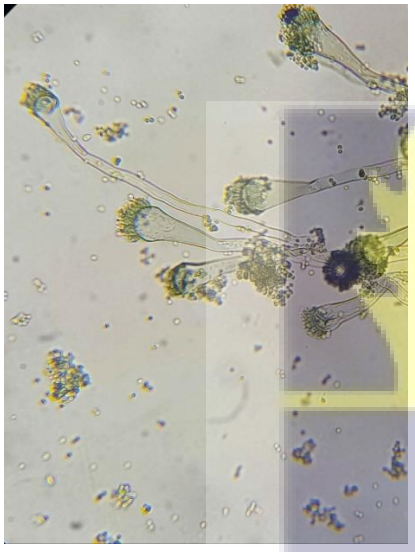


7. Mallam atta orange juice (MM OJ

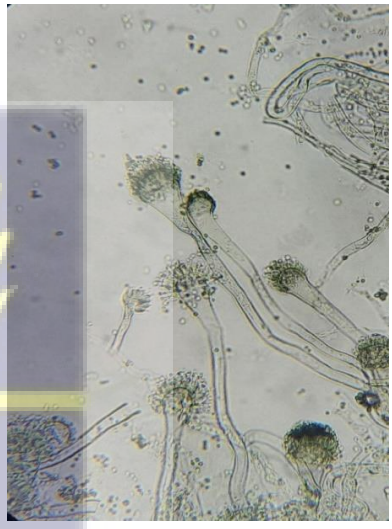


8. Mallam Atta Mango juice (MM_MEC)

9. Mallam Atta tomato ecto (MM TEC)



10. Mallam Atta tomato juice (MM TJ)



11. Dry cocoa (D COCOA)

Plate 4. Photomicrographs of culture of *Aspergillus fumigatus* showing the varying morphology of conidiophores, conidial vesicles and arrangement of their phialides obtained from the indicated fruits (7-10) and testa of dry cocoa beans (11) substrates purchased from different geographical markets locations and Breman Asikuma in the Central Region (×400)

Experiment 4 (a-d)

Statistical analysis (boxplot and Tukey HSD test and analysis of variance) to ascertain the significant differences between the micro-morphological (conidiophore diameter, vesicle diameter, and spore count and diameter) of *Aspergillus fumigatus* isolated from different environments.

a) Diameter of conidiophore diameter, vesicle diameter, spore diameter, and spore count.

Table. 5 summarizes the data obtained as well as the comparative statistical analysis of variance. The widest diameter of the conidiophore was 15. 51 µm collected from the Airspora of Mallam Atta Market (MM AIR) followed by the sample from the external (peel/skin) surface of Mallam Atta orange (MM_OEC) (14.72 µm). conidiophores of Airspora from Madina Market (M_AIR) were diminutive (7.55 µm) and about half the size of the diameter of air spores-producing conidiophores from Mallam Atta Market. Statistical analysis of variance with a P-value of 0.000779 showed that the differences observed were statistically significant (Table. 5).

The Box-plot (also known as the whisker plot), is a graphical representation of the distribution of a dataset. It summarizes key statistics such as the median, quartiles and potential outliers in a concise and visual manner. Using the data obtained in this study, the boxplot (materials and methods section) of the conidiophore dimensions at various samples sites were statistically different ($P \leq 0.05$) when we compare the medians, dispersal or spread of data and outliers together with the skewness which gives us the direction and magnitude of the lack of symmetry. Fig. 6 summarizes the boxplot of the dimensions of the conidiophores from all the sampling spots and indicates significant differences.

Table 5. Micro-morphological characteristics of *Aspergillus fumigatus* isolated from fruits and Airspora from different geographic locations

Sample (Fruits)	Average conidio-phore diameter (µm)	Average vesicle diameter (µm)	Average spore diameter (µm)	Average spore count per mi-croscopic field
Madina orange Ecto	12.438^{abc}	29.061^{abc}	1.208^{ab}	128
Madina Or-ange Juice	8.853^{bc}	20.825^{cd}	1.094^{b}	186
Madina Mango Ecto	12.893^{abc}	32.408^{a}	2.034^{ab}	247
Madina Air-spora	7.546^{c}	17.775^{d}	1.062^{b}	275
Mallam Atta Airspora	15.509^{a}	31.465^{ab}	2.374^{a}	138
Mallam Atta Orange ecto	14.719^{ab}	26.337^{abcd}	1.825^{ab}	173
Mallam Atta Orange juice	10.179^{abc}	22.466^{bcd}	1.181^{ab}	260
Mallam Atta tomato ecto	11.891^{abc}	24.187^{abcd}	1.471^{ab}	278
Mallam Atta tomato juice	9.889^{abc}	21.052^{bcd}	1.361^{ab}	321
Mallam Atta Mango juice	8.653^{bc}	21.981^{bcd}	1.338^{ab}	166
Dry cocoa	10.118^{abc}	23.771^{abcd}	1.352^{ab}	140

*Ecto: - refers to the outer peel

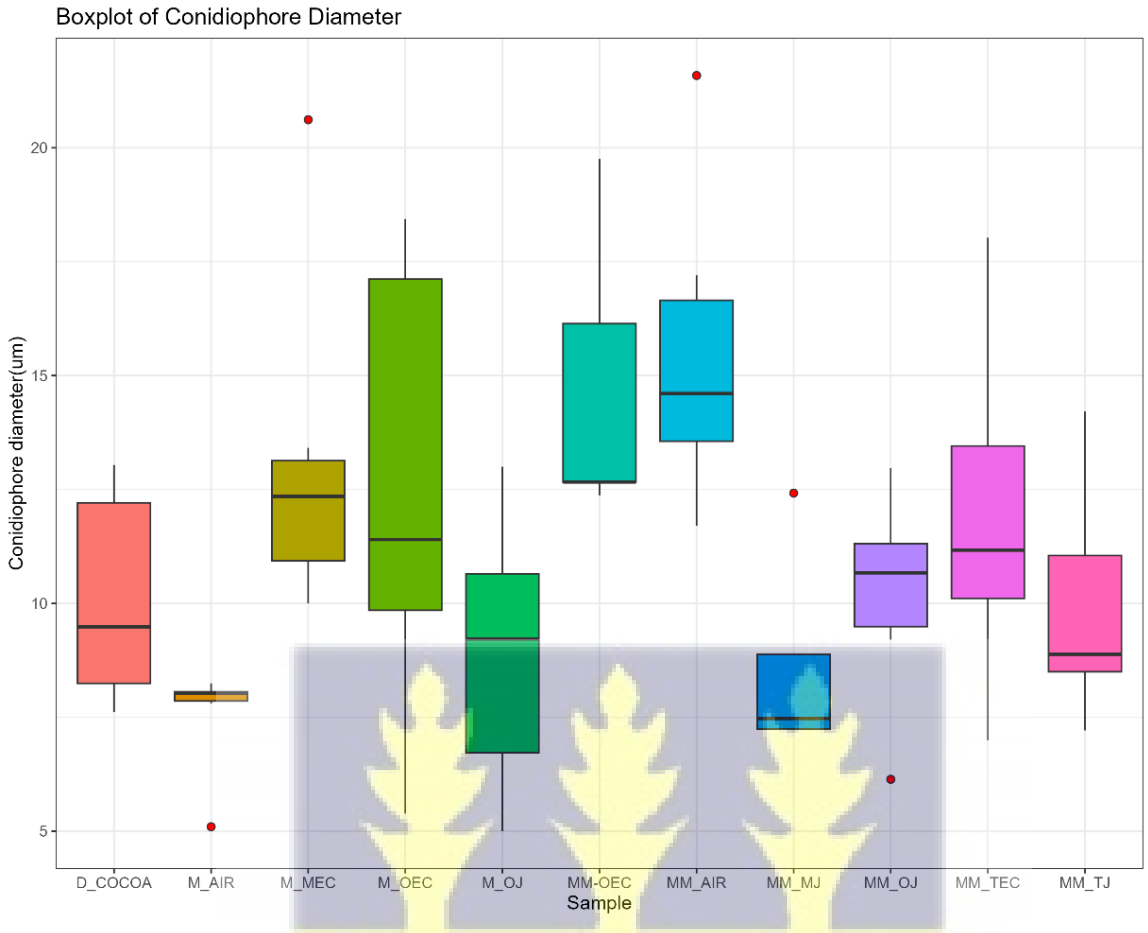


Fig. 6. A boxplot showing the micro-morphological characteristics of conidiophore diameter of *Aspergillus fumigatus* isolated from fruits and Airspora from different geographic locations.

