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Prevalence of *Aspergillus* species in Smoke-Dried Fish from Selected Markets in Uyo, Akwa Ibom State, Nigeria

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Abstract

Consumption of smoked and smoke-dried fish is common in Nigeria as smoking is one of the common methods of preserving fish. Sadly, due to unhygienic handling and processing techniques, coupled with poor storage facilities and sanitary conditions in the market environments, majority of the fish sold in markets are laden with fungal contaminants of the genus, Aspergillus. The purpose of this study was to determine the prevalence of Aspergillus species among the smoke-dried fish sold in Uyo markets. A total of 360 samples of smoke-dried fish from five breeds of fish were randomly collected from six Uyo markets for study. The samples were aseptically cultured and assayed for the presence of Aspergillus species in Obong University Microbiology Laboratory. Each of the fish samples was blended and 1g suspended in 9ml of sterile distilled water, and 10-fold serial dilutions were made to produce graded concentrations ranging from 10⁻¹ to 10⁻⁹. About 1ml of each dilution was spread on the surfaces of triplicate potato dextrose agar plates supplemented with 0.05 mg/ml of chloramphenicol and gentamicin and incubated at 25 ± 2 °C for 7 days. Representative colonies of Aspergillus species on PDA were sub-cultured on sterile PDA plates to obtain pure colonies, and were further confirmed by microscopic examination. The microbial colonies in each plate were counted and the average number of colonies in the triplicate was calculated. Out of the 360 samples collected, Aspergillus was isolated from 51 samples, resulting in a prevalence of 14.2%. Four species of Aspergillus isolated from this study were 22 isolates of A. fumigatus, 13 isolates of A. niger, 12 isolates of A. flavus and 4 isolates of A. ochraceus. A. fumigatus was the most encountered species of Aspergillus in each market while A. ochraceus was the least. Urua Ekpa market had the highest number of isolates of Aspergillus species, with 12 isolates, followed by Itam market with 10 isolates. According to the order of magnitude, catfish was the most contaminated fish samples, followed by mackerel (Titus), tilapia, Asa fish and finally, Shawa fish. The result also revealed the rich microbial population of Aspergillus species from the various breeds of fish samples with mean values ranging from 1.9×10^2 cfu/g to 2.4×10^2 cfu/g. The result obtained from this study shows that a lot of fish sold in Uyo markets are contaminated with Aspergillus fungi which are opportunistic pathogens of public health importance. Therefore, to cub the menace of aspergillosis



in Akwa Ibom state and possibly in Nigeria at large, fish contaminations should be avoided by employing appropriate professional techniques of fish processing and storage. This is the first systematic report on the prevalence and diversity of Aspergillus species in smoke-dried fish sold in Uyo, Akwa Ibom State. It provides baseline data for surveillance of fungal contamination in smoked fish and highlights the need for improved food hygiene in Uyo markets.

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1.0 Introduction

Fish is an important source of high-quality protein, and constitutes a vital portion of diet to man and animals especially in food-insecure regions of the world where it represents about 15-20% of all animal protein (Ojutiku et al., 2009; Benedict et al., 2023). While supporting the livelihood of millions of people worldwide, fish has been reported to provide 40-70% of the protein intake of the people living in West Africa, and represents a critical source of dietary protein not readily available in carbohydrate based staple foods (Ikutegbe and Sikoki, 2014). In Nigeria, fish is consumed more than other meat sources because it is relatively cheap and available as well as its high nutrient contents. It contains high quantities of calcium, phosphorus, lysine, vitamins A, B, and E, iodine and polyunsaturated fatty acids (Ike et al., 2020).

However, due to microbial contamination, fish products are one of the most perishable food items. In addition, the different abiotic factors like temperature, moisture, and relative humidity, as well as marketing areas are also the main cause and sources for contamination (Jonathan and Esho, 2010; Akwuobu *et al.*, 2019). It has been reported that about one third of the world's food production is lost yearly due to microbial spoilage (Daramola *et al.*, 2020).

Therefore, fish requires preservation immediately after harvest because of its high degradability. Several post-harvest processing and preservation techniques such as smoking, salting, freezing, drying and canning are often utilized to improve the shelf life of fish both in Nigeria and different parts of the world. In Nigeria, drying and smoking are the most popular methods of fish preservation because of anti-bacterial property. Smoke-drying accelerates the drying process of fish, prolongs its shelf life and serves as an antagonist to spoilage agents (Ngo-oum et al., 2021).

