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Research Article

STUDIES ON THE AFLATOXIGENIC AND AFLATOXIN PROFILE OF *ADANSONIA DIGITATA* LEAVES DRIED UNDER DIFFERENT TEMPERATURES

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ABSTRACT

Objectives: Isolation, identification, proximate analysis, and mineral composition profiling of aflatoxigenic fungi and aflatoxin B1 associated with dried baobab leaves (Kuka) (*Adansonia digitata*) dried at different temperatures Marketed in Sokoto metropolis. Nigeria.

Methods: The fungi associated with the oven-dried Kuka were analyzed using standard mycological methods. The total aflatoxin levels were determined using an enzyme-linked immunosorbent assay method. The proximate compositions of oven-dried *A. digitata* leaves dried at temperatures of 180°C, 220°C, 250°C, and 270°C were determined using the AOAC method. The sodium, phosphorus, and potassium levels were determined using a flame photometer, and calcium and magnesium through the ethylenediaminetetraacetic acid titration method.

Results: *A. digitata* leaves dried at 270°C had the highest ash, crude lipid, crude fiber, and crude proteins with values 12.5, 5.5, 18.0, and 15.84, respectively. Similarly, *A. digitata* leaves dried at 180°C also had a high carbohydrate content of 54.70. From the results, *A. digitata* leaves dried at 220°C had high sodium and magnesium values of 12.5 and 23.40, respectively. *A. digitata* leaves dried at 250°C had high sodium, potassium, and phosphorus values of 12.5, 900, and 6.8, respectively, while *A. digitata* leaves dried at 270°C had a high calcium value of 19.20. The fungal species identified in oven-dried *A. digitata* leaves included *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, and *Rhizopus stolonifera*. Among these, *A. niger* showed the highest percentage occurrence at 40% (4 out of 10), while *R. stolonifera* had the lowest occurrence at 10% (1 out of 10).

Conclusion: In this research, Kuka sourced from home recorded the highest total aflatoxin concentration at 40 ppb, whereas the leaves dried at 250°C exhibited the lowest concentration at 20 ppb. The findings suggest that drying temperature may influence the overall aflatoxin profile; however, it does not effectively eliminate aflatoxigenic fungi, highlighting the need for improved storage conditions.

Keywords: Kuka, Aflatoxins, Aflatoxigenic fungi, proximate analysis, Mycotoxins.

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INTRODUCTION

Mycotoxins are toxic chemical compounds produced by certain molds (fungi) that grow on food and crops, particularly under warm and humid conditions [1,2]. They can contaminate grains, nuts, spices, dried fruits, coffee, and other food products during production, storage, or transport. Mycotoxins pose a significant health risk to humans and animals if ingested, inhaled, or absorbed through the skin.

The most common genera of mycotoxigenic fungi include *Aspergillus* (*Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus ochraceus*), *Fusarium* (*Fusarium graminearum*, *Fusarium verticillioides*), *Penicillium* (*Penicillium verrucosum*, *Penicillium expansum*), *Alternaria* (*Alternaria alternate*), *Claviceps* (*Claviceps purpurea*), *Stachybotrys* (*Stachybotrys chartarum*), and *Monascus* (*Monascus purpureus*) [1,3].

Each of these genera includes species that thrive under specific environmental conditions, such as high humidity and warm temperatures, which must be controlled to minimize contamination in food and feed products.

Mycotoxins are toxic compounds that cause mycotoxicoses in humans and animals. Many mycotoxins exhibit toxicity toward vertebrates and other animal groups. Even at low concentrations, some mycotoxins can trigger autoimmune illnesses and exhibit allergenic properties, while others are teratogenic, carcinogenic, and mutagenic [3,4].

Aflatoxins (AFTs), a prominent group of mycotoxins, pose a significant threat to human health. These carcinogenic compounds, especially when combined with the Hepatitis B virus, are responsible for thousands of deaths annually in tropical countries [2,4]. AFTs are secondary metabolites, classified as difuranocoumarins, produced by *A. flavus* and *A. parasiticus.* These fungi frequently contaminate food and feed, leading to aflatoxicosis in livestock, domestic animals, and humans worldwide.

Humans are exposed to AFTs through the consumption of contaminated food. This can result in nutritional deficiencies, immunity suppression, and liver cancer [5]. AFT contamination has been reported in a variety of food items, including spices, cereals, oils, fruits, vegetables, dairy, meat, and other raw food products. Of the different types of AFTs, the major ones include aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), and the milk metabolites AFM1 and AFM2, predominantly produced by *A. flavus* and/or *A. parasiticus*. *A. flavus* strains span the non-toxic to highly-toxic, with a higher likelihood of producing AFB1 instead of AFG1 [6].