Keys:

M_OEC: - Madina orange Ecto/Peel

MM_TEC: - Mallam Atta Tomato Ecto/Peel

MM_MJ: - Mallam Atta Mange Juice

MM_TJ: - Mallam Atta Tomato Juice

M_AIR: - Madina Airspora

M_OJ: - Madina Orange Juice

M_MEC: - Madina Mango Ecto/Peel

D_COCOA: - Dry Cocoa Beans

MM_OJ: - Mallam Atta Orange Juice

MM_AIR: - Mallam Atta Airspora

M_OEC: - Mallam Atta Orange Ecto/Peel

b) Diameter of the vesicles of *A. fumigatus*

The widest diameter of the vesicles was recorded from the samples examined from the peel (ecto-derm) of mango fruit purchased from the Madina Market, M_MEC (32.41 µm) followed closely by vesicles from the culture derived from the Airspora from Mallam Atta (MM_AIR) is 31.4 µm. the lowest dimension of the vesicle of *A. fumigatus* measured 17.78 µm and derived from Airspora from Madina Market (M_ AIR). The other values of vesicle diameter differed significantly ($p < 0.05$) from the mango fruit peel (ectoderm) and Mallam Atta Airspora (Table 5). Generally, the differences observed in vesicle diameter across the board were statistically significant ($P \leq 0.05$).

The Boxplot of the data presented in Fig. 7 summarizes statistical representation of the distribution of the dataset. Using the dataset, for the plot, it can be seen that it, the median, quartiles and po-tential outliers appear in a concise and visible manner. The diameter of the vesicles at various sampling points were statistically different ($P < 0.05$) when compared to the medians, dispersal of spread of data, and outliers, not excepting the skewness which gives the direction and magnitude of the lack if symmetry (Fig.7).

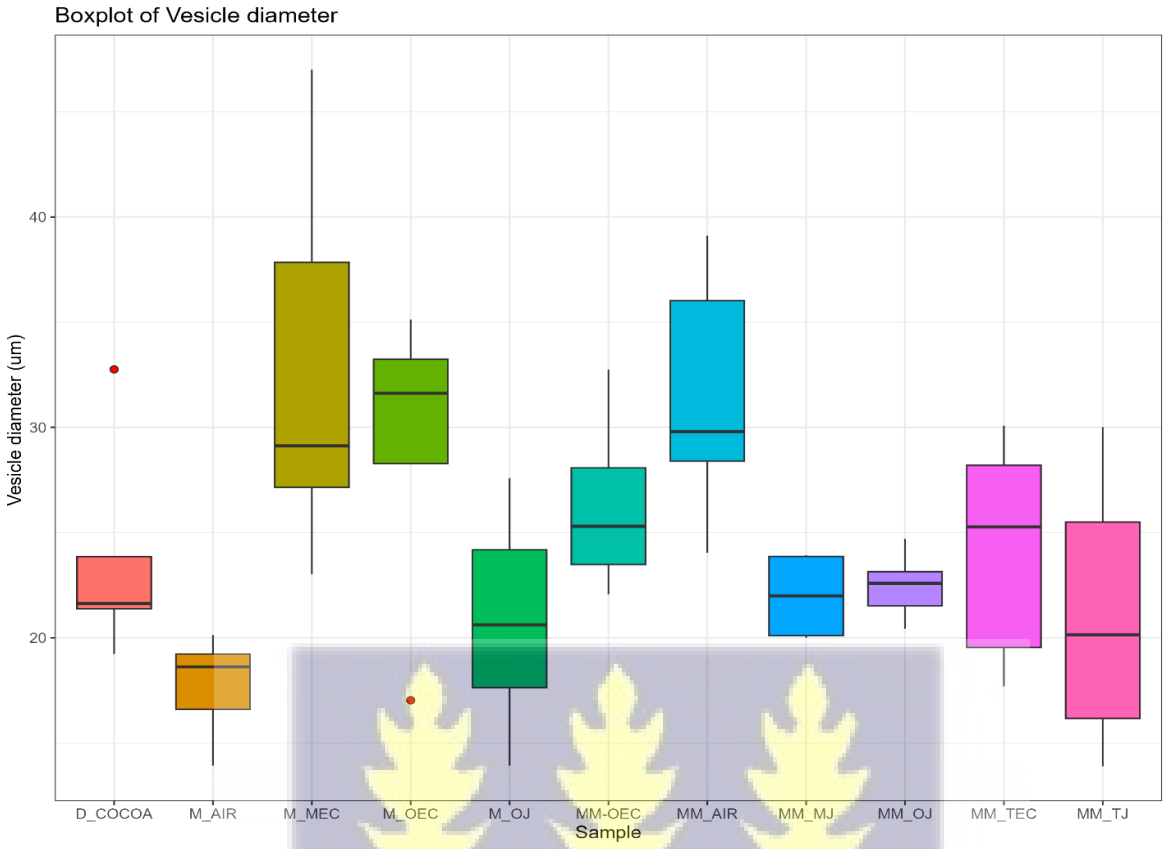


Fig. 7. A boxplot showing the micro-morphological characteristics of vesicle diameter of *Aspergillus fumigatus* isolated from fruits and Airspora from different geographic locations.

c) Spore diameter of *A. fumigatus*

The largest or biggest spore measured 2.37 µm in diameter and was obtained from the Airspora at Mallam Atta Market (MM_AIR). This was followed closely by 2.03 µm diameter of spores from the peel of mango fruit (M_MEC)) from Madina Market. These spores did not differ in size significantly from spores from Madina Airspora (M_AIR).

Spore diameter from MM_OEC (1.83 µm; Mallam Atta orange peel(ectoderm)), MM TEC (1.47µm; Mallam Atta tomato peel (ectoderm)), MM_TJ (1.36 µm; Mallam Atta tomato juice), D. COCOA (1.35 µm; Dry cocoa beans testa), MM_MJ, Mallam Atta mango juice(1.34 µm) and

Madina orange juice (1.094 µm) were not statistically different from the larger spore, 2. 034 - 2.274 µm from the external peel (ectoderm) of mango M MEC, and Mallam Atta Airspora using the statistical analysis of variance (Table 5).

The Boxplot of the data presented in Fig. 8 shows that the median, quartile, and potential outliers are concise. The diameter of the spores was different at different geographical substrates and location, and in the air and were statistically different when compared to median, dispersal of spread of data, and outliers, not excepting the skewness which gives the magnitude of the lack of symmetry (Fig. 8).

