

Molecular Detection of Aflatoxins-producing Fungi in Maize Sold in Gusau Major Markets. Implications for Food Safety and Public Health.

*Nusaiba Ibrahim Danmaliki, Ahmad Hamza Balarabe

Department of Biochemistry, Federal University Gusau, PMB 2109, Nigeria.

*Corresponding Author's Email: danmalikinusaiba@gmail.com

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ABSTRACT

A staple crop that is vital to maintaining food security, maize is consumed by millions of people worldwide. For this study, three samples of maize were gathered from important markets in the Gusau city of Tudun Wada, Sabon Gari, and Damba. The study's objective was to detect fungal contamination in maize samples acquired from specific vendors using the polymerase chain reaction (PCR) technique. Following the extraction of genomic DNA from cultured fungal isolates, PCR amplification and molecular analysis were carried out. Following amplification using universal primers and agarose gel electrophoresis, the internal transcribed spacer (ITS) gene was detected in all samples, yielding amplicons of roughly 700 base pairs (bp). The findings show that the examined maize samples had fungal contamination. These results show that PCR is a very specific and sensitive technique for identifying fungal pathogens in food samples.

Keywords: Gusau, ITS gene, fungus, universal

1.0 INTRODUCTION

1.1 Background Study

One of the most significant cereal crops in the world, maize (*Zea mays* L.) is the most produced cereal worldwide (Erenstein et al., 2022). The most popular cereal in Africa and a significant staple food, especially in sub-Saharan Africa, is maize (Benjamin et al., 2024). While mature grains are processed into flour, meal, porridge, and other food products, maize is consumed in a variety of ways in West Africa, such as roasted or boiled cobs (Benjamin et al., 2024).

However, fungal contamination can affect maize's quality, safety, and economic value during cultivation, harvest, processing, and storage, just like it can affect many other crops (Balan et al., 2024; Xue et al., 2025). Although maize (*Zea mays* L.) is a significant staple crop and a crucial part of the world's food security, its vulnerability to toxic fungi can lead to mycotoxin contamination along the entire food chain (Balan et al., 2024; Niaz et al., 2025). In addition to posing serious risks to public health and food safety, the presence of mycotoxins in maize can cause financial losses due to lower commodity quality and trade restrictions (Stoev, 2024; Niaz et al., 2025).

Fungi comprise a diverse group of microorganisms that play essential roles in ecosystems; however, some fungal species can cause plant diseases and contaminate agricultural commodities. In maize, toxigenic fungi, particularly species of *Aspergillus* and *Fusarium*, are important concerns because they can produce mycotoxins, which are toxic secondary metabolites that compromise food safety and quality (Awono Antoine Thierry et al., 2025; Xue et al., 2025). Among these mycotoxins, aflatoxins are of particular concern because of their high toxicity and

carcinogenicity. Aflatoxins are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* and can contaminate maize and other staple crops (Gemede, 2025; Xue et al., 2025).

Maize is an important dietary source of human exposure to aflatoxins because of its widespread consumption and susceptibility to contamination by aflatoxin-producing fungi. Human exposure occurs primarily through consumption of contaminated foods, particularly maize and other staple crops in regions where aflatoxin contamination is prevalent. Aflatoxins are highly toxic mycotoxins, and chronic dietary exposure, particularly to aflatoxin B₁, is strongly associated with hepatocellular carcinoma. Aflatoxin exposure has also been associated with adverse effects on immune function and other health outcomes, particularly in populations experiencing nutritional vulnerability (World Health Organization [WHO], 2023; Joint FAO/WHO Expert Committee on Food Additives [JECFA], 2017). Consequently, aflatoxin contamination of maize remains an important food-safety and public-health concern.

Given these concerns, rapid, sensitive, and reliable methods for detecting fungal contamination in maize are essential for accurate assessment of food safety. Molecular techniques, particularly polymerase chain reaction (PCR), provide sensitive and specific approaches for the detection and identification of fungal species based on their DNA. Compared with conventional culture-based approaches, PCR-based methods can facilitate more rapid and specific identification of target fungi. Therefore, this study aimed to detect and molecularly identify fungal contaminants in maize samples obtained from selected vendors using PCR-based analysis.

Traditional methods for the detection of fungi, including culture-based and morphological identification techniques, have long been employed in the analysis of maize and other food commodities. Although these approaches have proven useful, particularly in staple crops consumed by millions of people globally, they often lack the sensitivity required to detect low levels of fungal contamination. This limitation may result in false-negative outcomes and the unintended distribution of contaminated products. Furthermore, conventional detection methods are frequently time-consuming, labor-intensive, and costly, which limits their routine application in large-scale food safety monitoring programs (Leslie *et al.*, 2006).

In contrast, molecular detection methods offer significant advantages in overcoming these challenges. By targeting specific genetic markers within fungal species, molecular techniques such as polymerase chain reaction (PCR) enable rapid, highly sensitive, and specific identification of aflatoxin-producing fungi (Garcia *et al.*, 2022). These methods enhance early detection, thereby reducing the risk of contaminated maize entering the food chain.

