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Research Article

Fungi and Mycotoxins Contamination of Smoked *Micromesisitius poutassou* (Blue Whiting Fish) from Different Markets in Lagos, Nigeria

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ABSTRACT

Smoked fish contamination by fungi produces secondary metabolites called mycotoxins that may constitute potential public hazards to humans. This study was carried out to investigate fungi and mycotoxins contamination associated with smoked *Micromesistius poutassou* sold in some markets in Lagos State, Nigeria. Smoked *Micromesistius poutassou* were randomly purchased monthly for six consecutive months (Sept. 2020 – Feb. 2021) from selected markets in ten Local Government Areas in Lagos State, Nigeria. Isolation, identification and characterization of associated fungi were achieved using the "pour-plate method" and molecular techniques (Polymerase chain reaction and DNA sequencing). Mycotoxins were detected and quantified using AgraQuant kit (Enzyme-Linked Immunosorbent Assay (ELISA). Molecular characterization revealed 11 species belonging to 8 genera of fungi, namely; *Aspergillus giganteus, A. flavus, A. niger, Pichia kudriavzevii, Fusarium chlamydosporum, Asterodon ferruginosus, Cyberlindnera jadinii, Kodamaea ohmeri, Debaryomyces nepalensis, Debaryomyces hansenii and Asordaria mabokeensis.* Aflatoxin, ochratoxin, deoxynivalenol and T-2 toxin were detected in sampled fish and concentrations ranged from 1.33 ± 0.33 to 7.33±2.91, 0 to 5.00 ± 0.58, 0.0016 ± 0.0002 to 0.0032 ± 0.0004 and 0.00 ± 0.00 to 3.00 ± 1.50 ppb, respectively in sampled smoked fish. Although these concentrations observed in the study were within World Health Organization (WHO) permissible limit. However, human health may suffer significantly from continued exposure to mycotoxins. Adequate drying, hygienic post-processing techniques, and proper storage could all help control manifestation of these fungi and mycotoxins.

Keywords: Aflatoxin, Deoxynivalenol, Fungi, Mycotoxins, Ochratoxin, T-2 Toxin
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INTRODUCTION

Fish, which has become a staple food, is consumed by a large portion of the global population (Emere & Dibal, 2013). Comparing fish to meat and other protein-rich foods, it was shown to be the least expensive source of animal protein (Ricketts, 2019). Fish is becoming a more popular protein source among consumers as meat and other protein sources become more expensive. In Nigeria, a variety of fish preservation methods are employed. Chilling, freezing, salting, canning, drying, and smoking are a few of these methods. But in Nigeria, smoking fish is the most popular preservation technique (Adeyeye & Oyewole, 2016). From the production facility to the market areas where fish are exposed until they are bought, it has been noted that smoke-dried fish are frequently tainted with microorganisms like bacteria, yeasts, and fungi known as mould (Adebayo-Tayo et al., 2008; Wogu & Iyayi, 2011).

Fungi produce secondary metabolites known as mycotoxins. The main mycotoxigenic fungi concern to human health belong to three genera, *Aspergillus*, *Penicillium* and *Fusarium* (Wambacq et al., 2016). Essien et al. (2005) reported that common mycotoxins are aflatoxin, ochratoxin, deoxynivalenol, T-2 toxin, fumonisin and zearalenone. According to Singh and Nsokolo (2020), most mycotoxins are stable and not destroyed during food processing. These toxins can cause a range of harmful health effects, with varying

levels of toxicity. Mycotoxins can have various harmful effects on human health, including carcinogenicity (aflatoxin, ochratoxin A, fumonisin B1), estrogenic effects (zearalenone), neurotoxicity (fumonisin B1), nephrotoxicity (ochratoxin A), dermatotoxicity (trichothecenes), and immunosuppression (aflatoxin B1, ochratoxin A, T-2 toxin). Despite decades of research, fungal contamination remains a persistent problem (Bhat et al., 2010). This study aims to identify fungal species and mycotoxins associated with smoked *Micromesistius poutassou* (blue whiting) collected from selected markets in Lagos, Nigeria.