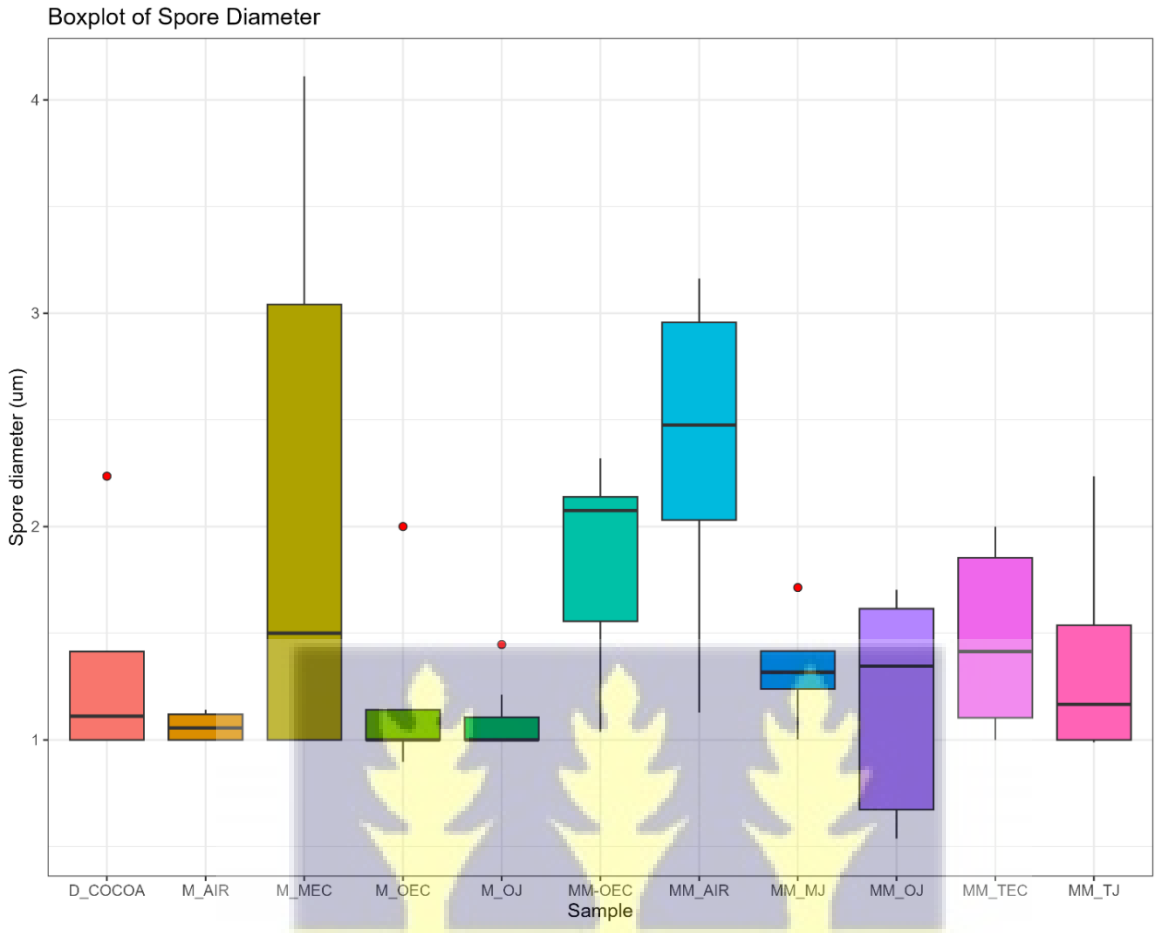


Fig. 8. A boxplot showing the micro-morphological characteristics of spore diameter of *Aspergillus fumigatus* isolated from fruits and Airspora from different geographic locations

d) Collation of Average spore counts at different geographical market locations and on different substrates using Heatmap Technique

Samples recording the highest number of spores/fields were from tomato fruit juice (MM_TJ) from Mallam Atta Market (321) followed by the pericarp of tomato MM_TEC (278) shows the darkest blue shades. Intermediate and low spore counts with moderate blue are MM_OEC (128), MM_MJ (166), M_AIR (138) and M_OJ (186). D.COCOA (140) (Table 5; Fig. 9) with higher blue shades. The Heatmaps therefore gave a comparative spore count criteria for the locations and substrates.

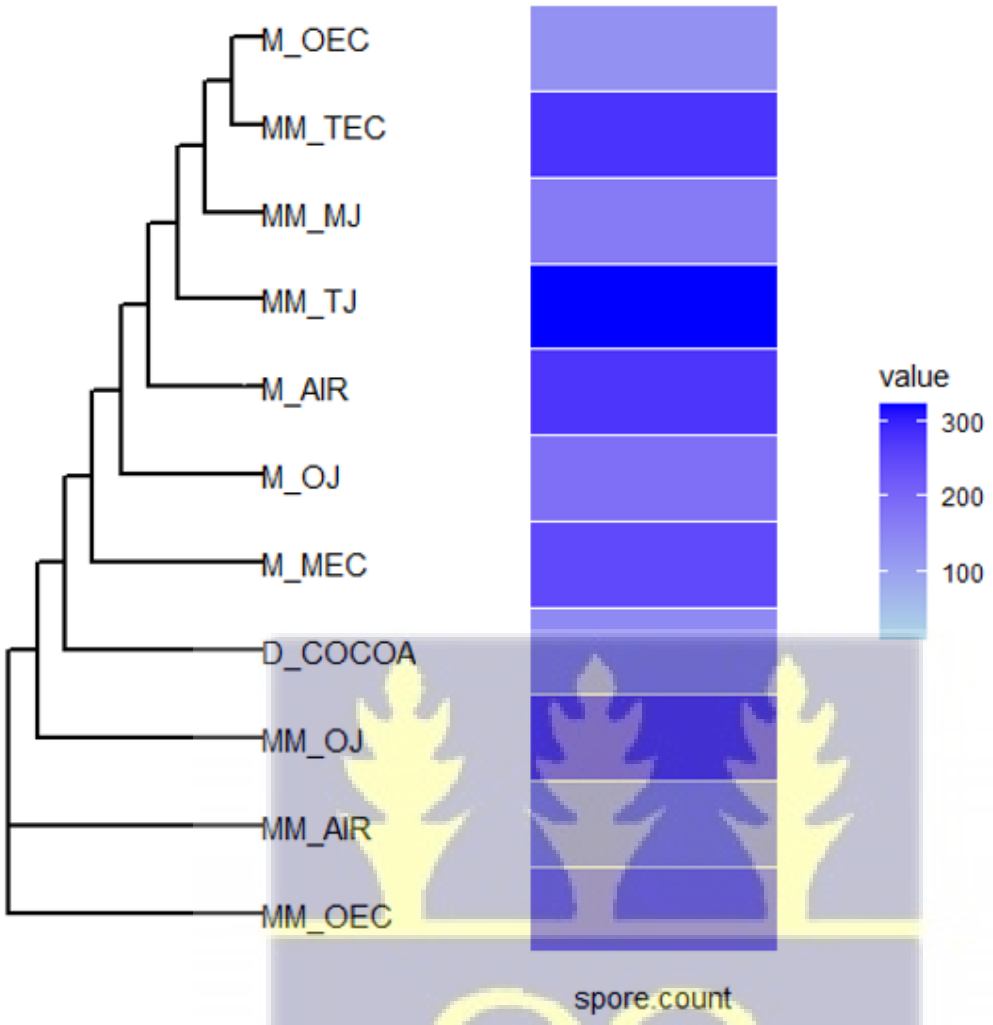


Fig. 9. A heatmap showing the micro-morphological differences of spore count of *Aspergillus fumigatus* isolated from fruits and Airspora from different geographic locations.

Keys:

M_OEC: - Madina orange Ecto/Peel

MM_TEC: - Mallam Atta Tomato Ecto/Peel

MM_MJ: - Mallam Atta Mange Juice

MM_TJ: - Mallam Atta Tomato Juice

M_AIR: - Madina Airspora

M_OJ: - Madina Orange Juice

M_MEC: - Madina Mango Ecto/Peel

D_COCOA: - Dry Cocoa Beans

MM_OJ: - Mallam Atta Orange Juice

MM_AIR: - Mallam Atta Airspora

M_OEC: - Mallam Atta Orange Ecto/Peel

Experiment 5

The Disease Progress Curve of the Pathogen *A. fumigatus* on Different Fruit Substrates

A disease progress curve (DPC) is a graph that shows the intensity of a plant disease over time. DPC'S are a key tool for understanding how pathogens, hosts, and the environment interact to cause disease in plants. The most common form is linear (rare and only early on), exponential (common in the beginning), saturation or monomolecular/monocyclic exponential (common in the beginning), or logistic curve (common).

A monocyclic disease is a plant disease caused by a pathogen that can complete one part of its disease cycle in a single year. Soil-borne pathogens are typically monocyclic e.g. tomato wilt, cotton wilt, and are also known as simple interest disease. The graphical disease caused by a monocyclic pathogen looks like an S-shaped or saturation curve (Anon, 2019).

