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FULL PAPER

Effect of Moringa leave Marinade on aflatoxin in fresh and smoked African catfish (*Clarias gariepinus*)

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Abstract

This study aimed at evaluating the effect of Moringa leaves Marinade (MOM) on aflatoxin contamination of *Clarias gariepinus*. A total of thirty fresh fish samples (n = 30) grouped into four; Fresh fish without smoking and storage, smoked fish +1% and 2% *Moringa oleifera* Marinade (MOM) respectively and Control (0% MOM) were subjected to microbiological and HPLC analysis while storing for 0-3 months. Mean CFU/g of 2.8 and 3.1 x 10^2 CFU/g for bacteria and fungi were recovered, respectively. Furthermore, four bacteria and fungi genera each of which *Aspergillus* spp. was the most predominant (57%) were recovered from the fishes. Aflatoxin concentration increased progressively in 0% MOM smoked fish as the storage period increased, while aflatoxin concentration reduced in the fishes treated with 2% MOM ($p \geq 0.05$). On average, between 1-40% reduction in aflatoxin concentration and increased keeping quality was enhanced with 2% MOM treatment. These findings recommend the possibility of the use of moringa leaves in the treatment of commercially smoked fish.

1. Introduction

African catfish *Clarias gariepinus* is one of the most popular fishes in Africa and generally known for its nutritional advantages and palatability. It is widely cultured because of its high ability to withstand stress, the minimal requirements with regard to management and its fast growth rate (Hams *et al.,* 2017).

Fishes are poikilothermic aquatic vertebrates with scales, fins and gills (Adebayo-Tayo *et al*., 2008). They are highly perishable and could become inedible within 12 hrs at tropical temperature (Pigott, 2015). Efforts have been made on improving and extending the storage quality of fish using diverse processing and preservation methods such as salting, smoking, drying and refrigeration (Adeyemi *et al.*, 2013; Pigott, 2015). Fish processing should be done as soon as the fish dies to prevent spoilage and microbial proliferation (Fish and fish product, 2011). Efficient processing of fish is necessary to achieve high yield and profits which could boost the nation's economy (Davies and Davies, 2009). The extent of microbial contamination in food produce differs with geographic location and the susceptibility of commodities to the invasion of fungi during processing and storage (Jonathan and Esho, 2010).

Aflatoxins are toxic fungal metabolite produced by *Aspergillus parasiticus*, *Aspergillus flavus*, and *A.nomius* that occurs in oilseeds, grains and fishes during production processes and storage (Herrmana *et al*., 2014). Aflatoxin B1 is considered the most potent of the aflatoxins and known as major causes of hepatocellular carcinoma and immune suppression which affect human and animals adversely (Williams *et al*., 2004). Effects of aflatoxin B1 in fish are generally linked to the age and species of fish and the aflatoxin quantity in the feed (Eaton and Groopman, 1994). Fish are often contaminated primarily by different microorganisms such as fungi, yeast and bacteria (D'Andrea, 2012).

Smoking is a common traditional method of fish preservation that prevents contamination in most developing countries. Fish preservation by smoking is done after they are out of water. Smoking is believed to confer good taste and odour on food products generally and it enhances longer shelf-life through its oxidative and anti-bacterial properties, lowering of pH and speeding up the drying process and acting as an antagonist to spoilage organisms (Eyo, 2012).

Wood smoke is a mixture of complex chemical gases and volatile substances and the volatile substance is

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responsible for the characteristic aroma (Abowei and Tawari 2011). Wood smoke contains up to 100 polycyclic aromatic hydrocarbons (PAHs) whose derivatives are mostly carcinogenic (Stołyhwo and Sikorski, 2005). The outer part of heavily smoked fish contains up to 50 µg/kg of PAHs while mild hot-smoked fish contains only about 0.1 µg/kg or even less (Abowei and Tawari, 2011).

Moringa oleifera is a small-sized tree, fast-growing and evergreen, drought-resistant tree of the family Moringaceae, native to tropical and subtropical regions of south Asia, (GRIN, 2017). *M. oleifera* is commonly known as "Drumstick" (Trapti *et al*., 2009) and has been proven as a good antimicrobial agent (Chen and Verdes, 2009). *M. oleifera* has great potential for prevention of different diseases like nutrient deficiency, cancer, and anemia as well as for dirty water purification. (Hermawan *et al*., 2012). *Moringa oleifera* was also promoted by the World Health Organization (WHO) as an alternative to an imported food source to treat malnutrition (Mishra *et al*., 2012). The leaves are the most nutritious part of the plant (Peter 2008), they are a good source of minerals, vitamins A, B and C (Talhaliani and Kar, 2000; Abdull Razis *et al*., 2014). According to Kar *et al*. (2003) *M. oleifera* leaves are also reported to possess anti-diabetic and hypertensive agent.