AFB1, the most cytotoxic and potent carcinogen among AFTs, has been implicated in hepatocellular carcinoma across various animal species [7]. Other AFTs generally exhibit significant toxicity only at higher concentrations. AFT-related deaths have been documented in countries such as India, Kenya, and Malaysia [8,9]. Moreover, reports on the natural occurrence of mycotoxins in vegetables have emerged from several regions globally [10].

Vegetables, commonly consumed parts of plants, are an integral part of the human diet. In Africa, traditional vegetables serve as

affordable sources of proteins, vitamins, minerals, amino acids, and antioxidants [11]. These vegetables are utilized for both dietary and medicinal purposes and are consumed fresh or dried. Drying, a common practice in Nigeria, enhances durability and availability across seasons. However, susceptibility to fungal infestation and contamination during drying, storage, handling, transportation, and marketing is prevalent [11]. Contaminated vegetables are often found to harbor AFTs 7.

Adansonia digitata, commonly known as Baobab, is referred to as "Kuka" in the Hausa language. Baobab leaves are rich in antioxidants (particularly Vitamin C) and are excellent sources of potassium, calcium, magnesium, protein, and fiber. The fiber in these leaves may help mitigate aflatoxicosis by maintaining a healthy gut microbiome. Baobab trees are abundant in northern Nigeria, home to approximately 61.4 million people, predominantly Hausa and Fulani (NPC, 2018). The leaves are conventionally dried and ground into a powder used to prepare a popular soup called "Miyankuka," consumed daily in most households.

Research indicates that dried Baobab leaves infested with fungi contain high levels of mycotoxins, with aflatoxin contents ranging from 18.17 to 185.5 ppb – values exceeding the maximum acceptable limits for food, especially those intended for children. Further investigation into the aflatoxigenic fungi and AFB1 profiles of dried Baobab leaves in the Sokoto metropolis is crucial, as AFB1 is among the most carcinogenic AFTs. Regulatory guidelines mandate that infant food should be entirely free of this compound.

METHODS

Sample collection

Samples of the Kuka powder were obtained from six major sellers in one location within Usmanu Danfodiyo University, Sokoto, into six clean polythene bags and thereafter transferred to the mycology research laboratory for follow-on analysis.

Media preparation

Sabouraud dextrose agar (SDA)

SDA, a selective growth medium, is primarily used for the isolation and cultivation of both non-pathogenic and pathogenic fungal species, including yeasts. It |contains dextrose (glucose, 40 g), peptone (10 g), and agar (15 g). To prepare the medium, 6.5 g of SDA was dissolved in 150 mL of deionized water, following the manufacturer's instructions. The mixture was then heated until fully dissolved and was sterilized by autoclaving at 121°C for 15 min. Aflatoxicosis streptomycin was subsequently added to prevent bacterial growth [12].

Neutral red desiccated coconut agar (NRDCA)

NRDCA is a specialized growth medium for detecting and isolating aflatoxigenic fungi, particularly *Aspergillus* species such as *A. flavus* and *A. parasiticus*. And also for detecting aflatoxin, which is composed of desiccated coconut, which serves as a carbon and nutrient source, and neutral red, a pH indicator that changes color based on the pH of the medium.

The neutral red dye allows for a visual indication of fungal growth. In this medium, aflatoxigenic fungi are often characterized by a specific color change or colony morphology. The desiccated coconut component provides a natural substrate that mimics the conditions under which these fungi grow in nature, making it particularly useful for both cultivating and studying fungi that produce AFTs.

The medium is commonly used in research to directly observe the presence of aflatoxigenic species, as it allows for easy differentiation based on colony morphology and the presence of pigmentations indicative of aflatoxin production.

To prepare this medium, 200 g of desiccated coconut were soaked in hot deionized water for 30 min, then blended aseptically using a blender and filtered. To the filtrate, 15 g of plain agar and 0.2% neutral red were added. The medium was sterilized at 121°C for 15 min [12] (desiccated coconut agar [DCA] serves as a medium for detecting aflatoxigenic fungi and for the direct visual determination of AFTs).

Isolation and culture conditions

The isolates were cultured on SDA at 25°C for 7 days and stored as spore suspensions in 20% glycerol before further analysis [12,13].