Results from this experiment show that the disease progress curves of mango, orange and tomato fruits infected with *A. fumigatus* was a sigmoid saturated curve characteristic of a monocyclic pathogen (Fig. 10). There were significant differences ($p \leq 0.05$) between the infection and susceptibility of the fruits of the pathogen. The pathogen grew fastest on mango peel attaining diameter/radius 4.5 cm (45 mm) in 7 days. This was followed by tomato (3.25 cm or 32.5 mm) in 7 days (Fig. 10). Plate 5 shows the extent of the disease spread on the fruit sample after 7 days. Visually, mango and tomato seem to be the worst affected by *A. fumigatus* inoculum.

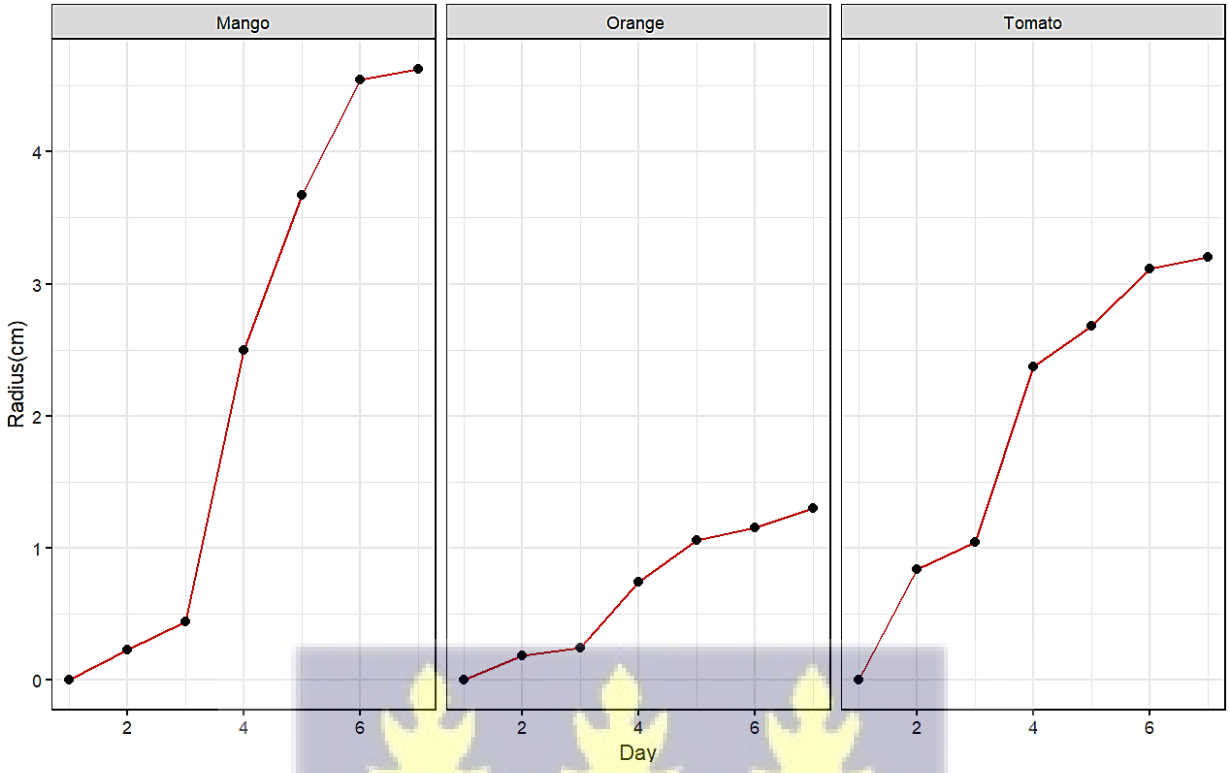


Fig. 10. Disease progress curves for mango, orange, and tomato fruits over a seven-day incubation period at 28 ± 2 ℃ showing the typical sigmoid curve of monocyclic disease.

The Area Under Disease Progress Curve (AUDPC) is a metric used in plant pathology to combine multiple observations of disease progress into a single value described by Simko and Piepho (2012) and Jackson (2022).

Fig. 11 shows the calculated AUDPC for the three types of fruits namely mango, orange, and tomato after infection by *A. fumigatus* in 7 days. The highest AUDPC was obtained on the data for mango infection (13.69 cm) followed by tomato (AUDPC; 11.17 cm) and the least recorded on orange (AUDPC; 3.98 cm). the AUDPC value reflects the variable disease severity over the observation period with higher values indicating severe rapid progressing disease. Statistical analysis shows that infection of the mango and tomato was significantly severe and faster ($P \leq 0.05$) with a

p-value of 0.0104 on mango and tomato than on orange. This observation agrees with the disease progress curve shown in Fig. 10 and the calculated AUDPC values in Fig. 11.

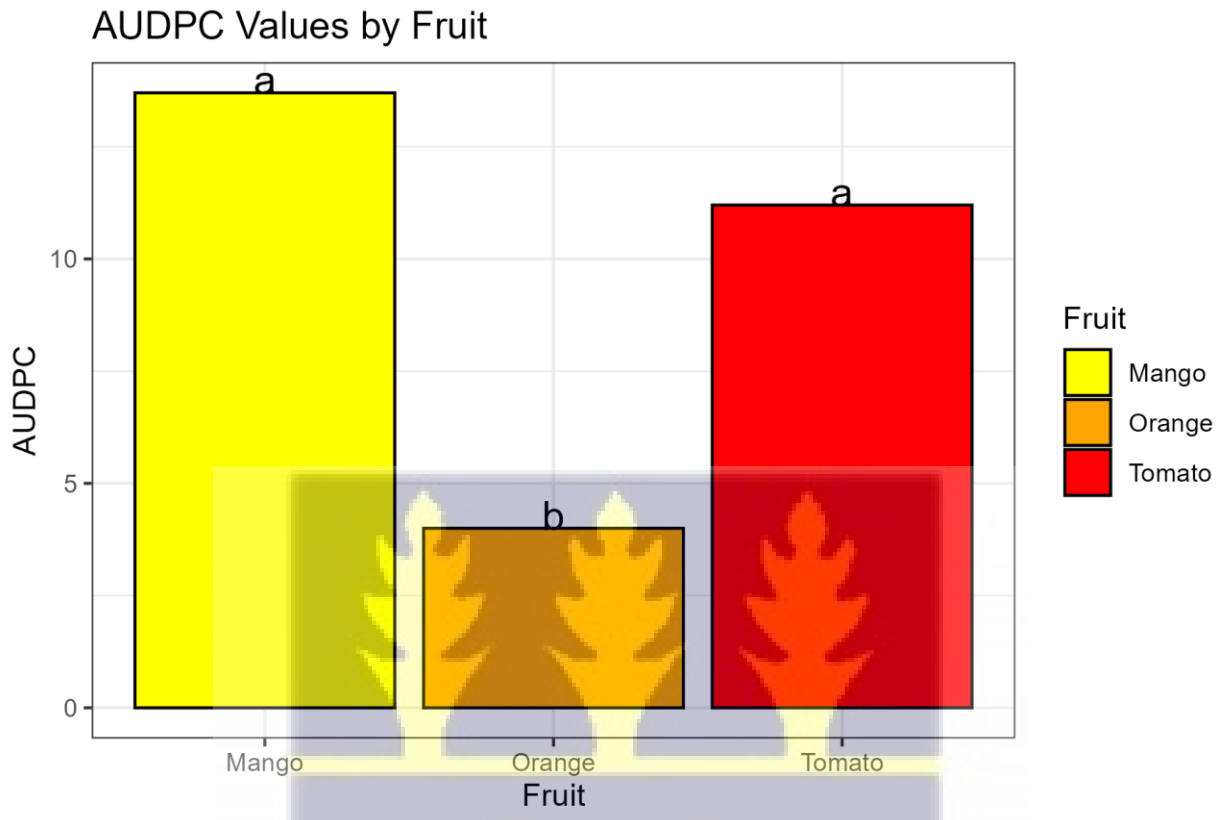


Fig. 11. A graphical comparison of disease severity (AUDPC) among mango, orange, and tomato fruits infected with *Aspergillus fumigatus* for 7 days at 28 ± 2 °C

Plate. 5. Photographs showing arrangement of uninfected fruits (left): top: oranges, middle: tomatoes, bottom: mangoes. The same arrangement is shown (right) of the extent of spread of *A. fumigatus* infection after incubation for 7 days at 28 ℃ (×0.5)

** Arrow indicating the growth of *A. fumigatus* on the fruit samples

Experiment 6 (a and b)

Molecular characterization of *Aspergillus fumigatus* variants obtained from different geographical locations and samples.

a) Gel Electrophoresis in the PCR showing ITS Characteristics of *A. fumigatus* isolated from different samples and locations.

