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Microbial Contaminants and Aflatoxin Level in Indigenous Okra (*Abelmoschus esculentus*), Bologi (*Senecio bialfrae*) and Ugwu (*Telfairia occidentalis*) from Two Selected Markets in Oyo State, Nigeria

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ABSTRACT

Farm product are majorly consumed globally, however, their level of contamination from microbes and aflatoxins from fungi species is worrisome. This study aimed to study the microbes and aflatoxin level in Okra (*Abelmoschus esculentus*), Ugwu (*Telfairia occidentalis*) and Bologi (*Senecio bialfrae*) from two selected markets (Bodija and Ojoo) in Oyo State. Thirty (30) fresh samples of Okra, Ugwu and Bologi were purchased from the study area. Microbiological examination was carried out using standard microbiological procedures, aflatoxin quantification was assayed using High Performance Liquid Chromatography while aflatoxigenic potential of the fungal isolates was carried out on neutral red desiccated coconut agar. The result revealed the presence of six bacterial isolates (*Proteus mirabilis*, *Micrococcus luteus*, *Klebsiella pneumonia*, *Streptococcus* spp., *Bacillus cereus* and *Salmonella* sp.) and four (4) fungal isolates (*Aspergillus niger*, *Aspergillus parasiticus*, *Cladosporium* sp. and *Rhizopus* sp.) in the samples. The highest level of aflatoxin was observed in dried okra (14.000 µg/kg) from Bodija Market, while the lowest level was found in fresh Ugwu (0.001 µg/kg) from Ojoo Market. AFB1 was detected in all samples except for the fresh and dry Ugwu from Ojoo Market and the dry Ugwu from Bodija Market. Additionally, AFB2 was found in all samples. The non-aflatoxigenic fungi included *Aspergillus niger* found in okra from Ojoo Market, *Aspergillus flavus* in Ugwu from Bodija Market, and *Aspergillus parasiticus* in Ugwu from Ojoo Market. In contrast, *Aspergillus*

parasiticus in okra from Bodija Market is aflatoxigenic. In conclusion, the vegetables investigated from the selected markets are contaminated with bacteria and fungi, except for Bologi, and aflatoxigenic fungus was identified in okra from Bodija Market.

Keywords: Aflatoxins, HPLC, Microbial Contaminants, Vegetables, okra, bologi, ugwu

1. INTRODUCTION

Microbial contaminants and aflatoxins pose significant health risks in various food crops, including indigenous vegetables such as Okra (*Abelmoschus esculentus*), Bologi (*Senecio biafrae*), and Ugwu (*Telfairia occidentalis*). These vegetables are staple foods in many African countries, contributing to the nutritional needs of the population (Malik et al., 2024). However, due to improper handling, storage, and environmental conditions, they can harbour microbial contaminants and toxic metabolites like aflatoxins, which are produced by fungi, mainly *Aspergillus flavus* and *Aspergillus parasiticus* (Ishola et al., 2020).

Aflatoxins are mycotoxins produced by *Aspergillus species* as secondary metabolites and are considered some of the most important mycotoxins, classified as Group I carcinogens by the International Agency for Research on Cancer (Oni et al., 2023). Aflatoxins, especially aflatoxin B1, are potent carcinogens and have been linked to liver cancer, immune suppression, and stunted growth, particularly in regions with high dietary exposure (Udomkun et al., 2017). Microbial contamination, including bacteria and fungi, also poses risks to food safety and can result in foodborne illnesses. Recent studies have highlighted the importance of proper cultivation practices, hygienic handling, and effective storage techniques in reducing contamination levels in these vegetables (Onakpa et al., 2021). The presence of microbial contaminants and aflatoxins can be exacerbated by environmental factors such as high humidity and temperature, which are conducive to fungal growth (Adebayo-Tayo & Odu, 2018).

A comparative analysis of these vegetables could reveal variations in contamination susceptibility, depending on factors like moisture content, storage methods, and geographical location. Such findings can inform best practices for reducing health risks and ensuring the safe consumption of these vital food sources. The overall objective of this research is to quantify and compare the aflatoxin in fresh Okra (*Abelmoschus esculentus*), Bologi (*Senecio biafrae*) and Ugwu (*Telfairia occidentalis*) from two selected markets in Oyo State, Nigeria [15-21].

2. METHOD AND MATERIALS

2. 1. Study Area

Thirty (30) fresh samples of Okra, Bologi and Ugwu vegetables were purchased from two selected markets (Bodija and Ojoo) in Oyo State. The samples were labelled, packaged in sterile sample collection bags, and taken to the laboratory for processing and further analysis.