MATERIALS AND METHODS

Fish Sample Collection

Smoked fish samples of *Micromesistius poutassou* were purchased monthly from September 2020 to February 2021. The samples were obtained from selected markets in ten Local Government Areas in Lagos State, Nigeria. Smoked fish samples were purchased randomly from three different sellers in each market. Smoked fish samples were purchased randomly from three different sellers in each market. A total of 540 smoked fish samples were collected throughout the study. The fish samples were placed in sterile Ziploc bags and transferred to the postgraduate laboratory at the Department of Marine Sciences, University of Lagos, for immediate analysis.

Isolation and Identification of Fungi

The culture medium used for growth and maintenance of fungal isolates was Potato Dextrose Agar (PDA) following manufacturer's instructions. Fungi were isolated from each collected fish samples (Robert & Pihet, 2008). The cultures were period of 5 days. Identification of isolated fungi was morphologically examined by colour, shape, size and hyphae. A portion of the growth colony were mounted on slides, stained with lacto-phenol cotton blue and examined under light microscope at objectives x40 (Walsh, 2018).

Molecular Identification

The fungal isolates that could not be identified using traditional methods were aseptically moved from the stock culture into tubes filled with potato dextrose broth (PDB) and allowed to sit at room temperature for five days. Pure culture mycelium and spores were collected in order to extract DNA. The DNA extraction procedure of Zymo Research Bacterial/Fungi kit by Inqaba Biotec (South Africa) was adopted. A Cleaver Scientific Mini micro-centrifuge was used to spin down and slightly vortex the DNA samples. The concentration and purity of the samples were measured using 1 µl of the elution buffer as a blank. The concentration was measured in relation to the absorbance at 260 and 280 nm. An agarose gel stained with 1% (w/v) ethidium bromide was used to evaluate the purity of the isolated genomic DNA. Lambda DNA Hindlll, a 1 kb DNA ladder, was used as a molecular weight indicator. To position the well at the cathode, the electrodes were fastened to the power pack. For one hour, the run's settings were 90 volts. The OMNIDOC System from Cleaver Scientific was used to record the gel.

Polymerase Chain Reaction (PCR) and Sequencing

The New England Biolabs ITS1 was followed in the ribosomal subunit amplification process. Using One Taq quick load 2x master mix with standard buffer and the 16S-27F (5'AGAGTTTGATCMTGGCTCAG 3') and 16S-1492R (5'CGGTTACC TTGTTACGACTT 3'). The PCR reaction mixture (30 ml) containing 15 ml PCR master mix (New England Biolabs), 1 ml (10mM) of each primer, 7.5 ml nuclease free water and 4.5 ml template DNA 1.5 µl (10nm) of (ITS1 and ITS4). PCR amplification was performed in cleaver scientific G-TC 965 following conditions 94 ℃ for 30 seconds; 94 ℃ for 30 seconds; 52 ºC for 1 minute; 68 ºC for 1 minute; 68 ºC for 5 minutes; 4 ºC hold in Cleaver Scientific GTC 96 S. PCR products were separated on 2% agarose gel, stained with ethidium bromide.

All fungal isolates' PCR amplicons were sequenced at Inqaba Biotec in South Africa. Sequencher 5.4.6. Build 46289 was used to edit and trim the sequences. Sequence Homology was done using the NCBI-BLAST tool.

Mycotoxins Extraction and Quantification in Fish Samples

Total aflatoxin, ochratoxin, deoxynivalenol and T2 toxin were determined by Enzyme-Linked Immunosorbent Assay (ELIZA) method using AgraQuant kit by Romer Labs. Representative samples were selected and 10 g each of fish sample was ground and mixed. Aflatoxin, Ochratoxin, deoxynivalenol and T-2 toxin were extracted by adding 5 g of each of

ground sample into 25 mls of 70% methanol and filtered using the whatman no.1 filter paper. Filtrate was added to enzyme conjugated total aflatoxin, ochratoxin, deoxynivalenol and T-2 toxin respectively from the AgraQuant® kit for antibody binding sites. After a washing step, substrate was added to produce blue colour indicating the presence of the tested mycotoxins. Microliter plates were measured optically using an ELX800 BioTek ELISA plate reader with absorbance filter of 450 nm (OD450) and reference wavelength of 630 nm and compared with the standards. Concentrations of mycotoxins detected were measured by extrapolation from the standard curve in parts per billion (ppb).

