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Fungal Diversity in Wet and Dry Ripe Plantains : Isolation, Identification, and Implications for Public Health

I. C. Adekanmbi*, A. P. Ogunsakin, P. O. Fabowale
Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria
Corresponding Author : I.C. Adekambi (adekanmbiiyabode@gmail.com)

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ABSTRACT

The persistent inadequacy of storage facilities in in globally and more prevalent in developing nations leads to substantial annual losses of harvested agricultural produce, severely impacting the economic stability of local farmers. This investigation focuses on the critical public health implications arising from the fungal contamination of stored plantain chips, a delicacy snack in Nigeria, West Africa. Notably, the fungal species isolated, which are Aspergillus flavus, Aspergillus niger, Penicillium chrysogenum, Saccharomyces cerevisiae, Aspergillus fumigatus, Fusarium spp., Rhizopus stolonifer, and Mucor spp. are critical from a public health perspective. Species such as Aspergillus flavus and Aspergillus niger are particularly concerning due to their capacity to produce aflatoxins, which are among the most carcinogenic substances known and have been linked to liver cancer and immune system suppression. The prevalence of these mycotoxins not only compromises the nutritional integrity and safety of the plantain chips but poses severe risks to consumers, highlighting a broader issue of food security and safety. This study brings to the fore the pressing need for interventions that enhance the storage conditions and decrease fungal contamination, thereby reducing the exposure of the population to mycotoxins. The implications of this research extend beyond the economic damage to encompass significant public health concerns, emphasizing the critical need for regulatory, technical, and infrastructural developments to uphold the microbial quality of plantain both in wet and dry states, ensuring safe consumption.

Keywords : Mycoflora, Plantain, Food Safety, Public Health, Aflatoxins, Fungal Contamination

I. INTRODUCTION

Plantains (*Musa parasidiaca*), a vital staple in the diet of millions, particularly in Africa, are fundamental for both sustenance and economic stability. These crops serve as a significant source of carbohydrates, proteins, and dietary fibers for millions, thus fulfilling essential nutritional requirements while also supporting agricultural livelihoods (Jonathan and Olowolafe, 2001; Akissoe et al., 2003). Plantains contribute not only to the caloric intake but also to the economic resilience of these regions by facilitating continuous agricultural activity and providing year-round food supplies (Onuoha et al., 2011). Despite their critical role, the post-harvest management of plantains is marred by significant challenges, primarily due to inadequate storage facilities that lead to substantial economic losses and expose the produce to microbial deterioration, notably fungal pathogens (Rashad et al., 2011).

The degradation of plantains during storage is predominantly attributed to contamination by fungi such as Aspergillus, Penicillium, and Fusarium species, which thrive under the humid storage conditions typical in these regions. These fungi not only undermine the nutritional quality and safety of the food but are also known for their production of aflatoxins—some of the most potent carcinogens known to affect human health, associated with severe risks such as liver cancer and immune suppression (Adebayo-Tayo et al., 2006). The frequent detection of these toxins in agricultural commodities underscores a critical public health concern that demands comprehensive study and effective intervention strategies (Jonathan and Olowolafe, 2001).

The primary aim of this study is to elucidate the fungal contamination dynamics in plantain storage facilities and assess their impact on the safety and quality of stored plantains. The research will focus on identifying and characterizing the fungal species predominantly impacting stored plantains, particularly their pathogenic and aflatoxigenic potentials (Deible and Swamson, 2001). Additionally, the study will quantify the levels of mycotoxins, especially aflatoxins, present in the storage environments and processed plantain products, thus assessing the health risks posed to consumers (Stinson et al., 2020). The effectiveness of current post-harvest storage and handling practices will be evaluated to determine whether they adequately control or inadvertently exacerbate fungal growth and toxin production (Fagbohun et al., 2010).

Furthermore, this investigation is intended to develop and propose enhanced storage solutions based on the findings, aimed at reducing fungal contamination and improving the safety, nutritional value, and shelf life of plantains (Akissoe et al., 2003). The study will provide significant contributions to the existing body of knowledge by offering data-driven insights into the microbial ecology of plantain storage. This will inform better agricultural practices and storage technologies, stimulating policy development to enhance food safety protocols in regions heavily reliant on plantains for food security and economic sustainability.