It has been reported that the majority of the microbial contaminants observed in fish after it has been processed could either be a result of poor handling and processing practices, or contamination from the water bodies, air, or degrading substances (Oparaku and Mgbemena 2012; Ayuba et al., 2013). Instead of resorting to professional food processing industry, most employ unprofessional fishermen and unhygienic methods to smoke-dry or sun-dry their fish. In addition to this, good storage practices are not observed by most producers and wholesalers of smoked and smoke-dried fishes (Akande and Tobor, 2012; Sani et al., 2016). This often leads to food safety issues because the drying conditions cannot be easily



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controlled. As practiced in many African countries, some fishermen prefer to dry their fish in open balconies of houses or streets (Gonkowski et al., 2018; Akwuobu et al., 2019). The quality of fish prepared in this way is usually compromised by the microbial, insect, and rodent contaminations (Ayuba et al., 2013). Fungal contamination is one of the major problems confronting fish industries in Nigeria and Africa in general (Ikeh et al., 2024). Fungi are diverse in nature and are causes of infection known as "mycosis" (Pal, 2017). Smoke-dried fish with medium to high moisture contents in humid tropical conditions are often predisposed to fungal contamination (Akwuobu et al., 2019). Studies have reported the decline in dried fish quality affected by fungal growth (Begum et al., 2011; Adeyeye, 2016). Microbial contamination

affects the fish quality by alteration of flavour,

texture and loss of nutrients, all of which can

threaten food safety with huge economic losses

to fish industries. Many fungi species such as

Aspergillus species, Mucor species, Penicillium

tropicalis and Fusarium moniliformis have been

reported in dried and smoked fish sold in Nigeria

(Ikeh et al., 2024). Their presence in product

could result in food-borne infection and food

poisoning which may constitute a serious public

species,

Candida

Cladosporium

species,

health hazard.

The numerous fungi species implicated in fish spoilage are also a serious threat to consumers' health due to their ability to produce mycotoxins, particularly aflatoxins, which are major food contaminants globally (Fallah *et al.*, 2014; John *et al.*, 2020). Aflatoxins are thermostable secondary metabolites which are highly toxic, carcinogenic and mutagenic. Swaminathan and Sparling (1998) reported that numerous pathogenic agents isolated from different types of fish were toxigenic, and their toxins were retained in fish even after salting and storage periods, which causes serious systemic dysfunctions and public health hazards (Sani *et al.*, 2016). Raghda *et al.* (2022) pointed out that

most mycotoxins are stable compounds that are not easily destroyed during food processing or home cooking, and several cases of human gastroenteritis, severe diarrhea and food poisoning outbreaks have been recorded after smoked fish consumption (Sani *et al.*, 2016).

It was also reported that the most dominant contaminant in dried food are toxigenic fungal species belonging to the genus Aspergillus, and these species can cause various diseases in man and animals (Hassan et al., 2011). Aspergillus species are filamentous fungi that are commonly found in food, where they thrive as saprophytes. Most species are found in a wide variety of environments and substrates on the earth throughout the year (Mousavi et al., 2016), and they usually grow fast in tropical and subtropical regions (Pitt and Hocking, 2009). Aspergillus species have the ability to survive in temperatures ranging from 12°C-48°C, with the optimal growth temperature ranging from 28°C - 37°C, with a high humidity of above 80% (Hassane et al., 2017).

Aspergillus species are able to produce a range of mycotoxins (Alshannaq and Jae-Hyuk, 2017), including e.g. aflatoxins, ochratoxins, fumonisins and patulin (Aristil *et al.*, 2017). Aflatoxins B1, B2, G1, G2 are the most toxic and carcinogenic mycotoxins, because of their extreme hepatocarcinogenicity. Ochratoxinis is carcinogenic, nephrotoxin, teratogenic, and immunotoxic. Fumonisins are hepatotoxic and nephrotoxic, while Patulin are genotoxic (Varga *et al.*, 2015).

In 2004, aflatoxins were reported to be responsible for the death of 123 people in the eastern province of Kenya (Lewis *et al.*, 2005). The aflatoxins have been detected in smokedried fish and other food items contaminated by Aspergillus species in Plateau state, Nigeria. (Job *et al.*, 2016). Last year, 49.7% prevalence of chronic pulmonary aspergillosis was reported at a diagnostic center, Lagos (Davies *et al.*, 2024), and a prevalence of 46.9% was also



reported in Otukpo local government area of Benue State, Nigeria (Odagboyi *et al.*, 2024). Despite numerous reports on fungal contamination of fish in Nigeria, limited data exist for Akwa Ibom State. This study therefore investigates the prevalence and diversity of Aspergillus species in smoke-dried fish sold in Uyo markets.