2. 2. Preparation of Culture Media

Preparation of the agar was done strictly by the manufacturer's prescription. 7gram of Nutrient Agar and 9.75g of Potato Dextrose Agar (PDA) were weighed and poured into a

conical flask containing 250 ml of distilled water, this was swirled gently to achieve homogeneity, covered with cotton wool and foil paper and then placed inside the autoclave for sterilization at 120 °C for 15 minutes, then allowed to cool. Exactly 0.2 g of chloramphenicol was added to the prepared PDA to inhibit bacteria growth.

One ml of each sample was aseptically introduced into 9 ml of sterile distilled water and properly mixed and a 10-fold serial dilution up to 10^{-5} to 10^{-7} was done. Exactly, 1 ml from the dilutions was pipetted into the sterile petri dish and 20 ml of freshly prepared cooled to 50 °C, nutrient agar and potato dextrose agar (PDA) was dispensed into the various plates then allowed to solidify. Plates for bacteria was incubated at 37 °C for 24 hours and those for fungi were kept at room temperature for 3-5 days. Discrete colonies were subcultured onto fresh agar plates for further identification.

2. 3. Phenotypic Identification of Isolates

The bacteria isolates were identified macroscopically, microscopically (gram staining) and through various biochemical tests (Catalase, Oxidase, Methyl red, Indole, Simmons Citrate Utilization, and Urease Test) The morphological characterization of fungi isolates was based on the macroscopic examination: colour, texture and pigment and microscopic examination were also carried out by using lactophenol cotton blue stain (Cheesebrough, 2006).

2. 4. Determination of Aflatoxigenic Potential of Fungi Isolates

2. 4. 1. Preparation of Media

Desiccated coconut agar was prepared by the modification of the method of Atanda *et al.*, (2011) Exactly, 100 grams of desiccated coconut was soaked in 1litre of hot distilled water for 30 minutes (pH 4.77), blended aseptically using a blender for 5 minutes and filtered through four layers of cheesecloth. Two percent agar (Oxoid) was added to the filtrate, heated to boiling, and cooled to about 50 °C, 0.1- 0.3% neutral red stain (pH 4.38). The media was sterilized at 121 °C for 15 minutes, cooled and poured uniformly (15 ml) into sterile petri dishes and allowed to solidify. Fervent care was taken to avoid trapping air bubbles in the media.

2. 4. 2. Determination and Inoculation of Media Characteristics

The plates were inoculated with suspected aflatoxigenic moulds and incubated at 30°C for 48 hours. Thereafter the plates were examined for some media characteristics: opacity, translucency and transparency. An uninoculated plate served as a control. The reverse side of each plate, which consists of a large colony, was observed under the long wave (365 nm) Ultra-violet light for blue/blue-green fluorescence and the media were visually assessed for colour and minimal time of pigmentation; intensity, minimal time, peak time and duration of fluorescence every four hours.

2. 5. Aflatoxins Extraction

Aflatoxins were extracted following the method outlined by Hell *et al.* (2000). Each sample of fresh and dried ground vegetables (10 g) was combined with 50 ml of 85% methanol solution, blended for 3 minutes, and then filtered using Whatman no 1 filter paper. The resulting filtrate, approximately 40 ml in volume, was mixed with an equal amount of 40 ml of 10% NaCl. This mixture was then transferred into a separator funnel and defatted using 25 ml of n-

hexane. The hexane layer was discarded, while the aqueous layer was partitioned twice with 25 ml of chloroform. The chloroform layers were combined, decanted over anhydrous sodium sulfate for drying, and subsequently evaporated to near dryness using a regulated water bath set at 50 °C. The remaining residue was placed in an amber vial and stored in a refrigerator at a temperature of -20 °C until analysis.

2. 5. 1. Quantification of Aflatoxin

The samples (Okra, Bologi, and Ugwu) were prepared for analysis according to the modified method described by Hassan et al. (2011). Exactly, 20 g of each sample was combined with 100 ml of a 50:50 v/v mixture of acetone and water. The resultant mixture was agitated for 30 minutes and then filtered using Whatman filter paper. To purify the mixtures, 40 ml of 10% NaCl, 25 ml of n-hexane, and finally 25 ml of dichloromethane were added. The extracts were gathered and allowed to evaporate to dryness in dark bottles. Subsequently, 100 microliters of chloroform were introduced to the samples or aflatoxin working standards, thoroughly mixed for 30 seconds, and filtered through No. 4 Whatman filter paper. Following this, 900 microliters of water: acetonitrile (9:1 v/v) solution were included and mixed well for another 30 seconds. A volume of thirty microliters from this mixture was injected into a preconditioned High-Performance Liquid Chromatography (HPLC) column that had been prepared with 5ml of methanol and 5ml of acetonitrile: water (9:1 v/v) mixture over 15 minutes.