The nuclear ribosomal Internal Transcribed Space (ITS) region of the rDNA array is the most prevalent marker used to identify fungi and is the primary fungal barcode to the consortium for the Barcode of Life.

The Polymerase Chain Reaction, PCR results showed two different target regions. The band is the sample lanes 28-43 spanning between 550 bp to 650 bp. Lanes 29-40 (Group 1) have bright, consistent bands closer to the 550 bp mark which suggests successful amplification of a target sequence around this size (Fig.12).

Lanes 41-43 (group 2) were similar to group 1 (Fig .12); these lanes also showed bright bands and appear closer to the 650 bp marker thus suggesting successful amplification of a slightly larger product than those of Group 1.

Lane 28 had a feint band and relatively smaller than the 550 bp

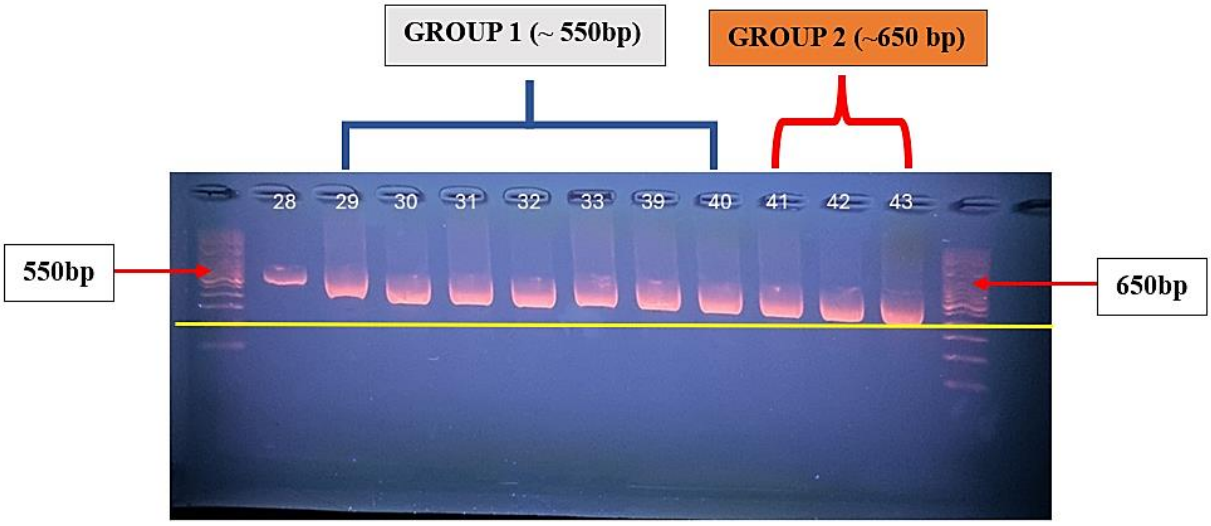


Fig. 12. Gel Electrophoresis Profile showing the ITS Characteristics (ITS 1 and ITS 4) amplification of *Aspergillus fumigatus* of different Samples from Different Locations (Lanes 28-43).

b) Plotting of the Phylogenetic Tree Showing the ITS Characteristics of *Aspergillus fumigatus* isolated from different locations and fruit samples.

The phylogenetic tree (phylogeny or evolutionary tree) is a diagram that represents evolutionary relationships among organisms. It is a branching diagram or a tree showing the evolutionary relationships among various biological species or other entities based upon similarities and differences in the physical or genetic characteristics (Anon, 2023).

The phylogenetic tree summarized in Fig. 13, shows the evolutionary relations between the indicated samples of *A. fumigatus* isolated from different geographic locations and fruits. The *A. fumigatus* strains can be delimited into two groups; those above the Standard Reference (AF293_REF) indicated in Red and those below the reference standard.

The MM_AIR (Mallam Atta Airspora) and MM_OEC (Mallam Atta orange ectoderm (peel) show a close phylogenetic relationship branching near the root of the phylogenetic tree with a high bootstrap value (~ 0.98) indicating that this group reflects a strong confidence level in the genetic similarity.

It can be deduced that *A. fumigatus* from the air sample of Mallam Atta Market (MM_AIR) and orange ectoderm (peel) (MM_OEC) share a recent common ancestry and may have similar evolutionary pressures or environmental adaptation (Fig. 13). In contrast, samples screened below the reference and including the rest of the *A. fumigatus*; D. COCOA; Dry cocoa testa, M MEC; Madina mango ectoderm (peel), M_OJ; Madina orange juice, M AIR; Madina Airspora from Madina Market. MM_MJ; Mallam Atta mango juice, MM_TJ; Mallam Atta tomato juice, MM OEC; Mallam Atta orange ectoderm (peel), MM _TEC; Mallam Atta tomato ectoderm(peel) show greater genetic diversity or divergence from the reference strain AF293_REF. This is shown by their bootstrap values ranging from ~0.49~0.98 (Fig. 13).

This is an indication of varying levels of confidence in their evolutionary relationships even though they are isolates of *A. fumigatus*. Presumably, these strains might have undergone different evolutionary paths and environmental pressures leading to a complex example of genetic diversity landscape.

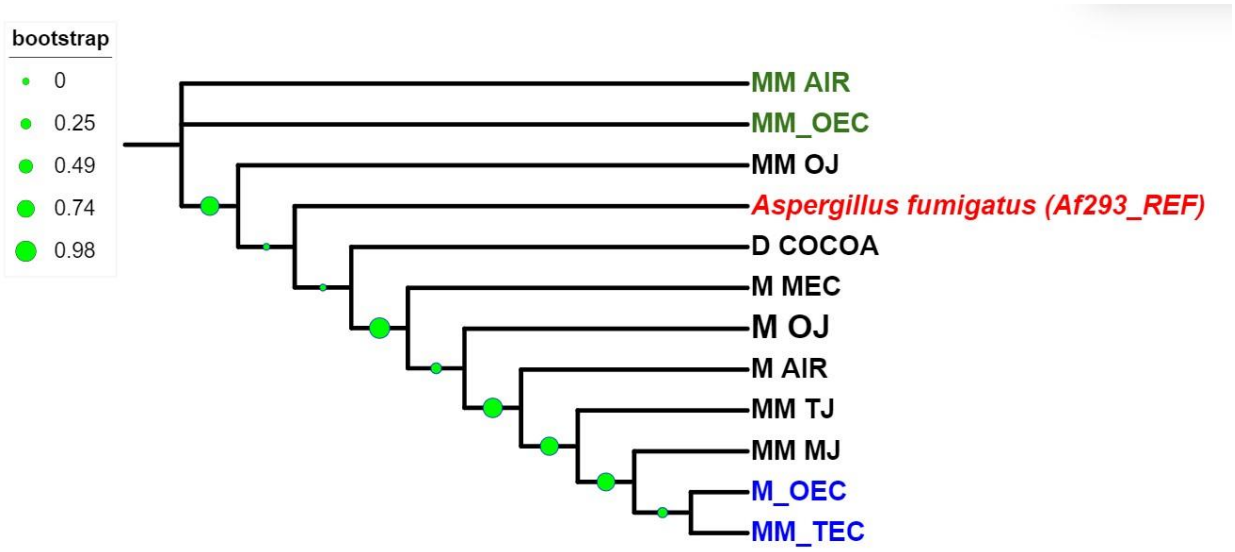


Fig. 13: A Phylogenetic Tree showing the ITS Characteristics of *Aspergillus fumigatus* of different Samples from Different Locations. *Aspergillus fumigatus* (Af293_REF) was a reference species genome we compared our used our samples with.