2.0 Materials and Methods

2.1 Experimental Design

This was a qualitative and quantitative study to show that Aspergillus species are common contaminants of fish sold in Uyo, Akwa Ibom State. This study employed the use of different breeds of smoke-dried fish commonly sold in various markets of Uyo metropolis by market women. The breeds of fish considered for this study were Asafish, Catfish, Mackerel (Titus), Shawa fish (Bonga fish), and Tilapia. The experiment was designed to isolate the various species of Aspergillus fungi from these breeds of smoke-dried fish as the possible pathogens of aspergillosis of man and animals, estimate the microbial load from each sample, and compare the results obtained to those obtained from other markets. Ethical approval was sought for, and obtained from Obong University Research Directorate.

2.2 Study Location

This study was carried out using six markets at different locations in Uyo town which included Akpan Andem market, Ekom Iman market, Ibom Plaza market, Itam market, Nsukara market and Urua Ekpa market. Uyo is the capital City of Akwa Ibom state and lies between latitudes 4°58'N and 5°04'N and longitudes 7°51'E and 8°01'E (Ekpenyong et al., 2020). It is bounded in the north by Ikono, Itu and Ibiono Ibom Local Government areas, in the East by Uruan LGA, in the west by Abak LGA and in the south by Ibesikpo Asutan and Nsit Ibom LGAs. The city covers an area of about 214.31 square kilometers (Ekpenyong et al., 2020). Akwa Ibom state is one of the south-southern states of Nigeria and has varying climate with cold dry season (harmattan) from October to February, hot wet season from March to May and a cold wet season from June to September (Anosike *et al.*, 2024). It borders Cross River State to the east, Rivers State to the west, Abia State to the north-west, and the Atlantic Ocean to the south. The state takes its name from the Qua Iboe River which bisects the state before flowing into the Bight of Bonny (Onyeakagbu, 2021).

2.3 Inclusion and Exclusion Criteria

Only smoke-dried fish sold in the Uyo markets were included in the study, irrespective of whether they were produced in Akwa Ibom state or not. Live fish or fish processed and preserved through other means were excluded from the study.

2.4 Sample Collection

Representative samples of the five breeds of smoke-dried fish under study were purchased from the six markets in Uyo city using simple random sampling. In each market, twelve (12) samples of smoke-dried fish from each breed were collected, and a total of sixty (60) samples of the five breeds of fish were collected from each market. In other words, a total of 72 samples of each breed of fish were collected from the six markets. A grand total of 360 smoke-dried fish samples from the five breeds of fish were randomly collected from the six markets under study. Each sample collected was put in a well-labeled sterile polythene bag and transported immediately to Obong University Microbiology Laboratory for processing and analysis for the presence of fungi. The sampling was done from March to August, 2025.

2.5 Preparation of Culture Medium

The agar medium used for culture and isolation of the organisms is potato dextrose agar (PDA). The culture medium was prepared according to the manufacturer's instructions and autoclaved at 121°C for 15 minutes. The medium was aseptically dispensed into the sterile petri dishes; agar surfaces flamed to remove air bubbles and then allowed to solidify.



The fish samples were aseptically processed for fungal isolation following the procedure described by Oyebamiji and Oyebimpe (2013). Each of the fish samples was macerated with and 1g suspended in 9ml of sterile distilled water and 10-fold serial dilutions were made. During the process, 1ml of each sample suspension was serially transferred into various test tubes containing 9ml of distilled water each with the aid of sterile pipettes to produce graded concentrations with dilution factors ranging from 10⁻¹ to 10⁻⁹.

2.7 Procedure for Fungal Culture and Isolation

One ml of each dilution (10⁻¹ to 10⁻⁹) was spread on the surfaces of triplicate potato dextrose agar plates supplemented with 0.05 mg/ml of chloramphenicol and gentamicin (to inhibit bacteria growth). Plates were incubated at room temperature (25 \pm 2°C) by placing them on a clean bench in the laboratory. The plates were then examined daily for fungal growth for 7 days. Uninoculated PDA plates were also incubated at room temperature along with the inoculated plates for 7 days. The absence of microbial growth on the uninoculated plates confirmed the PDA plates to be 100% sterile. Macroscopic features (cultural characteristics) used for species differentiation were colour of the colony from the surface and reverse of the plate, margins, elevation and texture of the colony. Representative colonies of Aspergillus species grown on PDA were subcultured on sterile PDA plates to obtain pure colonies. The species were further confirmed by microscopic examination.