The HPLC setup comprised an Agilent Technologies Pump Model 1200 Series along with a fluorescence detector used for quantification under specified conditions: FLD at 295 nm for excitation and 330 nm for emission; a Zorbax Eclipse Plus C18 Analytical column measuring 4.6 × 250 mm with a particle size of 5 microns; and a post-column UVE LC Tech, Photochemical Post Column Derivatizer at UVC 254 nm. The HPLC analyses were conducted under isocratic conditions utilizing a mobile phase composed of acetonitrile: methanol: water in a ratio of 30:15:55 v/v/v, with a constant flow rate of 1.0ml/min. Before use, the mixture was filtered using a membrane filter and degassed in an ultrasonic bath for 25 minutes, and the volume injected was 30 microliters.

3. 6. Data Analysis

Data were presented in tables and graph using Microsoft (MS) Excel version 2013

3. RESULTS

3. 1. Identification of Bacterial and Fungal Isolate

Table 1. Morphological Identification of Bacteria Isolate in samples.

S/N	Isolate code	Shape	Colour	Suspected organism
1	BU	Circular	Creamy yellow colonies	<i>Micrococcus</i> sp.
2	BO	Rod	Mucoid yellow	<i>Klebsiella</i> sp.
3	BB	Spherical	White-greyish	<i>Streptococcus</i> sp.

4	OU	Opaque/ circular	Whitish-yellow	<i>Bacillus</i> sp.
5	OO	Straight-rod	Fade yellow	<i>Salmonella</i> sp.
6	OB	Irregular	Greyish-white	<i>Proteus</i> sp.

Keys: BU – Ugwu from Bodija, BO – Okra from Bodija, BB – Bologi from Bodija, OU – Ugwu from Ojoo, OO – Okra from Ojoo, OB – Bologi from Ojoo

Table 2. Biochemical identification of bacteria isolated from the samples.

S/n	Isolate code	Gram Reaction	Cat	H ₂ S	Mot	Ind	Cit	Suspected Organism
1	BB	+cocci	-ve	+ve	-ve	-ve	-ve	<i>Streptococcus pneumoniae</i>
2	BO	-rod	+ve	-ve	-ve	-ve	+ve	<i>Klebsiella</i> sp.
3	BU	+cocci	+ve	-ve	-ve	-ve	+ve	<i>Micrococcus luteus</i>
4	OB	-rod	+ve	+ve	+ve	-ve	+ve	<i>Proteus mirabilis</i>
5	OO	-rod	+ve	+ve	+ve	-ve	-ve	<i>Salmonella</i> sp.
6	OU	+rod	+ve	-ve	+ve	-ve	+ve	<i>Bacillus cereus</i>

Key: +ve = Positive, -ve = Negative, CAT = Catalase, H₂S = Hydrogen sulphide, MOT = Motility, IND = Indole, CIT = Citrate,

Table 3. Identification of fungi isolate found in the samples.

S/n	Isolate code	Septation	Type of spores	Shape of spores	Suspected organism
1	BO	Septate	Conidia	Round and globose	<i>Aspergillus niger</i>
2	BU	Aseptate	Sporangia	Ovoid	<i>Rhizopus</i> sp.
3	OO	Septate	Conidia	Spherical rough and thick	<i>Aspergillus parasiticus</i>
4	OU	Septate	Conidia	Cylindrical	<i>Cladosporium</i> sp.
5	BB	-	-	-	NG
6	OB	-	-	-	NG

Keys: BU – Ugwu from Bodija, BO – Okra from Bodija, BB – Bologi from Bodija, OU – Ugwu from Ojoo, OO – Okra from Ojoo, OB – Bologi from Ojoo, NG = no growth

A total of six (6) bacteria and four (4) fungi were isolated from the samples, Table 1 shows the morphological characteristics of the bacteria isolates such as colour and shape while Table 2 shows the biochemical characteristics of the bacteria isolates. The microscopic and macroscopic characteristics of the fungal isolates are shown in Table 3.