To safeguard public health, regulatory agencies worldwide have established strict maximum permissible limits for aflatoxins in food and feed products. The United States Food and Drug Administration (FDA) has set a maximum limit of 20 parts per billion (ppb), while the European Food Safety Authority (EFSA) enforces a more stringent limit of 4 ppb for total aflatoxins in food products. The World Health Organization (WHO) recommends regulatory limits generally ranging from 4 to 15 ppb, depending on the commodity and level of exposure (FDA *et al.*, 2021). These regulatory standards aim to minimize health risks associated with aflatoxin exposure and ensure food safety compliance in both domestic and international markets.

2.0 MATERIALS AND METHODS

2.1 SAMPLE COLLECTION

Three maize samples were collected from major selling points in Tudun Wada, Sabon Gari, and Damba within the Gusau metropolis. The samples were carefully packed in sterile containers and transported to the laboratory for further analysis.

2.2 DNA Extraction

A QIAamp DNA extraction kit (Qiagen) was used to extract DNA in accordance with the manufacturer's instructions; the sample volume and processing parameters are detailed below. All samples and reagents were brought to room temperature prior to extraction. In short, a sterile 1.5-mL nuclease-free microcentrifuge tube was filled with 200 μ L of spore

suspension and 400 μ L of Buffer AVL. After 15 seconds of vortexing, the mixture was allowed to sit at room temperature for ten minutes. Following a brief centrifugation of the tubes, 400 μ L of absolute ethanol was added, and the mixture was vortexed for 15 seconds. After that, the tubes underwent a quick centrifugation.

After that, the mixture was carefully moved to a 2-mL collection tube containing a QIAamp Mini spin column. The flow-through was disposed of after the column was centrifuged for one minute at 8,000 rpm. After moving the QIAamp Mini column to a sterile 2-mL collection tube, 500 μ L of Buffer AW1 was added. After centrifuging the column for one minute at 8,000 rpm, the flow-through was disposed of. After adding 500 μ L of Buffer AW2, the column was centrifuged for three minutes at 14,000 rpm. After discarding the flow-through, the column was centrifuged once more for three minutes without the addition of any reagent in order to eliminate any remaining wash buffer.

Next, a sterile 1.5-mL nuclease-free microcentrifuge tube was filled with the QIAamp Mini column. To elute the DNA, 30 μ L of room-temperature-equilibrated Buffer AVE was carefully added to the center of the silica membrane. After five minutes of room temperature incubation, the column was centrifuged for one minute at 8,000 rpm. After discarding the spin column, the extracted DNA-containing tube was sealed and kept at -20°C until PCR amplification was needed.

2.3 PCR Amplification

PCR amplification of the fungal internal transcribed spacer (ITS) region was performed in a final reaction volume of 25 μ L. Each PCR reaction contained 12.5 μ L of Transgenic Master Mix, 6.7 μ L of PCR-grade water, 0.4 μ L of the forward primer ITS1F, 0.4 μ L of the reverse primer ITS4, and 5.0 μ L of template DNA.

The PCR reactions were performed using a Bio-Rad MyCycler thermal cycler. The amplification programme consisted of an initial denaturation at 94°C for 1 min, followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 51°C for 1 min, and extension at 72°C for 1 min. A final extension was performed at 72°C for 8 min, followed by a hold at 10°C until the samples were removed from the thermal cycler.

2.4 Agarose Gel Electrophoresis

Agarose gel electrophoresis of the amplified PCR products was performed using a submarine gel electrophoresis system following the method described by Sambrook and Russell (2001), with minor modifications. A 1.5% (w/v) agarose gel was prepared by dissolving 0.75 g of agarose in 50 mL of 1× Tris-borate-EDTA (TBE) buffer. The mixture was heated until the agarose was completely dissolved and a clear

solution was obtained. The solution was allowed to cool to approximately 50°C, after which 10 µL of ethidium bromide (0.5 µg/mL) was added and mixed thoroughly. The molten agarose was then poured into a gel-casting tray fitted with a comb and allowed to solidify for approximately 30 min.

Following gel solidification, the comb was carefully removed and the gel was placed in the electrophoresis tank containing 1× TBE buffer. For electrophoresis, 8 μ L of each PCR product was mixed with 2 μ L of 6×

DNA loading dye (New England Biolabs, UK) and loaded into the wells. A 100-bp DNA molecular-weight ladder (New England Biolabs, UK) was loaded into the first well to estimate the sizes of the amplified DNA fragments. Electrophoresis was carried out at 70 V for 60 min, with DNA migration monitored using the tracking dye. Following electrophoresis, the gel was visualized under ultraviolet (UV) illumination using a Bio-Rad gel documentation system, and the resulting banding patterns were recorded.