2.8 Determination of Microbial Load

After incubation at 25°C for 7 days, appropriate dilution factor was chosen from plates of Aspergillus growth which was neither too numerous to count nor too few to count. The number of microbial colonies in each plate was counted and the average number of colonies in the triplicate was calculated. Colony forming unit (CFU) was obtained by multiplying the

average number of the colonies with reciprocal of the dilution factor. Samples of various breeds of fish which were positive for Aspergillus were numbered.

2.9 Wet Mounts Preparation for Microscopic Examination of Fungal Growth

A single colony of fungal growth was collected with a sterile wire loop from PDA and placed on a microscope slide. A drop of lactophenol cotton blue was added to the colony and a cover slip was carefully placed over the mixture, avoiding air bubbles. The slide was placed under the microscope and examined for microscopic features such as size of conidia and conidiophores and their arrangements.

2.10 Identification of Aspergillus species

Isolates were identified using cultural characteristics, microscopic morphologies and comparison with already identified species in relevant fungal atlas (Hungerford *et al.*, 1998; Madigan *et al.*, 2009) as follows:

- i. White fluffy colonies which became velvet and bright bluish green in colour, with dome-shaped vesicles bearing phialides upon which green conidia (spores) were attached indicated the presence of Aspergillus fumigatus.
- ii. Whitish young colonies that became black as they got older, with large spherical vesicles bearing large metulae that supported small phialides with black conidia indicated the presence of Aspergillus niger.
- iii. Yellow-green colonies with whitish powdery edge, whose reverse were colorless, and the phialides directly attached to globular vesicles in the absence of metulae were indicative of Aspergillus flavus.
- iv. Powdery, buff-coloured colonies, whose reverse were colourless, with rough conidiophores, globular vesicles, and phialides carried by metulae were indicative of Aspergillus ochraceus.



3.0 Results

3.1 Features of the Isolates

Table 1 shows the macroscopic and microscopic features of the isolates. The macroscopic features of the colonies which revealed the presence of Aspergillus fumigatus were fluffy colonies that turned velvet and bright bluish green in colour. Their microscopic features were dome-shaped vesicles bearing phialides upon which green conidia (spores) were attached. Whitish young colonies that became black as they got older, with large spherical vesicles bearing large metulae that supported small phialides with black conidia indicated the presence of Aspergillus niger. The yellow-green colonies with whitish powdery edge, whose reverse were colorless, and the phialides directly attached to globular vesicles in the absence of metulae were indicative of Aspergillus flavus. Powdery, buff-coloured colonies, whose reverse were colourless, with rough conidiophores, globular vesicles, and phialides carried by metulae were indicative Aspergillus of ochraceus.

3.2 The Prevalence of Aspergillus among the Fish Samples

Table 2 shows the prevalence of Aspergillus in the study area. Out of the 360 samples collected, Aspergillus was isolated from 51 samples, resulting in a prevalence of 14.2% in the study area. Also, out of 60 samples of the five breeds of fish collected from each market, 7, 8, 7, 10, 7, and 12 samples from markets of Akpan Andem, Ekom Iman, Ibom Plaza, Itam, Nsukara and Urua Ekpa respectively were contaminated with Aspergillus. This shows that Urua Ekpa market had the highest number of samples contaminated with Aspergillus, followed by Itam market, and then, Ikom Iman market. Akpan Andem and Nsukara markets were the least infected.

3.3 Breeds of Fish Samples Contaminated with Aspergillus species

Table 2 also shows the samples of the various breeds of fish contaminated with Aspergillus species and their distribution among the six Uyo markets. These include 9 samples of Asa fish, 14 samples of catfish, 11 samples of mackerel, 7 samples of Shawa fish and 10 samples of tilapia. According to the order of magnitude, catfish was the most contaminated fish samples, followed by mackerel, tilapia, Asa fish and finally, Shawa fish, the least infected breed.