By addressing these critical issues, the research directly supports the health and economic well-being of millions in plantain-dependent regions. The anticipated improvements in handling and storage practices are expected to significantly reduce the incidence of fungal infections and mycotoxin contamination. Such advancements will ensure safer plantain consumption, stabilize and potentially increase the income of farmers by reducing crop losses, and enhance product marketability on both a local and global scale. The public health implications of reducing exposure to aflatoxins are profound, offering a clearer pathway to achieving broader goals

of food security and public health safety in the developing world.

II. MATERIALS AND METHOD

2.1 Plantain Sampling

Ripe plantains were sourced from the local Akungba-Akoko market in Ondo State, Nigeria. To minimize contamination, the plantains were transported to the Microbiology laboratory at Adekunle Ajasin University in clean polyethylene bags. Upon arrival, the fruits underwent a rigorous cleaning process where they were washed thoroughly under tap water to remove any adhering substances. The cleaned fruits were then peeled and sliced using a sterile knife, and the slices were placed in a clean tray for further processing according to the protocol of Amande et al. (2012).

2.2 Preparation of Media

The media required for fungal isolation were prepared according to the manufacturer's specifications and then sterilized. Sterilization was achieved by autoclaving the prepared media at 121°C for 15 minutes, ensuring the elimination of all potential microbial contaminants. All glassware used in the experiment, including Petri dishes, test tubes, McCartney bottles, conical flasks, and beakers, as well as metal apparatus like spatulas, were thoroughly washed with detergents. These were then rinsed with water, drained, and dried. Sterilization was performed in an oven set at 180°C for 2 hours. Additionally, 95% alcohol was used to swab the laboratory bench and spatulas to maintain a sterile working environment.

2.3 Isolation Procedures

2.3.1 Direct Plating Method

From the dried plantain chips, ten slices were randomly examined for external moldiness. These slices were surface sterilized with ethanol and subsequently washed with sterile distilled water to remove any residual ethanol. Using a sterile blade, the surface of each dried plantain slice was scraped, and the scrapings were aseptically plated on Potato Dextrose Agar (PDA) plates. The plates were then incubated at room temperature for 5-7 days, as per the method described by Amusa (2001). Subculturing was conducted to obtain pure fungal colonies, employing successive hyphae tip transfer techniques (Egbebi et al., 2007; Fagbohun and Lawal, 2011).

2.3.2 Dilution Plating Method

This method was utilized to identify the fungi present in both dried and wet stored plantain chips. Approximately one gram of each sample was sterilized with ethanol, then ground with 10 ml of sterile distilled water. The resultant mixture was serially diluted, and 1 ml aliquots of the 10^{-3} and 10^{-5} dilutions were plated onto molten PDA. After gentle swirling to ensure thorough mixing, the plates were allowed to solidify and were incubated at room temperature for 5-7 days. Fungal colonies were counted every 24 hours, and pure cultures were obtained through successive transfers of hyphae tips.

2.4 Identification of Mycoflora

The fungi isolated from the plantain chips were identified based on their cultural and morphological characteristics. Initial identification was carried out under natural light to assess the color and texture of the cultures. Further microscopic examinations were conducted using the Needle Mount Preparation Method: small fragments from the sporing surface of cultures were teased out in a drop of alcohol on a sterilized glass slide using a botany needle, stained with Lactophenol blue, covered with a cover slip, and examined under a microscope using x10 and x40 objective lenses (Tuite, 1961; Crowley et al., 1969; Egbebi et al., 2007).

2.5 Characterization of Fungal Isolates

Following a 72-hour incubation period, the cultures on the potato dextrose plates were observed for growth. The identification and characterization of the

different types of fungi encountered were performed based on cultural characteristics observed on the growth medium and detailed microscopic studies. Each type of fungi was prepared for microscopy by placing a small sample on a glass slide, adding a drop of Lactophenol cotton blue, and spreading it thinly with inoculating needles. The prepared slides were then examined under the microscope to determine the specific morphological features of each fungal type.

III.RESULTS

The study effectively cataloged the diversity of fungal species present in plantain samples subjected to different drying methods, highlighting the influence of drying techniques on fungal colonization and growth. The fungi isolated varied depending on the drying process applied, indicating the impact of each method on fungal prevalence and diversity.

3.1 Fungi identified in the Wet Plantain

The fungi isolated from samples that remained wet are detailed in Table 1, which includes *Aspergillus flavus, Fusarium spp., Aspergillus niger, Mucor spp., and Penicillium chrysogenum*. This selection reflects the types of fungi that thrive in moist conditions, potentially leading to rapid deterioration and significant impact on the nutritional quality of the plantains.