RESULTS

This study revealed that smoked *Micromesistius poutassou* sold across ten markets from Ten Local Governments in Lagos State Nigeria were contaminated with different species of fungi with variations from one market to the other (Table 1). From 540 sampled fishes, fungi were isolated from 346 which represent 64.7 % of the total fish sampled. *Aspergillus flavus, Aspergillus niger, and Kodamaea ohmeri* were observed in all the markets. *Aspergillus* species were the most prevalent. All fungal isolates collected and extracted produced excellent DNA bands when the ITS region 1 and 4 primers were amplified using PCR. Molecular characterisation of 16 samples revealed 8 genera and 11 species. Three isolates each belonged to the *Aspergillus* (KA, KL and KO19), *Kodamaea* (KG, KH and KO) and *Debaryomyces* (KI, KK and KN) genera. Other isolates belonged to the *Asterodon* (KD and KE), *Pichia* (KB), *Fusarium* (KC), *Cyberlindnera* (KF) and *Asordaria* (KM) genera. Isolates KA and KL showed 99.82 % and 99.64% homologies with *Aspergillus giganteus* while KO19 showed 100 % homologies with *Aspergillus flavus*. Isolate KG showed 100 % homology with *Kodamaea ohmeri* while isolates KH and KO showed 100 % homologies each with *Kodamaea ohmeri*. Isolates KI and KN showed 99.83 % and 99.64 % homologies with *Debaryomyces nepalensis* while isolate KK showed 100 % homology with *Debaryomyces hansenii*. Isolates KD and KE showed 100 % and 99.82 % homologies with *Asterodon ferruginosus* respectively. Isolates KB showed 99.78% homology with *Pichia kudriavzevii,* Isolates KC showed 99.80% homology with *Fusarium chlamydosporum*. Isolates KF showed 100% homology with *Cyberlindnera jadinii,* Isolate KM showed 100% homology with *Asordaria mabokeensis* and isolate QO showed 100 % homology with *Aspergillus niger,* (Table 2). Total aflatoxin, ochratoxin, deoxynivalenol, and T-2 toxin were detected in the sampled fish, concentrations value ranged from 1.33 ± 0.33 to 7.33±2.91, 0 to 5.00 ± 0.58, 0.0016 ± 0.0002 to 0.0032 ± 0.0004 and 0.00 ± 0 to 3.00 ± 1.50 ppb, respectively in smoked *Micromesistius poutassou* across the sampled markets. Highest mean Aflatoxin, ochratoxin, deoxynivalenol and T2 toxin in smoked *Micromesistius poutassou* were observed in Ketu, Ikorodu, Ketu and Badagry markets while the lowest values were observed in Mushin, Ikotun, Epe and Ikorodu markets respectively (Figures 1 - 4)

DISCUSSION

In this study, *Aspergillus flavus*, *Aspergillus niger*, and *Kodamaea ohmeri* were identified across all market samples, with *Aspergillus* species being the most prevalent (Table 1). This aligns with the findings of Pitt and Hocking (2009), who noted that in tropical regions where fish drying and smoking are common, *Aspergillus* species typically dominate the spoilage microbiota of dried fish. The detection and prevalence of *A. niger* corroborate previous studies by Thiyagarajan and Jamal (2021), who reported fungal contamination in smoked dried fish sold in open markets across Benue and Kano states, Nigeria. Similarly, the presence of *A. flavus* in this study is consistent with findings from Junaid et al. (2010) and John et al. (2020), who identified this species in smoked dried fish sold in Uyo, Jos, and Bida.