3.4 Estimation of microbial Load from Fish Samples

Following the counting of the fungal colonies in each plate and calculation of the average number of the colonies from each triplicate culture, per fish sample, the result shows that the total count of fungal colonies in catfish ranged from 1.1 x 10^2 cfu/g to 3.2×10^2 cfu/g with a mean value of 1.9 x 10² cfu/g. The fungal colonies in mackerel ranged from 1.0 x 10² cfu/g to 3.3 x 10² cfu/g with a mean value of 2.4 x 10² cfu/g. The colonies in tilapia ranged from 1.3 x 10² cfu/g to 2.9×10^2 cfu/g with a mean value of 2.0×10^2 cfu/g. Fungal colonies in Asa fish ranged from 0.5×10^2 cfu/g to 3.0×10^2 cfu/g with a mean value of 2.0 x 10² cfu/g, and in Shawa fish, fungal colony counts ranged from 1.2 x 10² cfu/g to 2.9×10^2 cfu/g with a mean value of 2.1×10^2 cfu/g (Table 3). The result shows that mackerel has the highest colony count of 2.4 x 10² cfu/g while catfish has the lowest count of 1.9×10^2 cfu/g. Table 3 also shows that catfish (C1 to C14) was the most contaminated breed of fish followed by mackerel (M1 to M11), while Shawa fish (S1 to S7) was the least contaminated breed.

3.5 Relative Occurrence of Aspergillus species Isolated

Four species of Aspergillus were isolated from the study area. These include 22 isolates of A. fumigatus with 43% relative abundance, 13 isolates of A. niger with 25% relative abundance, 12 isolates of A. flavus with 24% relative abundance and 4 isolates of A. ochraceus with 8% relative abundance (figure 1). A. fumigatus was the most isolated species of Aspergillus while A. ochraceus was the least isolated species.



Microscopic Features Total **Isolates Macroscopic Features** Vesicles Phialides Metulae Conidia A. Dome-Velvety bluish-green colonies Absent Green conidia 22 Present fumigatus shaped Whitish colonies that turned black Present Present Spherical Black conidia 13 A. niger (large) as they got older (small) colonies Yelloy-green with Yellow-green A. flavus whitish powdery edge and Globular Present Absent 12 conidia colourles reverse A. Powdery buff-coloured colonies buff coloured Globular 4 Present Present with colourles reverse conidia ochraceus

Table 1: Macroscopic and Microscopic Features of the Isolates

Table 2: Prevalence of *Aspergillus* from fish samples

Market						
	Asafish	Catfish	Mackerel	Shawafish	Tilapia	 Total
Akpan Andem	1	3	0	1	2	7
Ekom Iman	3	2	1	0	2	8
Ibom Plaza	1	2	2	2	0	7
Itam	2	1	3	2	2	10
Nsukara	0	3	2	1	1	7
Urua Ekpa	2	3	3	1	3	12
Total	9	14	11	7	10	51(14.2%)

3.6 Species of Aspergillus Isolated from the Breed of Fish

Figure 2 shows the distribution of the various species of Aspergillus among the five breeds of fish under study. The result shows that A. fumigatus was the most encountered species of Aspergillus in each breed of fish, followed by A. niger, A. flavus and finally A. ochraceus. A. ochraceus was the least isolated species of Aspergillus among the breeds of fish studied, and was absent in mackerel.

3.7 Species of Aspergillus Isolated from Each Market

Figure 3 shows the distribution of the various species of Aspergillus among the six Uyo markets under study. The result shows that A. fumigatus was the most encountered species of Aspergillus in each market except at Ibom Plaza market where A. niger was the most isolated

species. On the other hand, A. ochraceus was the least isolated species of Aspergillus, and was completely absent in Akpan Andem, Ibom Plaza and Nsukara markets.

4.0 Discussion

The result obtained from this study shows that a lot of fish we consume in Uyo are contaminated with Aspergillus fungi. Of the 360 smoke-dried fish samples collected from the Uyo markets, 51 of them were contaminated with Aspergillus, giving a prevalence of 14.2%. Majority of the fish sold in Uyo comes from different parts of Akwa Ibom state and beyond, which shows that Aspergillus is prevalent in Akwa Ibom state and wide spread in Nigeria. A lot of factors have been reported as the associated courses of widespread contamination of fish in Nigeria. Majority of the contaminants arises from poor handling and processing practices (Oparaku and



Mgbemena 2012; Ayuba *et al.*, 2013). Instead of resorting to professional food processing industry, most fishermen employ unhygienic and local methods of processing smoked-dried fish. As practiced in many African countries,

some fishermen prefer to dry their fish on streets or in open balconies of houses (Gonkowski *et al.*, 2018; Akwuobu *et al.*, 2019), and these provide ample opportunities for microbial, insect, and rodent contaminations