Table 1 : Fungi isolated from wet plantain

Cultural Characteristics	Microscopic observation	Suspected organisms
Army green mycelia growth was seen in colonies on the growth medium.	Conidiosphores upright, bearing phialides at the apex.	*Aspergillus flavus*
White and fluffy mycelia growth spread through the entire growth medium.	Conidiosphores richly branched, bearing phialides which proliferate.	*Fusarium spp.*
Brownish mycelia spread through the plate; it is fast growing.	Conidiosphores arising from long, broad thick smooth wall and mostly brownish.	*Aspergillus niger*
Greyish mucoured on plate.	Round sporangia	*Mucor spp.*
Green on plate.	Conidiosphores richly branched, bearing phialides which proliferate.long, broad thick smooth wall and mostly brownish	*Penicillium chrysogenum*

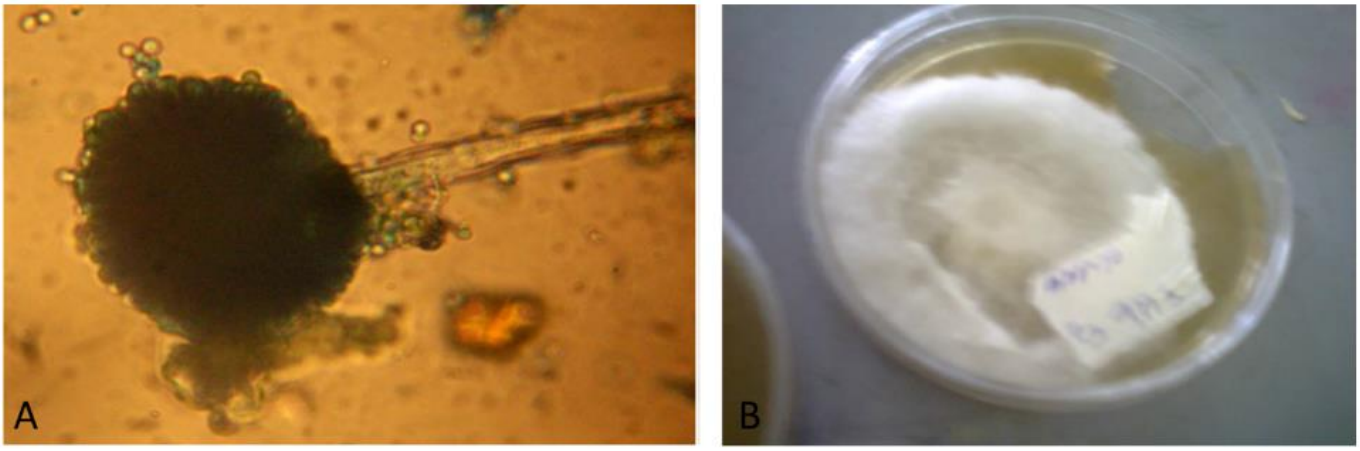

Figure 1. *Fusarium spp.* isolated from the wet plantain. **A.** Microscopic view at a magnification of x400. **B.** Macroscopic view in a petri-dish

3.2 Fungi identified in the Oven-dried Plantain

Plantain samples subjected to oven drying hosted a different fungal profile, as outlined in Table 2. This profile includes Aspergillus fumigatus, Aspergillus niger, Rhizopus stolonifer, Saccharomyces cerevisae, and Fusarium spp. The elevated temperatures used in oven drying may select for fungi capable of withstanding heat, possibly affecting the safety and shelf life of the dried product.

Table 2: Fungi isolated from oven dried plantain

Cultural Characteristics	Microscopic observation	Suspected organisms
Grey-Brown colour. Colonies are fast growing.	Unbranched sporangiosphores with rhizoides	Rhizopus stolonifer
Powdery, white at first then turned greenish.	Cover entire vesicle, form “radiate” head.	Aspergillus fumigatus
Creaming with lots of tiny colonies.	Hyphal growth is not extensive.	Saccharomyces cerevisae
White and fluffy.	Conidiosphores richly branched, bearing phialides which proliferate.	Fusarium spp.
Brownish mycelia spread throughout the plate	Conidiosphores arising from long, broad thick smooth wall and mostly brownish.	Aspergillus niger