Culture of aflatoxigenic fungi

A serial dilution was performed, and 1.0 mL from each dilution was inoculated onto SDA using the spread method. The plates were incubated at 25°C for 5 days, and the fungal colonies were counted. Observed colonies were then subcultured to obtain pure cultures [13].

Isolation of aflatoxigenic fungi

Morphological and growth analyses were conducted on SDA, while physiological analysis was carried out on DCA and A. *flavus/parasiticus* agar [14].

Identification of fungi

The color, colony morphology, diffusible pigmentation, growth rate, and texture of each sample were examined visually. A tease mount with lactophenol cotton blue was prepared, and the microscopic characteristics, such as spore and hyphal morphology, were observed and compared to the standard color atlas as outlined by Frisvad *et al*. [15].

RESULTS

The proximate compositions of *A. digitata* leaves dried under different oven temperatures of 180°C, 220°C, 250°C and 270°C were determined (Table 1). From the results, *A. digitata* leaves dried at 270°C had the highest ash, crude lipid, crude fiber and crude protein with values of 12.5, 5.5, 18.0, and 15.84, respectively. Similarly, *A. digitata* leaves dried at 180°C had a high carbohydrate content of 54.70, as indicated in Table 2.

The mineral compositions of *A. digitata* leaves dried under different oven temperatures of 180°C, 220°C, 250°C and 270°C were determined (Table 3). From the results, *A. digitata* leaves dried at 220°C had high sodium and magnesium values of 12.5 and 23.40, respectively. *A. digitata* leaves dried at 250°C had high sodium, potassium, and phosphorus values of 12.5, 900, and 6.8, respectively, while *A. digitata* leaves dried at 270°C had a high calcium value of 19.20.

The fungi profiles of *A. digitata* leaves dried at different oven temperatures were evaluated and fungi were identified as *Aspergillus niger, Aspergillus fumigatus, A. flavus,* and *Rhizopus stolonifera* (Table 2). Table 4 shows that *A. niger* had the highest percentage occurrence of 4 (40%) while *R. stolonifera* had the least percentage occurrence of 1 (10%).

Table 5 shows that *A. fumigatus* had the highest percentage of aflatoxin level of 25 ppb while *R. stolonifera* had the least value of 5 ppb.

Table 1: Proximate analysis of fresh and oven-dried *Adansonia digitata* leaves

Proximate composition	Fresh	Oven dried *Adansonia digitata* leaves			
		180°C	220°C	250°C	270°C
Moisture	68.0	-	-	-	-
Ash	2.50	11.0	11.5	11.0	12.5
Crude lipid	1.50	4.5	4.5	5.5	5.5
Crude fibre	1.00	16.5	17.0	17.0	18.0
Crude protein	6.48	13.3	14.09	15.66	15.84
Carbohydrate	20.52	54.70	52.91	50.91	48.16

Table 2: Morphological and microscopy identification of fungi isolated from oven-dried *Adansonia digitata* leaves

Organisms	Colony description	Microscopy
Aspergillus niger	It is a black, velvety, or powdery mass	The conidiophore terminates in swollen vesicles, and the conidia are in chains
Aspergillus flavus	It is yellow-green in color and powdery	Smooth branched conidiophores ending in vesicles.
Aspergillus fumigatus	Powdery and bluish-green in color and powdery.	Septate hyphae with even, short-walled conidiophore, conidiophore intermixed with serial hyphae.
Rhizopus stolonifera	It is grayish black and powdery; it is deeply cottony. The reverse is pale white	They have filamentous, branching hyphae coenocytic, bearing gem-shaped sporangiophores.

Table 3: Mineral analysis of fresh and oven-dried *Adansonia digitata* leaves

Mineral composition	Fresh	Oven-dried *Adansonia digitata* leaves			
		180°C	220°C	250°C	270°C
Sodium	5.0	5.0	12.5	12.5	10.0
Calcium	5.45	17.95	18.95	19.15	19.20
Magnesium	9.65	21.90	23.40	21.65	22.10
Potassium	105	600	500	900	800
Phosphorus	5.2	6.5	6.7	6.8	6.6

Table 4: Percentage occurrence of the isolated fungi from oven-dried *Adansonia digitata* leaves

Organisms	Occurrence	Percentage occurrence
Aspergillus niger	4	40
Aspergillus flavus	2	20
Aspergillus fumigatus	3	30
Rhizopus stolonifera	1	10
Total	10	100