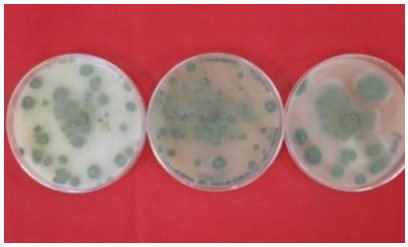
Genome source: https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=330879

Experiment 7

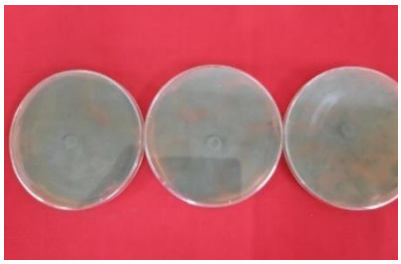
Use of some antifungal agents in the control of *Aspergillus fumigatus.*

A wide range of antifungal agents are used in combating biodeterioration and preventing or treating fungal diseases of plants. In these contexts, they are commonly referred to as fungicides. Others are used in treating diseases in animals and humans and are referred to as antifungal drugs or, if produced by means of microbial fermentation, an antifungal antibiotic (Carlile, Watkins, and Gooday, 2005).

In this Experiment 7, three antifungal agents were tested at different concentrations (1:25 v/v, 1:100 v/v and 1:200 v/v) on their ability to prevent the growth of *A. fumigatus* in a solid culture. Griseofulvin and Cefuroxime at all concentrations did not prevent the growth of *A. fumigatus* (Plate 6). On the other hand, Itraconazole prevent prolific growth even at 1:25 v/v dilution (Plate 6). The triazole drugs, viz, fluconazole, itraconazole, voriconazole have broad spectrum of anti-fungal activity. Itraconazole had been used for treatment of wide range of fungal infection (Carlile, Watkins and Gooday, 2005).

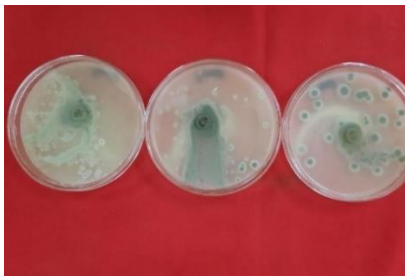


1:25 v/v Griseofulvin

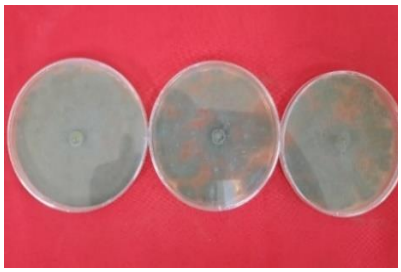


1:25 v/v Cefuroxime

1:25 v/v Itraconazole

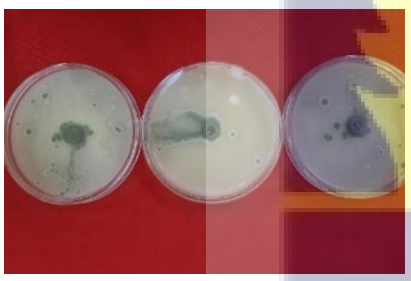


1:100 v/v Griseofulvin

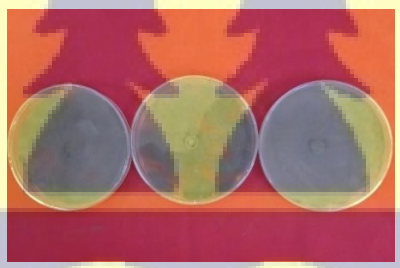


1:100 v/v Cefuroxime

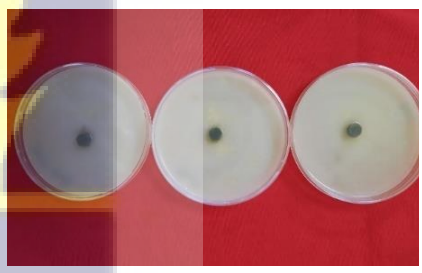
1:100 v/v Itraconazole



1:200 v/v Griseofulvin



1:200 v/v Cefuroxime



1:200 v/v Itraconazole

Plate 6. The antifungal effects of griseofulvin, cefuroxime, and itraconazole on *aspergillus fumigatus* isolated from fruits: concentration ratios (1:25 v/v, 1:100 v/v, 1:200 v/v) after 7 days of incubation at 30 ± 2 °C.

CHAPTER FOUR

4.0 DISCUSSION

Fungi are ubiquitous, and found in almost all environments, especially on fruits, vegetables, inert surfaces, etc. Post-harvest losses due to fungal contamination can reach up to 30 % in some crops (Alegbeleye *et al*., 2022; FAOSTAT, 2020; Hocking, 2014). Fungal deterioration affects the profitability of farmers all over the food supply chain (Honger *et al*,2018). *Aspergillus* species are among the most notorious post-harvest pathogens.

In Experiment 1, the mycoflora residents in mangoes, oranges, and tomatoes were updated from the two Central Markets in Accra namely Madina and Mallam Atta Markets. The total fungal populations in the fruit juices of orange, mango, and tomato varied from one market to another depending on the type of fruit (log_{10} 3.0 CFU/g-log_{10} 3.34 CFU/g). The differences were marginal. The goods for sale on these markets are obtained from the same hinterland source by traders but diverted for sale at different markets where storage and handling of goods determine their shelf-life.

Differences in mycofloral load of the fruit juice of tomato from Mallam Atta and Madina Markets could be attributed to handling practices while mango fruits juices were apparently more laden with fungal spores presumably because of the penetration of the mycelium from the pericarp into the fruit was easier for mango. On the contrary, the orange fruit juice was the least contaminated (log_{10}3.08 CFU/g) as the pericarp was acting as a barrier to penetration by the advanced mycelium from the exterior (Fig.1). Generally, the exterior pericarp of the fruits harbour lower fungal population (log_{10} 1.0 - log_{10} 1.4 CFU/g as compared to the extracted juice samples (log_{10} 3.08CFU/g)

Fig. 2. This may be attributed to limitation to penetration due to the chemical barriers embedded in the pericarp as a natural defense mechanism.

Seemingly, both markets had oranges, mangoes, and tomatoes on sale with comparable fungal populations on their skins. These fruits were presumably from the same location in the hinterland, sourced by a group of experienced market vendors well-versed in this trade. This is augmented by the data in Fig. 3. The aeromycoflora population was only marginally higher (log_{10} 0.08 CFU/g) at Mallam Atta Market than what existed at Madina Market (log_{10} 1.43 CFU/g).

Aspergillus fumigatus was isolated from all samples from Mallam Atta Market in contrast with only orange and mango from Madina Market. The mango juice of samples purchased from Madina Market did not record contamination by *A. fumigatus* both in juice and peel/skin (Fig. 4). On the contrary, tomatoes obtained from Madina Market were devoid of *A. fumigatus* spores. Such contrasting evidence may be due to an intrinsic chemical environment unsuitable for its growth *in vitro* or a strain of *A. fumigatus* with peculiar growth characteristics. There was another significant observation where the percentage incidence of *A. fumigatus* in the samples from Mallam Atta and Madina Markets were not the same for the juice extracts and the peels when plated on PDA medium. Data from this thesis cannot be explained but further investigation is required to elucidate this.

The general trend of the isolation of *A. fumigatus* from the juice and epicarp/peel of mango, tomato and orange are shown in Fig. 4. The variation in the ability of the fungus in the juice and peel of tomatoes, oranges and mangoes to support the growth and development of *A. fumigatus* is a subject for future investigations. The isolation of 6 % *A. fumigatus* on dry cocoa beans from Breman Asikuma is another finding with health implications for consumers should *A. fumigatus* be found in cocoa products and chocolate bars.