The occurrence of *Fusarium* species in the samples parallels earlier reports by Akani and Nwakwo (2019) and Deng et al. (2021). Less common fungal isolates included *Aspergillus giganteus*, *Pichia kudriavzevii*, *Asterodon ferruginosus*, *Cyberlindnera jadinii*, *Debaryomyces nepalensis*, *Debaryomyces hansenii*, and *Asordaria mabokeensis*. These fungi, though infrequent, pose potential public health risks. For example, *A. giganteus*, *A. mabokeensis*, and *P. kudriavzevii* are typically soil fungi (Magnusson et al., 2014; Nji et al., 2023); *A. ferruginosus* inhabits decaying wood (Nji et al., 2023); *C. jadinii* is linked to decomposing plant material and industrial fermentation (Sousa-Silva et al., 2021); while *D. nepalensis* and *D. hansenii* are found in saline environments and food products (Kholife et al., 2019).

The high prevalence of *Aspergillus* and *Fusarium* suggests potential mycotoxin production, posing significant health risks (Kholife et al., 2019). Contamination may stem from poor handling, inadequate drying, improper storage, and unhygienic

Table 1: Frequency of occurrence of fungal isolates in smoked *Micromesistius poutassou* in ten markets in Lagos State (September 2020 - February 2021).

Fungal Isolates	Agege	Badagry	Eko	Epe	Ikorodu	Ikotun	Ketu	Mushin	Oyingbo	Shomolu	Total	Frequency of Occurrence (%)
Kodamaea ohmeri	6	6	10	10	10	4	6	4	10	6	72	20.8
Aspergillus flavus	6	10	6	8	7	7	8	12	11	10	85	24.5
Aspergillus niger	14	9	12	12	10	15	5	16	13	16	122	35.2
Fusarium chlamydosporum	6	5	-	6	4	3	1	3	-	2	30	8.7
Aspergillus giganteus	-	-	-	5	-	2	-	-	4	-	11	3.1
Pichia kudriavzevii	-	-	1	-	-	-	2		-	1	4	1.2
Asterodon ferruginosus	2	-	-	-	1	-	-	-	1	-	4	1.2
Cyberlindnera jadinii	-	-	1	-	-	1	-	-	2	2	6	1.7
Debaryomyces nepalensis	1		-	-	1	-	-	1	1		4	1.2
Debaryomyces hansenii	-	2	-	-	-	-	1	-	-	1	4	1.2
Asordaria mabokeensis	-	-	1	1	-	1	-	1	-	-	4	1.2
Total	35	32	31	42	33	33	23	37	42	38	346	100
Percentage	10.2	9.3	9	12.1	9.5	9.5	6.7	10.7	12.1	10.9		

Table 2: Percent sequence similarity and GenBank accession numbers.

S/N	Isolate Code	Identity and NCBI-GenBank Code	Nucleotide sequences (bp)	Accession Number	Percentage identity/Homology
1	KA	*Aspergillus giganteus* OOSF19	559	MZ928432	99.82
2	KB	*Pichia kudriavzevii* OOSF20	462	MZ930149	99.78
3	KC	*Fusarium chlamydosporum* OOSF21	509	MZ928433	99.80
4	KD	*Asterodon ferruginosus* OOSF22	571	MZ928434	100
5	KE	*Asterodon ferruginosus* OOSF23	570	MZ928435	99.82
6	KF	*Cyberlindnera jadinii* OOSF24	569	MZ928436	100
7	KG	*Kodamaea ohmeri* OOSF25	367	MZ928437	100
8	KH	*Kodamaea ohmeri* OOSF26	371	MZ928438	100
9	KI	*Debaryomyces nepalensis* OOSF27	602	MZ928439	99.83
10	KK	*Debaryomyces hansenii* OOSF28	606	MZ928440	100
11	KL	*Aspergillus giganteus* OOSF29	559	MZ928441	99.64
12	KM	*Asordaria mabokeensis* OOSF30	546	MZ928442	100
13	KN	*Debaryomyces nepalensis* OOSF31	553	MZ928443	99.64
14	KO	*Kodamaea ohmeri* OOSF32	365	MZ928444	100
15	KO19	*Aspergillus flavus* OOSF33	542	MZ928445	100
16	QO	*Aspergillus niger* OOSF14	555	MT065684	100