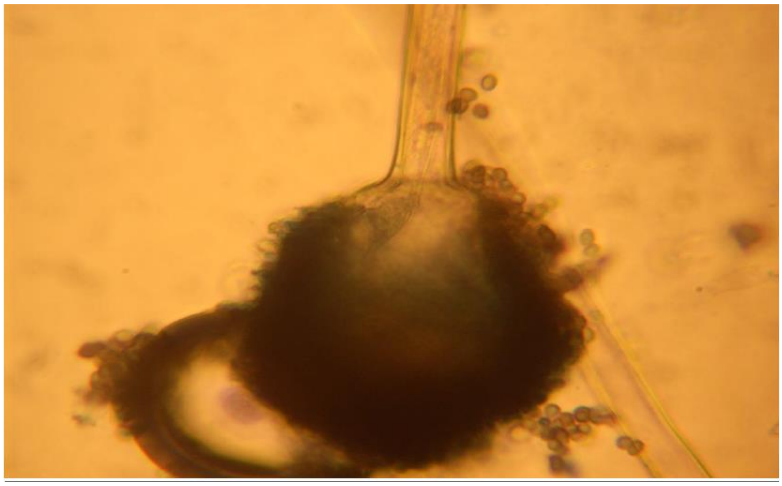

Figure 2 : Microscopic view of Aspergillus niger isolated from the oven-dried plantain, at magnification of x400.

3.3 Fungi identified in the Sun-dried Plantain

Sun-drying resulted in yet another distinct set of fungi, listed in Table 3. The species identified were Penicillium chrysogenum, Aspergillus niger, Saccharomyces cerevisae, Rhizopus stolonifer, and Aspergillus flavus. Sun-drying, which involves exposure to environmental conditions, may allow for a broader range of fungal species due to varying temperatures and humidity levels throughout the drying process.

Table 3 : Fungal isolated from sundried plantain chips

Cultural Characteristics	Microscopic observation	Suspected organisms
Started growing by appearing creamy in tiny colonies, turned yellow-green in 3 days.	Conidiosphores upright, bearing phialides at the apex.	Aspergillus flavus
Grey-Brown with black pigment, colonies are very fast growing.	Sporangiophores with rhizoides, and connected by a stolom	Rhizopus stolonifer
Creaming with lots of tiny colonies.	Hyphal growth is not extensive.	Saccharomyces cerevisae
Deep green	Conidiosphores richly branched, bearing phialides which proliferate.long, broad thick smooth wall and mostly brownish	Penicillium chrysogenum
Brownish mycelia spread throughout the plate	Conidiosphores arising from long, broad thick smooth wall and mostly brownish.	Aspergillus niger

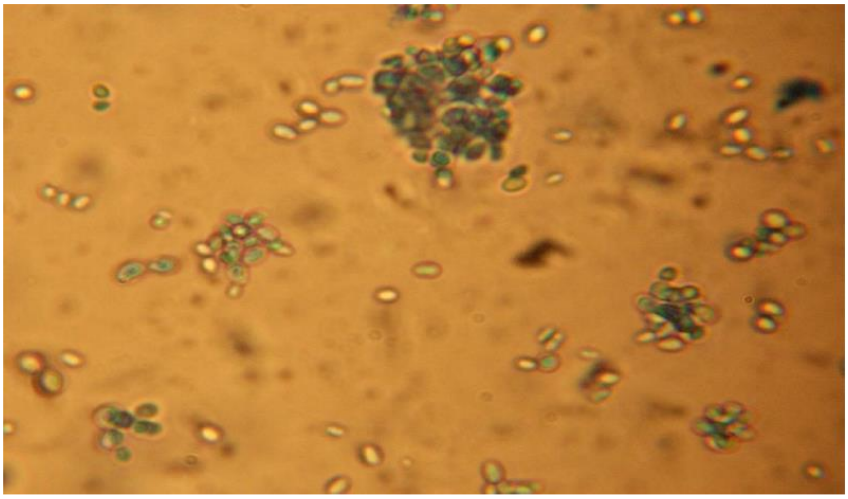

Figure 3 : Microscopic view of Saccharomyces cerevisae isolated from the sun-dried plantain, at magnification of x400.

IV. DISCUSSION

In the comprehensive mycological analysis of plantain samples, both wet and dry, a total of eight fungal species were isolated, each identified by their unique cultural and morphological traits. The fungal spectrum encompassed *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Mucor spp., Penicillium chrysogenum, Fusarium spp., Rhizopus stolonifer,* and *Saccharomyces cerevisae*. These isolates, stratified across various sample types, are represented in Tables 1, 2, and 3, which delineate the mycological landscape of plantain conservation.