A. fumigatus can secrete molecules *in vitro* that interfere with the host immune system, such as gliotoxins, which influence T-cell proliferation, mast cell activation, cytotoxic T-cell responses, monocyte apoptosis, and neutrophil function (Abad *et al*, 2010). The same fungus *A. fumigatus* also produces a mycotoxin, fumagillin, and pseurotin A (Guruceaga *et al.,* 2020). Recently International Cocoa and Coffee Organization, ICCO has included quality control to screen *A. fumigatus* in cocoa and coffee because of its potent toxicity on humans (Guruceaga *et al.,* 2020).

Quantitatively, the incidence of Airspora of *A. fumigatus* at Mallam Atta Market (12.4%) was nearly double what was obtained at Madina Market (7.3 %) (Fig. 5). It is conjectured that the abundance of *A. fumigatus* at Mallam Atta Market could be attributed to the timing of sampling, human activity, increased organic wastes contributed by market waste products or more favourable environmental conditions for the survival of spore at Mallam Atta Market or an interplay of these factors. Time limitations prevented an in-depth study of the contributions of these listed factors may lead to the prevalence of aeromycoflora of *A. fumigatus* at Mallam Atta Market. This could form part of future studies on this subject matter to complement current data obtained. Air sampling for Airspora can also be predetermined at specific intervals throughout the day when sampling occurs, and the results can be correlated with windspeed and impaction. This aspect can be incorporated into subsequent work.

In Experiments 3 and 4, the complex *A. fumigatus* microstructure including hyphae, conidia, conidiophores, and vesicles were studied with the view to using their parameters as criteria for identification. Most fungal species have been recognized and classified in the past on the basis of their morphological form of sporophores.

The *A. fumigatus* isolated from the air at different markets in Accra and Breman Asikuma had the formation of flask-shaped or cylindrical phialides in a single series on the surface for vesicles at

the apex of a conidiophore (Tables 4 and 5). Conidia were deciduous and globose, oblong to elliptical, and presented typical blue-green with a suede-like surface consisting of a dense felt of conidiophores. Colonies on Potato Dextrose Agar at 25-30 ℃ were smoky grey-green with a slight yellow reverse (Tables 4 and 5). Analysis of variance of conidiophore diameter, vesicle diameter, spore diameter, and spore count indicate that the differences observed were statistically significant ($p < 0.05$) and that they are variants of *Aspergillus fumigatus* (Table 5).

The Box Plot, also known as the box and whisker plot, is a graphical representation of the distribution of the dataset in Table 5. It summarized the key statistical parameters such as the median, quartile, and potential outliers in a concise and visual manner.

The Box Plot analysis showed that the media, quartiles, and potential outliers were concise and that the differences observed in the dimensions of the conidiophore diameter, vesicle diameter and spore diameter were statistically significant ($P \leq 0.05$). This data agrees with conclusions from Experiments 4 and 5 that the fungus was a variant of *A. fumigatus*. It is further augmented by the varying spore counts of the *A. fumigatus* spores isolated from different locations and products (Table 5 and Fig 9). The heatmap showing the micromorphological differences of the spore counts of *A. fumigatus* isolated from fruits and Airspora underscores the results obtained that the *A. fumigatus* isolated may be variants of the same species.

The Disease Progress Curve (DPC) is the graph that shows the intensity of a plant disease over a specified period. This is an indication of how the pathogens, host, and the environment interact to cause disease in plants. The disease progress curves obtained for mango, orange, and tomato in Fig. 10 were sigmoid shaped implying a monocyclic disease caused by a pathogen in a single cycle. They are also known as simple interest diseases (Anon, 2019). There were however significant differences ($P \leq 0.05$) between the infection and susceptibility of fruits to the pathogen. The

pathogen grew fastest on mango fruit, followed by the tomato while infection on orange lagged behind (Fig. 10; Plate 5). Visually, mango and tomato seem to be the worst affected, presumably because of the variation in substrate composition supporting growth and degradation enzyme formation, and the presence of strong chemical infection barriers in the peel of oranges.

The Area Under Disease Progress Curve, AUDPC, is a metric used in plant pathology to combine multiple observations of disease progress curves into a single value (Simko and Piepho, 2012; Jackson, 2022). The highest AUDPC for mango infection was 13.69 followed by tomato (11.7) and orange AUDPC=3.98 (Fig. 11). Statistical analysis shows that the infection of mango and tomato was significantly severe and faster with a p-value of 0.0104 on mango. The AUDPC values reflect the variable disease severity over the observation period of 7 days; the higher values indicate severe rapid progress. This agrees with the disease progress curve shown in Fig 10.

The external morphological features used for identifying *A. fumigatus* differences were clear-cut and palpable. The statistically significant differences ($P \leq 0.05$) in conidiophore size, vesicle diameter, spore diameter, and spore production by the mycelium suggest that the microscopically examined isolates represent variants within the *A. fumigatus* species rather than distinct

species.

Until recently, most fungi could be identified only by sporophore observations. But this has recently become a great obstacle to progress in identification of species. However, DNA technology is now enabling a fungal species to be identified in the absence of sporophores (or mycelium). In this thesis, the Gel Electrophoresis in the PCR showing ITS characteristics gave two different target regions. Lanes 29-40 located to 550 bp marker (Group 1) and lanes 41-43 (Group 2) located to the 650 bp marker with successful amplification (Group 2) compared to (Group 1). The size

differences presumably came from variability in the genomic target regions among the sample or from polymorphism that results in length variations. Such genomic variations are common in complex genomes where insertions, deletions or repeated sequences can modify amplicon size (Thompson *et al.,* 1997). Slight differences in the primer handling sites or secondary structures within the DNA sequence template have influenced the PCR outcomes.

The phylogenetic tree or evolutionary tree (Fig. 13) represents evolutionary relationships among the samples. It is a branching diagram or tree that shows the evolutionary relationships among various biological species and other entities, based on similarities and differences in their physical or genetic characteristics (Anon, 2023). The *A. fumigatus* strains can be divided into two groups; those above the standard reference (AF293_REF); indicated in red, and those below the reference standard.

The samples MM_AIR (Mallam Atta Airspora) and MM_OEC (Mallam Atta orange peel/skin) showed a close genetic relationship branching near the root of the phylogenetic tree with a high bootstrap value (~0.98) indicating that this group has a strong confidence level in genetic similarity with the reference, AF293_REF and likely to share common ancestry (Fig. 13). It could be conjectured that these two varieties of *A. fumigatus* might have been subjected to similar evolutionary pressure and environmental conditions shaping their similar genetic profiles.

On the other hand, below the reference group (AF293_REF) there was a broader range of *A. fumigatus* variants namely D. COCOA (samples from dry cocoa beans testa) M_MEC (Madina mango peel). M_OJ (Madina orange juice), MM AIR (Mallam Atta Airspora), MM TJ (Mallam Atta tomato juice) MM_MJ (Mallam Atta mango juice), M_OEC (Madina orange peel), MM_TEC (Mallam Atta tomato peel) (Fig 13) which exhibited greater genetic divergence from the reference *A. fumigatus* strains.

Their more distant branching on the phylogenetic tree indicates their divergence from the standard. Their bootstrap values ranged between ~0.49 - ~0.98 (Johnson *et al*, 2021); implying a different confidence level in the evolutionary relationship among these variants. It can be deduced that this variability within groups may suggest that the isolates (variants within the group may have followed distinct evolutionary paths influenced by unique nutritional, environmental and selective pressure (Lee *et al*., 2022).

It is evident that relatively complex genetic landscape was observed in *A. fumigatus* variants. Strains below the Reference Group underscores the considerable diversity present within the Ghanaian *A. fumigatus* populations. It would be worthwhile to extend work in this thesis countrywide to ascertain the views expressed by data from this limited study. Clearly, understanding the evolutionary dynamics in fungal diversity is crucial in comprehending how different strains and varieties respond to environmental changes and challenges especially when control measures are prescribed to curtail spread of disease.