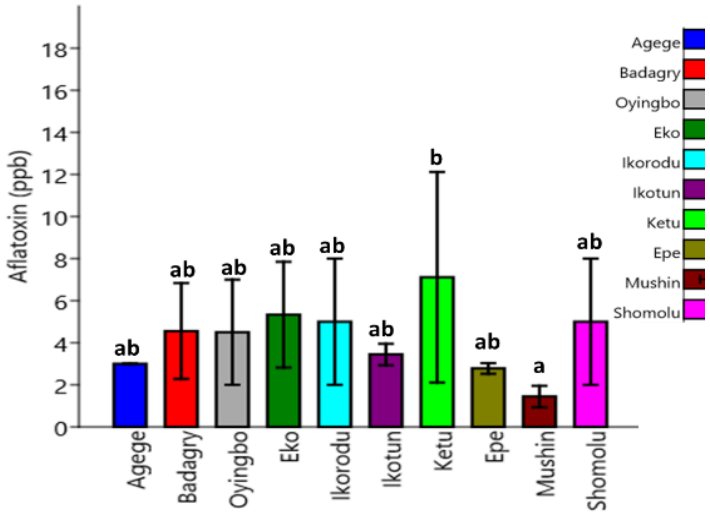

Figure 1: Mean variations of total Aflatoxin (ppb) in the muscle of smoked *Micromesistius poutassou* across the markets. Letters with similar alphabet indicate no significant differences ($p>0.05$)

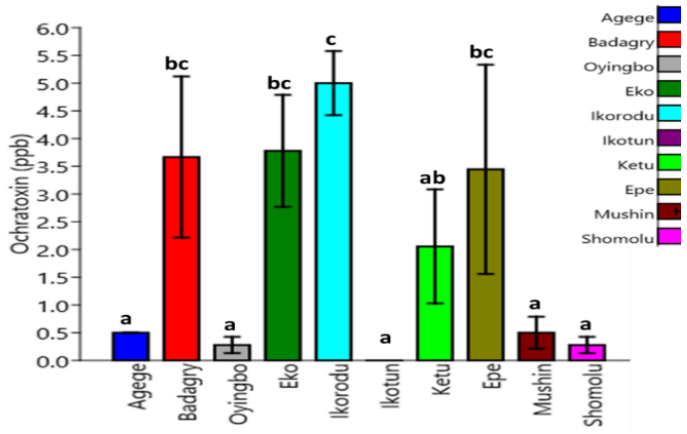

Figure 2: Mean variations of Ochraaztoxin (ppb) in the muscle of smoked *Micromesistius poutassou* across the markets. Letters with similar alphabet indicate no significant differences (p>0.05)

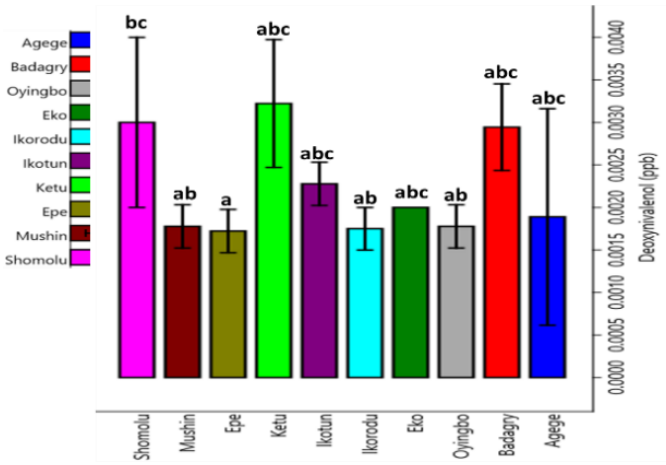

Figure 3: Mean variations of Deoxynivalenol (ppb) in the Muscle of smoked *Micromesistius poutassou* across the markets. Letters with similar alphabet indicate no significant differences (p>0.05)