3.0 RESULTS

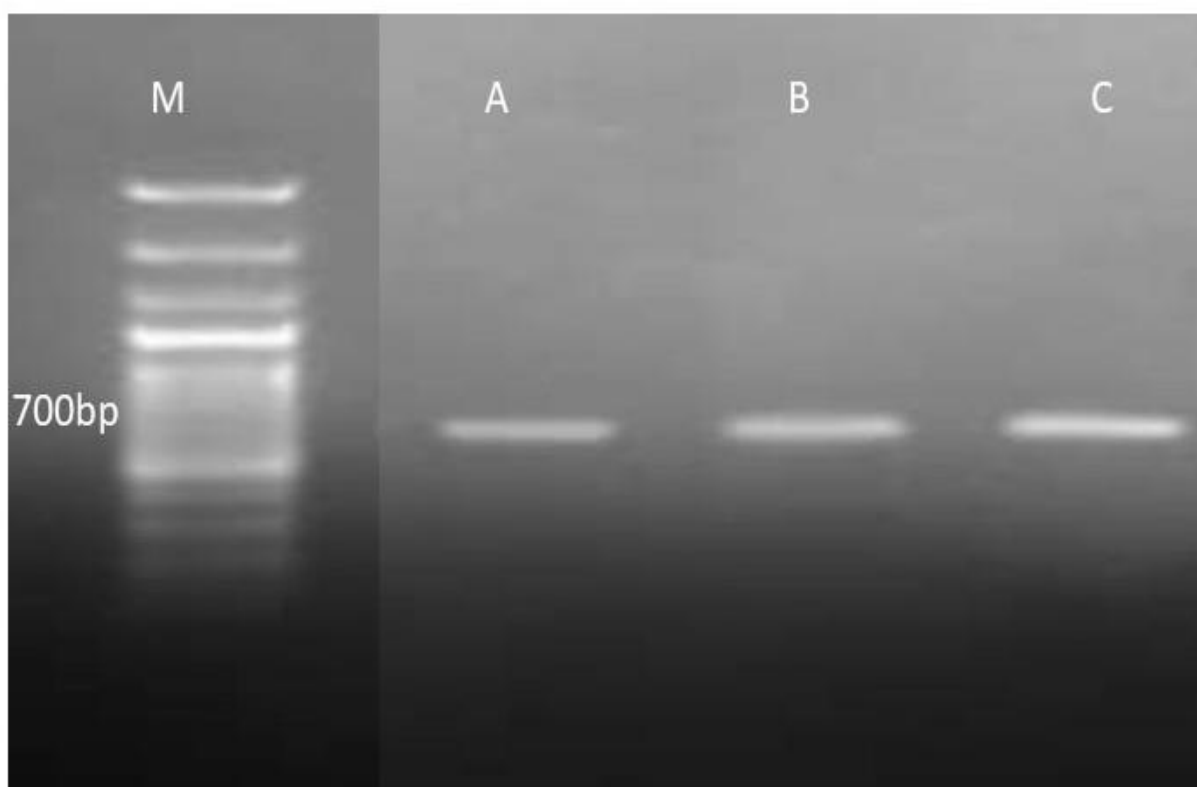


Fig. 1: The result of gel electrophoresis showing positive detection of ITS gene. Well A-C showed positive bands of ITS gene representing samples analyzed in this study. Lane M = 100bp ladder,

4.0: DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1 Discussion

Fungi can produce mycotoxins, which could be hazardous to consumers' health, in addition to lowering the quality and shelf life of maize. These results emphasize the need for proper post-harvest handling, storage, and preservation practices to lower fungal contamination and safeguard public health. Additionally, because PCR is a sensitive and specific tool for quick fungal detection, the study demonstrates that it is a useful method for routine monitoring and quality control in maize and other staple food commodities. Future research should focus on

identifying specific fungal species and measuring mycotoxin levels in order to better assess the risk to consumers and direct regulatory strategies.

This study's findings about fungal contamination in maize are in line with earlier findings (Renu et al., 2018; Agwanande et al., 2016). These results highlight how crucial it is to minimize fungal contamination by using appropriate preservation methods and maintaining suitable storage and transportation conditions. Effective handling and preservation techniques can improve the safety of maize meant for human consumption and drastically lower the risk of fungal food poisoning.

4.2 Conclusion

In conclusion, the successful molecular detection of the universal fungal ITS gene using PCR indicates contamination across maize obtained from different vendor locations within the Gusau metropolis, confirming the presence of fungal species in all analyzed maize samples. This discovery emphasizes how vulnerable maize is to fungal contamination, especially in situations with high moisture content, poor storage, and unfavorable environmental elements like high humidity and temperature.

In addition to reducing maize's quality and shelf life, fungi can produce mycotoxins, which could be harmful to consumers' health. These findings highlight the necessity of appropriate handling, storage, and preservation procedures after harvest in order to reduce fungal contamination and protect public health. Additionally, the study shows that PCR is a useful method for routine monitoring and quality control in maize and other staple food commodities because it is a sensitive and specific tool for fast fungal detection. To better evaluate the risk to consumers and guide regulatory strategies, future research should concentrate on identifying particular fungal species and measuring mycotoxin levels.

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