The moist environment of wet plantains proved conducive to the proliferation of *Aspergillus niger, Aspergillus flavus, Fusarium spp., Mucor spp.,* and *Penicillium chrysogenum*. These species are notorious for their surface colonization of agricultural products, leading to decay—a phenomenon reinforced by their ability to penetrate the food matrix deeply, as noted by Amusa et al. (2002). The prevalence of such fungal species raises the specter of not only post-harvest losses but also the alarming potential for mycotoxin production within consumer goods.

Upon examining dried plantain samples, a correlative increase in fungal diversity was noted, echoing the findings of Fagbohun et al. (2009). The aerobiological origin of these fungi, predominantly airborne contaminants, underscores the vulnerability of dried plantains to microbial infestation during storage. Particularly, *Aspergillus flavus* poses a significant concern, being a common environmental mold known to afflict stored grains with spoilage. Its pathogenicity is not restricted to plant degradation; it also manifests as aspergillosis in the human respiratory system, causing infections in corneal, otomycotic, and nasoorbital areas. Strains of this species can produce aflatoxin, a toxin with both carcinogenic and acutely toxic properties. The ubiquity of *Aspergillus flavus* is attributed to its prolific spore production and resultant dispersal via air currents, thriving optimally within a water activity range of 0.86 to 0.96, as reported by Vujanovic et al. (2001).

Aspergillus niger, another species identified, is notorious for causing 'black mold' on fruits and vegetables and is a frequent food contaminant. Its presence in soil and environments indoors often leads to mistaken identification due to visual similarity with stachybotrys species, also known as 'black mold' The impact of Penicillium species is not to be underestimated. Infections caused by these fungi can lead to a range of conditions including keratitis, pneumonia, and various systemic infections, demonstrating an alarming versatility in afflicting

human health (Lueg et al., 1996; Mitchell et al., 1996; Kontogiorgi et al., 2007). The ability of Rhizopus stolonifer to cause rhinocerebral mucormycosis among other severe infections marks it as a pathogen of particular concern. Its capacity for vascular invasion often results in tissue necrosis and, subsequently, high mortality rates. Prevention of Rhizopus infections hinges on minimizing contact with contaminated matter and maintaining rigorous hygiene practices (Welsh and Kaplan, 1998).

The breadth of fungal species isolated in this study is a clear indicator of the substantial mycological risk present in plantain storage, which can directly affect both the shelf life of the product and the health of consumers. The implications of these findings demand urgent attention to storage protocols and conditions, a reassessment of the current practices, and a rigorous scientific inquiry into innovative methodologies to curb the prevalence of these fungal contaminants.

V. CONCLUSION AND RECOMMENDATION

Given the substantial role that plantains play in the sustenance of populations and the stimulation of economies, especially in tropical regions, the need for rigorous control of fungal proliferation cannot be overstated. This study has uncovered not only the ubiquity of fungal pathogens in various storage conditions but also the consequential risk posed by the metabolites of these organisms, such as aflatoxins, fumonisins, and ergot alkaloids, which are particularly hazardous to immunocompromised individuals.

In conclusion, it is imperative that the preservation of plantains receives dedicated attention, aligning with international standards for food safety. The establishment of optimal storage conditions, incorporating controlled humidity and temperature, alongside regular mycological assessment, should be a priority to forestall the onset and spread of fungal contamination. Moreover, implementing good manufacturing practices (GMP) in the handling and processing of plantains is crucial to minimize the risk of contamination. To this end, advancing storage technology and refining food processing protocols are essential.

Further recommendations include the adoption of integrated pest management practices to reduce the incidence of fungal infestation from field to storage, the utilization of antifungal treatments that are safe for both consumers and the environment, and the development of plantain varieties with improved resistance to fungal pathogens through agricultural research initiatives. Regular training and education programs for farmers and food processors on the significance of fungal risks and effective contamination mitigation strategies would also be beneficial. Ultimately, enhancing the quality and safety of plantains necessitates a concerted effort from multiple stakeholders, including farmers, food scientists, health professionals, and policymakers. By implementing these strategic recommendations, we can secure the integrity of plantains as a food source and uphold their economic potential in the global market.

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