Table 5: Total aflatoxin level of oven-dried *Adansonia digitata* leaves

Oven-dried Kuka	Total aflatoxin (ppb)
180°C	25
220°C	25
250°C	20
Kuka obtained from home	40

DISCUSSION

A. digitata is a highly-consumed vegetable food among Nigerians, especially in northern Nigeria, where it is usually used in eating *Tuwo*. It is mostly used in its dried form and preserved for consumption. During the storage process, it may become toxic with a high level of aflatoxin from fungal colonization. In this research, *A. fumigatus*, *A. flavus*, *A. niger,* and one *R. stolonifera* were isolated (Table 1). *A. niger* had the highest percentage occurrence of 4(40%), while *R. stolonifera* had the least percentage occurrence of 1 (10%).

Similarly, the mineral compositions of *A. digitata* leaves dried at temperatures of 180°C, 220°C, 250°C, and 270°C were determined (Table 4). From the results, *A. digitata* leaves dried at 220°C had high sodium and magnesium values of 12.5 and 23.40, respectively. *A. digitata* leaves dried at 250°C had high sodium, potassium, and phosphorus values of 12.5, 900, and 6.8, respectively, while *A. digitata* leaves dried at 270°C had a high calcium value of 19.20.

A. fumigatus exhibited the highest percentage of aflatoxin level of 25 ppb, while *R. stolonifera* indicated the least value of 5ppb. This result of aflatoxin can be attributed to a conducive growth condition for the fungi with a good percentage of nutrients and minerals for survival in the sample. The level of aflatoxin is seen to be above the acceptable limit of the WHO, which expects it to be zero (0), but is still permitted to some extent [16]. A high degree to which such is consumed may pose some risks, such as liver cancer and other secondary dangerous outcomes [17].

The high level of moisture content in fresh *A. digitata* may be due to water availability, which can be attributed to the freshness of the sample because it had not undergone any processing. Such fresh samples with high water content favor the growth of fungi. The high Calcium content and presence of carbohydrate as an energy source enable the growth of fungi mycelium, catalyzing fungi growth, and the generation of more mycotoxins in the *A. digitata.*

The high percentage of water in fresh Kuka is in agreement with the work of [18] who described fresh leaves to contain a high percentage of water, unlike the dry leaves or vegetables, which must have lost their water content through either evaporation or direct heat in the course of drying.

The crude protein percentage is enough to sustain the fungi. A high carbohydrate content can be attributed to the form in which energy stores its food in the course of converting CO_2 to energy, which is later stored in the plant as starch. The carbon in the carbohydrate is an energy source to the fungi and other uses in their total Aflatoxinochemical function and secondary metabolite production. The mycotoxins get released into food due to the fungi's activities. The high crude protein, ash content, and carbohydrate may be the cause of aflatoxin present in the sample, which is in line with the work of [19] who attributed the presence of aflatoxin in his soyabean sample to all the contents present from proximate analysis. The high moisture content in a fresh sample of Kuka in this research can be linked to the work of [20], who affirmed that high moisture content above 13% acted as a source of nutritional aid to fungi.

Elements such as calcium, potassium, magnesium, and Sodium are needed in humans, animals, and microorganisms, therefore fungi, as eukaryotes, also need these minerals for their survival and can therefore be a good source of minerals for the fungi and subsequently results in producing AFTs.

Ekhuemelo and Abu [21] evaluated AFTs contamination and proximate composition of groundnut and found *A. niger* in its samples, which is in agreement with this research work that identifies *A. niger a*s one of the AFTs-producing fungi that deteriorate food in Nigeria. Although he recorded low levels of AFTs, the AFTs presence can also be attributed to high moisture content in the fresh Kuka sample.

CONCLUSION

The study has shown that the hawked *A. digitata* in the study area contained fungi that produce AFTs as secondary metabolites, such as the isolated *A. niger* and *A. fumigatus,* and the AFTs presented by these fungi are 40 and 25 ppb. There is high carbohydrate content in all the samples, with high moisture content in the fresh samples; these samples contained crude proteins and ash contents. The resultant implication is that the concentration of AFTs in this batch of Kuka exceeds the limit of acceptability, and therefore needs to be well monitored in the course of preparation to have a level of 0 or <20 ppb. By reason of the high levels of AFTs in Kuka sampled from the study area, there is a reduction in its quality. Intervention is therefore needed to educate producers on the long-term effects of AFTs and how it reduces food value.

CONFLICT OF INTEREST

None.

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