Table 3: Microbial Loads of the Fish Samples

Sample	Microbial Load (cfu/g)								
C1	1.2×10^2	M1	2.6×10^2	T1	1.6×10^2	A1	1.2×10^2	S1	2.9×10^2
C2	3.2×10^2	M2	1.2×10^2	T2	1.4×10^2	A2	0.5×10^2	S2	1.4×10^2
C3	2.6×10^2	M3	2.4×10^2	Т3	2.0×10^2	A3	2.4×10^2	S3	1.8×10^2
C4	2.0×10^2	M4	3.1×10^2	T4	2.4×10^2	A4	1.3×10^2	S4	2.6×10^2
C5	1.6×10^2	M5	2.5×10^2	T5	2.6×10^2	A5	2.6×10^2	S5	2.1×10^2
C6	1.4×10^2	M6	1.0×10^2	Т6	1.8×10^2	A6	2.9×10^2	S6	1.2×10^2
C7	3.1×10^2	M7	1.8×10^2	Т7	1.6×10^2	A7	1.5×10^2	S7	2.7×10^2
C8	2.3×10^2	M8	2.7×10^2	Т8	2.2×10^2	A8	2.6×10^2	Mean	2.1 x 10 ²
С9	2.2×10^2	M9	3.2×10^2	Т9	2.9×10^2	A9	3.0×10^2		
C10	1.3×10^2	M10	2.7×10^2	T10	1.3×10^2	Mean	2.0×10^2		
C11	1.1×10^2	M11	3.3×10^2	Mean	2.0×10^2				
C12	1.2×10^2	Mean	2.4×10^2						
C13	2.4×10^2								
C14	1.5×10^2								
Mean	1.9 x 10 ²								

Key: A = Asa fish, C = Catfish, M = Mackerel, S = Shawa fish, T = Tilapia



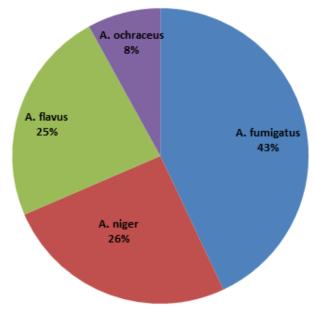


Figure 1: Species of Aspergillus isolated and their relative abundance

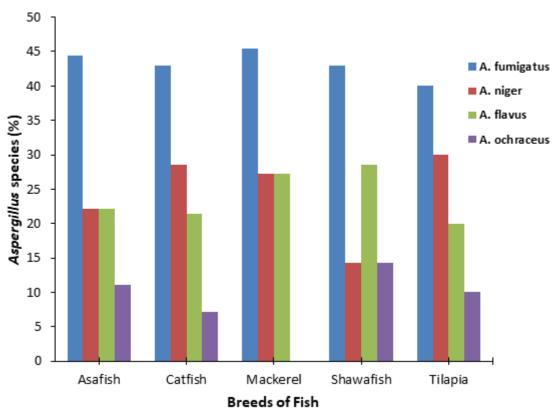


Figure 2: Distribution of Aspergillus species among the Breeds of Fish

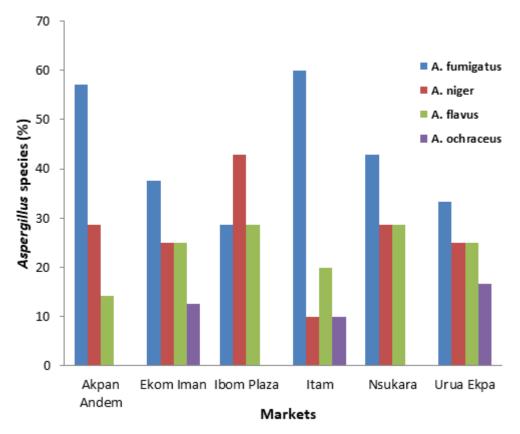


Figure 3: Distribution of Aspergillus species among the six Uyo markets

This study shows that out of the five breeds of fish that were sampled, catfish was the most contaminated breed, followed by mackerel (Titus). Catfish (*Clarias gariepinus*) is the major breed of fish reared in Nigeria. Aquaculture diseases appear to be one of the major threats to fish production in Nigeria (Agnew et al., 2009). Therefore, the high contamination of catfish with Aspergillus species could be an aquaculture infection that persisted within the fish samples even after the fish have been harvested and processed by smoke-drying. In other words, the fish could have been infected in the pond during the rearing stage. This is in line with the report that fish reared in a polluted aquatic environment have a high susceptibility to different illnesses and contamination than those reared in nonpolluted environments (Ngo et al., 2021). Therefore, the high contamination of catfish with Aspergillus species in this study could be an aquaculture infection that persisted within the fish samples even after the fish have been harvested and processed by smoke-drying. The smoking process does not ensure complete