3. 2. Aflatoxigenic Potential of the Fungi Isolates

Aspergillus parasiticus, *Aspergillus niger* after 2 days (48 hours) of incubation on Neutral red desiccated coconut agar blue/bluish-green fluorescence after exposing the plate to ultraviolet light 365 nm. Tables 4 and 5 show the aflatoxin quantification of the fresh and dried samples while the comparison between the fresh and dried samples is presented in Figure 1. The fresh Okra from Bodija had a total aflatoxin of 2.900 and 5.000 µg/ml when dried while fresh Ugwu from Bodija had a total aflatoxin of 0.600 and 3.500 µg/ml when dried. Furthermore, fresh Okra from Ojoo had a total aflatoxin of 3.000 and 14.000 µg/ml when dried while the dry Ugwu from Ojoo had a total aflatoxin of 0.001 and 1.000 µg/ml when dried.

The sample appears in a downward order of dried OO> dried OB>dried UB>fresh OO>fresh OB>dried UO>fresh UB>fresh UO. AFB1 was recorded in the samples except for fresh and dry samples of Ugwu from Ojoo market, and dry samples of Ugwu from Bodija. Furthermore, AFB2 was recorded in all the samples while AFG1 & 2 were not detected or below the detectable limit in the samples. The aflatoxigenic potential of the fungal isolates is presented in Table 6.

This reveals that *Aspergillus niger* in Okro from Ojoo, *Aspergillus flavus* in Ugwu from Bodija and *Aspergillus parasiticus* in Ugwu from Ojoo are non –aflatoxigenic while the *Aspergillus parasiticus* in Okro from Bodija is Aflatoxigenic.

Table 4. Concentrations of Aflatoxins in Fresh Samples.

SAMPLES	AFB1	AFB2	AFG1	AFG2	Total Aflatoxin (µg/ml)
Bodija Okra	1.000	1.900	-	-	2.900
Bodija Ugwu	0.000	0.600	-	-	0.600
Ojoo Okro	1.000	2.000	-	-	3.000
Ojoo Ugwu	-	0.001	-	-	0.001

Keys: AFB = Aflatoxin B and AFG = Aflatoxin G

Table 5. Concentrations of Aflatoxins in the dried samples (after a month of storage).

Samples	AFB1	AFB2	AFG1	AFG2	Total Aflatoxin (µg/ml)
Bodija Okra	2.000	3.000	-	-	5.000
Bodija Ugwu	-	3.500	-	-	3.500
Ojoo Okro	9.000	5.000	-	-	14.000
Ojoo Ugwu	-	1.000	-	-	1.000

Keys: AFB = Aflatoxin B and AFG = Aflatoxin G

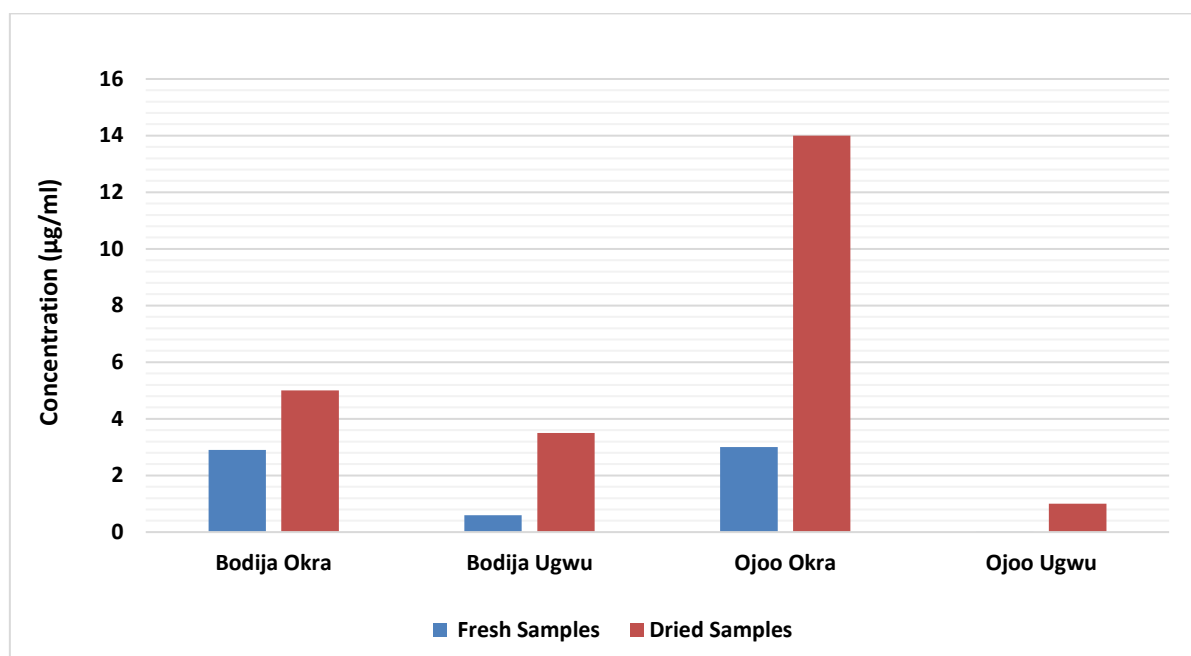


Figure 1. Comparison between total aflatoxin level of fresh and dried samples

Table 6. Aflatoxigenic Potential of the Fungal Isolates.