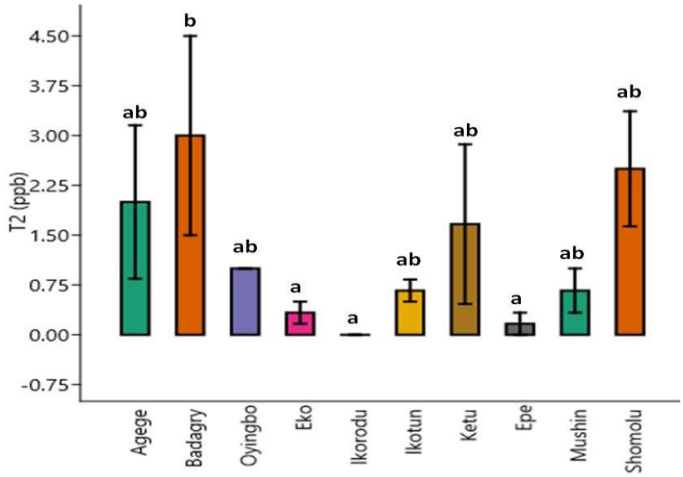

Figure 4: Mean variations of T2 (ppb) in the muscle of smoked *Micromesistius poutassou* across the markets. Letters with similar alphabet indicate no significant differences (p>0.05)

market conditions (Edema & Agbon, 2010; Oyebamiji & Oyebimpe, 2014; Olajuyigbe et al., 2017; Babalola et al., 2018;).

Mycotoxin analysis revealed aflatoxin concentrations ranging from 0.50 to 6.3 ppb, aligning with values reported by Olajuyigbe et al. (2017) and Singh & Nsokolo (2020) for smoked fish from Ibadan, Lagos, and Gambia. These levels are below the WHO recommended limit of 5–15 ppb. However, they contrast with Shazla (2013), who reported much higher aflatoxin levels (up to 53.6 ppb) in Maldive fish. Ochratoxin levels in this study (1.2 ppb) were comparable to those recorded by Shazla (2013) and Deng et al. (2021), but higher than those reported by Farag et al. (2011) and Osibona et al. (2018). Deoxynivalenol levels were lower than those reported by Deng et al. (2021), while T-2 toxin levels matched their findings but exceeded the EU's 1 ppb permissible limit.

Aflatoxins, particularly aflatoxin B1, are among the most potent known human carcinogens, causing hepatocellular carcinoma even at low, chronic exposure (Zain, 2011; Negash, 2018; Mahdjoubi et al., 2020). Ochratoxins, especially Ochratoxin A, are nephrotoxic and potentially carcinogenic (IARC Group 2B), associated with Balkan endemic nephropathy (Pavlovic, 2013). Deoxynivalenol can induce gastrointestinal and neurological symptoms (Payros et al., 2020), while T-2 toxin is a highly toxic trichothecene with known immunosuppressive effects.

Co-contamination with multiple mycotoxins was observed in the samples, reflecting the ability of fungi like *Aspergillus* and *Fusarium* to produce more than one toxin. This co-occurrence can lead to additive or synergistic toxic effects in humans (Mahdjoubi et al., 2020; Karsauliya et al., 2022), increasing the overall health risk.

In conclusion, smoked *Micromesistius poutassou* samples from the study areas were contaminated with mycotoxigenic fungi capable of producing aflatoxins, ochratoxins, deoxynivalenol, and T-2 toxin, posing serious health threats to consumers. While some detected levels were below international safety thresholds, the cumulative and synergistic effects of chronic exposure and co-contamination are concerning. Ensuring proper drying, hygienic handling, clean storage, and controlled transportation is essential to mitigate these risks.

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