elimination of all the fungal infections. Aspergillus spores and Aflatoxins are heat stable, and are not easily destroyed in the process of smoke-drying. Aspergillus spores can survive high temperatures of above 70°C (de Massaguer et al., 2014; Raghda et al., 2022). If the heat is not evenly distributed, spores will remain viable and this, together with the toxins can pose a serious threat to public health. Also, aquaculture infection may arise from poorly controlled feed use and waste products, and lack of technical knowledge relating to proper husbandry and health management. Almeida et al. (2011) reported that many fish farmers use locally made fish feed usually produced from available plant and animal wastes and stored in large quantities. ingredients are often subjected to contamination by moulds due to poor storage conditions during pre-harvest period.

As pointed out by Akande and Tobor (2012) and Sani *et al.* (2016), it was discovered during sampling that almost all the smoked fish sold in the markets by retailers were kept in unhygienic environments. Good storage practices are not



observed by most vendors of smoke-dried fish. Urua Ekpa market has the highest number of smoke-dried fish contaminated with Aspergillus species followed by Itam market where large quantities of fish are stacked in various locations. Prolonged storage and high temperature and humidity conditions facilitate fungal development and production of attendant mycotoxins in smoke-dried fish. Unstable conditions brought about by sudden changes in weather, such as heavy prolonged rain can adversely affect the storage and preservation of fish. As pointed out earlier, Akwa Ibom State is located in south southern geopolitical region of Nigeria, classified as high rain forest zone. The region is characterized by prolonged rainfall, high humidity and hot climate (Anosike et al., 2024). This hot and humid environment facilitates active fungal colonization.

The species of Aspergillus isolated from the breeds of fish in Uyo markets were A. fumigatus, A. niger, A. flavus and A. ochraceus. These species were obtained from different locations of the six major markets in Uyo town. In this study, A. fumigatus was the most encountered species with 43% relative abundance, followed by A. niger with 25% and then A. flavus (24%) and A. ochraceus (8%). A. ochraceus was the least encountered species. In the same vein, A fumigatus was mostly isolated from fish samples obtained from Akpan Andem market followed by the samples from Itam market. These species of Aspergillus are pathogens or causative agents of aspergillosis in man and animals, and their presence in fish may constitute a serious public health hazard and may also lead to economic losses.

The four species of *Aspergillus* isolated in this study are mycotoxin producing *Aspergillus*, and are causative agents of opportunistic infections in man and animals. *Aspergillus fumigatus* is the most common airborne fungal pathogen and can lead to invasive pulmonary aspergillosis, which is often fatal in immune-compromised patients

(Caroline et al., 2021). A. niger is also associated with lung infections, particularly in individuals with compromised immune systems (Koteswara et al., 2016). Aspergillus flavus frequently infects and contaminates food items and produces carcinogenic secondary metabolite aflatoxin (Suanthie et al., 2008), while A. ochraceus produces a carcinogenic nephrotoxin mycotoxin known as ochratoxin (Heussner et al., 2015). Several authors have reported that these species of Aspergillus produce various life-threatening mycotoxins (Baker et al., 2003; Patel et al., 2018; Udovicki et al., 2018; Rushing and Selim, 2019; Aduly et al., 2020). Mycotoxins are potential carcinogens and can cause some degree of acute toxicity when consumed in food, more especially, those consuming smoked and smoke-dried fish without further cooking.

The present finding is consistent with other researches about fungal infections on fish. Chauhan (2013) and Podeti and Benarjee (2015) found that Aspergillus fumigatus was the highest fungi pathogen isolated from fish samples with 41.3% incidence. However, the finding from the present study disagrees with the finding of Resmila (2018) which reported A. flavus as the most encountered species of Aspergillus. As discovered in this study, Podeti and Benarjee (2015) also reported the occurrence of mixed infections; and stated that Aspergillus fumigatus was found in combination with Aspergillus niger and/or other fungal species. Co-contamination can occur when a single fish is inhabited by a number of different fungal species resulting in the production of different multiple toxins (Gonçalves et al., 2018).