Fungal Isolates	Sources	Aflatoxigenicity
<i>Aspergillus niger</i>	Ojoo Okro	Non-aflatoxigenic
<i>Aspergillus flavus</i>	Bodija Ugwu	Non-aflatoxigenic
<i>Aspergillus parasiticus</i>	Ojoo Ugwu	Non- aflatoxigenic
<i>Aspergillus parasiticus</i>	Bodija Okro	Aflatoxigenic

4. DISCUSSION

Generally, the microorganisms isolated from the samples include *Micrococcus* spp., *Streptococcus* sp., *Bacillus* spp., *Proteus* spp., *Salmonella* sp., and *Klebsiella* spp., *Aspergillus niger*, *Aspergillus parasiticus*, *Rhizopus* sp. and *Cladosporium* sp. The presence of this organism is not surprising as their source could be from soil, dust or the vegetables themselves at the point of harvest (Malik et al., 2024). Additionally, this can also be linked to exposure in various stages such as harvesting, transportation, processing, distribution, and marketing, the open display method of vegetables for sale, their storage techniques, and cleanliness practices (Umoren et al., 2024). The existence of both gram-positive and gram-negative bacterial strains, particularly harmful bacteria such as *Proteus* spp., *Salmonella* sp., *Klebsiella* spp., and

Micrococcus spp., is recognized to present significant health hazards (Oni et al., 2023). Furthermore, the presence of the genus *Aspergillus* which is composed of fungi found mostly in soil, dust and compost (Umoren et al., 2025).

The presence of *Rhizopus* sp. in samples has been identified as a factor leading to the spoilage of tropical crops and incidents of food poisoning (Oni et al., 2019). Moreover, *Rhizopus* sp. is recognized for its capability to cause angio-invasive conditions. Human contact with their spores can lead to neurological and gastrointestinal mucormycosis through either inhalation or ingestion (Umoren et al., 2025), and *Cladosporium* sp. are among the most abundant fungi in outdoor and indoor environments (Visagie et al., 2014; Bensch et al., 2018), it is acknowledged for its potential to act as an opportunistic pathogen in individuals with weakened immune systems.

The contamination of aflatoxin poses a significant issue in tropical regions of the world where humidity levels are high and temperatures are favorable for mould growth and aflatoxin production (Oni et al., 2019). Aflatoxins B1 and B2 were the most commonly found in the samples, which aligns with the findings of Oni et al. (2019), who noted the presence of Aflatoxin B1 and the absence of Aflatoxin G1 in their study on pepper samples. The high concentration of Aflatoxin detected in dry Okro (*Abelmoschus esculentus*) may be due to the moisture content, and the humidity condition of the storage method which is optimal for fungal growth. Additionally, the higher aflatoxin concentration in dry samples compared to fresh samples can be linked to fungi infestation resulting from inappropriate handling and storage methods often associated with poor hygiene (WHO, 2018). Vegetables with low aflatoxins may contain some natural chemicals which inhibit aflatoxins (Schmidt-Heydt et al., 2009).

5. CONCLUSION

The study investigates the microbial contamination and aflatoxin levels in select Okra (*Abelmoschus esculentus*), Ugwu (*Telfairia occidentalis*) and Bologi (*Senecio bialfrae*) from two selected markets (Bodija and Ojoo) in Oyo State. The study reveals the presence of microbial contaminants in the vegetables and aflatoxin levels in Okra (*Abelmoschus esculentus*) and Ugwu (*Telfairia occidentalis*). Furthermore, the *Aspergillus parasiticus* isolated in Okro from Bodija Market are Aflatoxigenic which is of significant health concern to the consumers.

As a result, it is essential to focus on quality assurance practices throughout the production and distribution of indigenous vegetables. Regulatory bodies implement strict quality control measures to safeguard the health and safety of consumers. The detection of aflatoxins at hazardous levels in these vegetables requires the application of proper agricultural practices, along with appropriate processing, handling, and storage methods.

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