In the concluding experiment of this project in the thesis some three antifungal agents were tested on the growth of *A. fumigatus in vitro*; using Griseofulvin, Cefuroxime and Itraconazole.

A. fumigatus was isolated from the fruits purchased from the markets. Itraconazole, even at the highest dilution of 1:200 v/v, was effective in preventing growth of *A. fumigatus* (Plate 6). Itraconazole drug have been used for the treatment of a wide range of fungal infection (Carlile *et al.,* 2005) and is among the triazole drugs (fluconazole, voriconazole) that have a broad-spectrum antifungal activity. What remains to be demonstrated is its suitability to control all the various *A. fumigatus* varieties that have been shown to be present in our environment which might play a role in pathogenesis in the field and in the storage as well as in the case of the use in treatment of aspergillosis in humans. Although azole antifungals are often more effective against *A. fumigatus*,

there are cases where resistance is not a factor (CDC, 2024; Mers *et al.,* 2016). The emergence of azole-resistant strains from among those screened here highlights the need to continue this novel surveillance reported in this thesis and possibly in order to develop new antifungal agents based on their efficacy against our local *A. fumigatus*, particularly in post-harvest deterioration, when this is pursued vigorously, this thesis would have been the springboard background work to have spurred on this search.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

Fungi are ubiquitous and have useful and harmful benefits they impact man, plants, and animals alike. Because they are found in the environment, you can come across them in the air, food, and agricultural produce worldwide causing about 30-40 % losses in agricultural produce, especially in Africa (Alegbeleye *et al*., 2022; Odamtten, 2018).

The studies underscore that our local fruits and vegetables are prone to infection and the genera *Aspergillus* and *Penicillium* play a major role in the post-harvest deterioration of harvested crops in Ghana (Odamtten, 2018). In this thesis, the mango fruit, oranges, and tomatoes (whole fruit and peel) obtained from two major markets in the Greater Accra Region (Mallam Atta and Madina), as well as dry cocoa beans from Breman Asikuma (Central Region), were variably contaminated with mycoflora with special reference to *Aspergillus fumigatus*. Mycoflora population varied from $\log_{10}$ 3.0 CFU/g- 3.34 $\log_{10}$ CFU/g in the fruit juice sample and was also on the exterior pericarp ($\log_{10}$ 1.0-1.4$\log_{10}$ CFU/g). The Breman Asikuma (Central Region) cocoa beans were also infected with *A. fumigatus* (6%).

The quantitative incidence of *A. fumigatus* at Mallam Atta and Madina Markets were 12.4 % and 7.3 % respectively. The microstructures of the conidia, conidiophore, vesicles of *A. fumigatus* strains encountered in the air and products had dimensional characteristics and colour that were distinguishable statistically ($P \leq 0.05$) and show variations. The Boxplot or Whisker plot and the Heatmaps derived from the measured data agree with conclusions of the physical micro-graphical measurements and therefore the *A. fumigatus* isolated could be variants of same species.

The Disease Progressive Curve and Area Under Disease Progress Curve obtained for mango, orange and tomato were sigmoid, implying a monocyclic disease caused by a pathogen in a simple interest disease. Visually, mango and tomato seem to be the worst affected presumable owing to the penetration mechanism of the post-infection process of the pathogen as compared to orange with formidable chemical barrier to penetration of the peel.

The DNA Technology employed to distinguish between the *A. fumigatus* variants isolated from mango, orange and tomato (PCR, Gel Electrophoresis and ITS Characterization) as well as the Evolutionary Tree showed that:

1. Lanes 29-40 with 550 bp were Group 1.
2. Lanes 41-43 with 650 bp were Group 2.
3. There was a close relationship between Group 1 and Group 2 with the reference *Aspergillus fumigatus* and could share a common ancestry.
4. D.COCOA sample, M MEC, M OJ, MM AIR, MM_TJ, MM_MJ, M_OEC, and MM_TEC all showed genetic divergence from the reference *A. fumigatus* (AF293_REF) with a bootstrap of 0.49-0.98.

This variability within the *A. fumigatus* variants suggests that the isolated variants within this group may have followed a distinct evolutionary path influenced by unique nutritional and environmental requirements and other selection pressures.

Among the three antifungal agents tested (Griseofulvin, Cefuroxime and Itraconazole), Itraconazole at 1:200v/v dilution was effective in preventing in-vitro growth of *A. fumigatus* attributed to the triazole broad-spectrum component activity on pathogenic fungi. The emergence of azole resistance strains of isolated *A. fumigatus* strains in Ghana underscores the need to extend the

search to *A. fumigatus* on other agricultural produce in other regions and to develop a potent anti-fungal agent effective against local strains of post-harvest *A. fumigatus* strains (variants).

5.2 RECOMMENDATIONS

1. The study should be extended nationwide to better understand the genetic diversity of *A. fumigatus* populations in Ghana and further investigate the varying growth characteristics of *A. fumigatus* on different fruits and environments.
2. In future studies, farmers and traders/vendors should be provided with training on improving post-harvest management practices e.g., by the implementation of programs such as hazard analysis and critical control point (HACCP) in places where the fruits and vegetables are produced, stored and sold to develop better handling practices for fruits, especially mangoes which showed higher vulnerability to fungal penetration.
3. Regular monitoring and surveillance should be done through routine assessments of fungal populations in markets to identify contamination hotspots and implement targeted interventions to ensure proper quality control measures at markets to reduce fungal contamination.
4. Investigate environmental factors contributing to higher fungal populations at specific markets, such as Mallam Atta Market and study the role of intrinsic fruit chemical environments in resisting fungal growth, particularly for oranges, which showed lower contamination levels due to their strong chemical barriers.
5. Conduct further research into exploring eco-friendly methods to prevent post-harvest losses caused by *Aspergillus fumigatus* infections of fruits and vegetables and investigate the resistance mechanisms of *A. fumigatus*.

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APPENDICES

Appendix 1. The fungal population isolated from fruits from Mallam Atta and Madina Market expressed in log_{10}cfu/g (Juice)

Samples	Location	
	Mallam Atta (log_{10}) CFU/g	Madina(log_{10}) CFU/g
Orange	3.08	3.01
Mango	3.07	3.34
Tomatoes	l3.21	3.07

Appendix 2. The fungal population isolated from fruits from Mallam Atta and Madina Markets and Breman Asikuma expressed in log_{10}cfu/g (Ectoderm/peel)

Samples	Location	
	Mallam Atta (log_{10}) CFU/g	Madina (log_{10}) CFU/g
Orange	1.42	1.38
Mango	1.27	1.52
Tomatoes	1.36	1.43
Airspora	1.51	1.43
	CR Breman Asikuma (log_{10}) CFU/g	
Dry Cocoa beans	1.42	0.99

Appendix 3. Table showing the percentage occurrence of *A. fumigatus* on selected fruits and vegetables from Mallam Atta and Madina markets and Breman Asikuma

Sample	Location	
	Mallam Atta (%)	Madina (%)
Orange Juice	22.5	6.5
Mango Juice	5.4	0
Tomato Juice	2	0
Orange Ecto	3.8	10.7
Mango Ecto	8.2	4.9
Tomato Ecto	2.8	0
Airspora	12.4	7.3
	CR- Breman Asikuma	
D.Cocoa beans	6	

Appendix 4. A table showing the daily radial growth of *A. fumigatus* on fruit samples

Fruits	Reps	Day 1(cm)	Day 2 (cm)	Day 3 (cm)	Day 4 (cm)	Day 5 (cm)
Orange	1	0	0.15	0.23	0.68	1.00
	2	0	0.20	0.25	0.73	1.05
	3	0	0.20	0.25	0.83	1.13
Mango	1	0	0.20	0.25	1.88	2.50
	2	0	0.23	0.45	2.98	4.76
	3	0	0.25	0.63	2.65	3.75
Tomato	1	0	0.63	0.85	2.33	2.85
	2	0	0.93	1.18	2.36	2.56
	3	0	0.95	1.10	2.43	2.68