The present study also reveals the rich microbial population of *Aspergillus* species from the various breeds of fish samples with mean values ranging from 1.9×10^2 cfu/g to 2.4×10^2 cfu/g. Their population dynamics however varied with the sample situations. Mean values obtained from the samples in this study show that the



loads of Aspergillus species were reasonably high. The result of this study is validated by the finding of Resmila (2018) in which microbial load count of Aspergillus species on smoked fish ranged from 0.5 x 102 cfu/g to 3.5 x 102 cfu/g. However, the result is lower than the one previously reported by Oku and Amakoromo (2013) in Yenagoa, Nigeria with Aspergillus load count of 1.0×104 cfu/g to 4.0×105 cfu/g on smoked fish. These reveal the dangers of consuming not only smoke-dried fishes but other food items prepared and stored in unhygienic ways such as rice, maize, wheat bran, blood meal, cottonseed cakes, groundnut cakes (kwulikwuli), sunflower seedcakes, soybeans and cassava (Liti et al., 2005).

According to Deryck (2006) three different disease states of aspergillosis are observed in man and animals. Firstly, aspergillosis can arise secondary to immunosuppression caused by primary infections e.g. colonization of lung cavities due to tuberculosis, HIV/AIDS and neoplasms, and this may involve any organ or system in the body. Secondly, allergic reactions to Aspergillus species can cause serious health implications as seen in allergic bronchopulmonary aspergillosis, sinusitis and disseminated aspergillosis. Thirdly, toxicity may occur as a result of aflatoxins produced by Aspergillus species. Ingestion of aflatoxins can lead to hepatocellular carcinoma, also known as liver cancer.

As reported by WHO (2015), cases of food poisoning are seen more in children under the age of 5 years, the elderly people and the immune compromised patients. Foodborne pathogens can complicate diseases such as diabetes mellitus, tuberculosis, HIV/AIDS and other life- threatening diseases with resultant increase in mortality. Odagboyi *et al.* (2024) studied the distribution of *Aspergillus* species among tuberculosis patients in Otukpo Local Government Area of Benue State, Nigeria. Of the 461 patients sampled, 216 were infected with pulmonary aspergillosis, giving a prevalence

rate of 46.9 %. Also, Davies *et al.* (2022) studied the prevalence of chronic pulmonary aspergillosis (CPA) among patients in the tuberculosis treatment clinics, Lagos, and the result showed CPA prevalence rate of 49.7%, with 6.1% incidence rate.

5.0 Conclusion

The result obtained from this study shows that a lot of fish sold in Uyo markets are contaminated with Aspergillus fungi. Majority of the fish sold in Uyo comes from different parts of Akwa Ibom state and beyond, which shows that Aspergillus is prevalent in Akwa Ibom state and wide spread in Nigeria. The four species of Aspergillus isolated in this study are causative agents of aspergillosis in man and animals. The high rate of occurrence of Aspergillus species among the various breeds of fish studied in Uyo markets could be attributed to poor sanitary conditions in environments market coupled the unhygienic handling and processing techniques, as evidenced by high microbial load counts. Furthermore, prolonged storage, high ambient temperature, heavy and prolonged rainfall and humidity conditions can facilitate fungal development with production of attendant mycotoxins in smoke-dried fish. These constitute a serious public health hazard and economic losses. Therefore, to reduce the prevalence of aspergillosis in Akwa Ibom State and possibly in Nigeria at large, there should be continuous national surveillance of Aspergillus infections. with proper implementation of public health policies to improve food hygiene in Uyo markets.

5.1 Recommendations

1. There is need for further studies to identify the circulating strains or serotypes of *Aspergillus* species both from contaminated fish and infected individuals in Uyo, and possibly from other neighbouring states. This will help to develop proper diagnostic and preventive strategies in order to reduce the outbreak of aspergillosis in Nigeria.



- 2. Fish species or strains that are naturally resistant to fungal infections can be selected to reduce the risk of disease transmission in aquaculture and good hygiene practices should be encouraged during processing and storage.
- 3. Control of the aflatoxin during the production and processing of fish is very important for human safety. According to the European Commission, the overall allowable level for the specific toxins in food is 0.5–200 ppb and this should be enforced in Nigeria.
- 4. Processed fish should be packaged and stored with zinc oxide nanoparticles (ZnO-NPs). ZnO-NPs is a food additive that has selective toxicity against wide range of microbes, and has good interaction properties, moisture absorption, monolayer moisture, and solubility.
- 5. Yeasts and bacteria (e.g. *Bacillus* species) which produce volatiles and diffusible compounds of generally regarded as safe (GRAS) should be explored as biocontrol strategies because of their antagonistic activity against mycotoxin-producing fungi.

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