Post-harvest losses have been estimated to claim 40% of total fish in Nigeria. It was estimated that 20 to 50% of the fish produced in the rural areas are lost due to improper handling, processing and preservation practices adopted by fish processors and farmers (Eyo, 2012). Fungal diseases are the second most serious cause of losses in aquaculture. Fish fungal contamination is a serious problem that could affect fish farmers and consumers in Africa (Eyo, 2012). Fungal and aflatoxin contamination of fish in Nigeria (Adebayo-Tayo *et al.,* 2008; Adeyemi *et al.,* 2013) and other countries (Paul *et al*., 2018) has been reported but report on mitigation strategies to reduce fungal proliferation and aflatoxin contamination is scarce. However, control of aflatoxins in fish using different remedies such as ozone and clay has been reported but information on the effect of dried Moringa leaves infusion on fungal growth, aflatoxin contamination of catfish and the potential effects of aflatoxin in fish on human is reported for the first time in this region (Ogun State, South-West Nigeria) to the best of our knowledge.

This study aimed at evaluating the effects of Moringa leaf infusion on aflatoxin contamination of fresh and smoked catfish.

2. Materials and methods

2.1 Sample collection and study area

The experiment was carried out in September 2019 at the Microbiology Laboratory of the Federal University of Agriculture, Abeokuta which is located at 7°9'39" North 3°20'54" East. Abeokuta is the largest city and state capital of Ogun in Southwest Nigeria. It is situated on the East bank of the Ogun river, near a group of rocky outcrops in a wooded Savannah (Hoiberg, 2010).

2.2 Preparation of Moringa leaf marinade

Fresh leaves were collected from the *Moringa oleifera* plant from Federal University of Agriculture Farm, Abeokuta Ogun State. It was ensured that the plant was physically healthy and uninfected. The leaves were washed under running tap water to remove dust and other foreign materials and dried. Fresh leaves of *Moringa oleifera* (35-40 g) were shade-dried to constant weight for 4 days at ambient temperature (32±2°C). The dried leaves were ground into powder using Christis® Commercial Food Blender for 3 mins.

Moringa oleifera marinade (MOM) was prepared by adding separately specific quantity (2.5 g and 5 g) of *Moringa oleifera* leaf powder to 250 mL of distilled water to form 1% and 2% MOM and mixed properly (Adeyemi *et al.,* 2013).

2.3 Treatment of fish

A total of thirty pieces of fresh catfish (*Clarias gariepinus*) weighing between 95-100 g were purchased from ten different fish retailers in Lafenwa market (3 pieces per retailer) in Abeokuta, Ogun State. Samples were collected in sterile ziplock bag and then transferred to the laboratory for microbiological analysis.

The fishes were gutted using a sterile sharp knife by cutting laterally from the gill through the belly to the anus and thoroughly washed and rinsed. The total weight after gutting was 80 g. The fishes were randomly grouped into three for application of treatment (Control (0%), 1% and 2% MOM). The fishes but the control were soaked in the Marinade for 2 hrs. The fishes were arranged for smoking in the kiln consisting of three twin tiers and subjected to hot smoking for 6 hrs using charcoal as a heat source. The fishes were interchanged every 3 hours to ensure even distribution of heat. The smoke-dried fishes were afterwards stored for 3 months in air-free netted boxes placed on a raised platform in the laboratory in a cool dry environment at room temperature (20-25°C) to prevent flies contamination throughout the storage period (Adeyemi *et al.,* 2013). Aflatoxin concentration analysis of the fish using HPLC

was done at 0-, 1-, 2- and 3-months interval. Prior to soaking in the marinade, 4 cut pieces of the fresh fish were selected randomly per retailer and these were used for the microbiological and HPLC analysis of the fish without storage and treatment (This is referred to as Fresh fish in this manuscript). This was done to know the initial microbial load and concentration of aflatoxin in the fish before processing.

2.4 Isolation of fungi and bacteria from fish samples

A piece of the cut fish was randomly selected from each of the three categories of treatment (Fresh fish, control, 1% and 2% MOM and storage duration 0, 1, 2 and 3 months), the whole fish abdominal muscle for each treatment per sample was aseptically crushed using sterile mortar and pestle. One gram of the crushed fish was weighed from each homogenous lot by placing a sterile foil directly on a clean laboratory weighing balance (ACZET Cx series) and the sample decimally diluted six times (10^6) as previously described (Klich, 2002; Samson, 1995). A total of 20 mL of sterilized molten nutrient agar and Potato Dextrose Agar (PDA) supplemented with 0.01% chloramphenicol were cooled to 45°C and poured separately unto plates containing 1 mL aliquots of each sample in triplicate and the plates gently swirled and allowed to solidify. The nutrient agar plates were incubated at 30°C for 48 hrs for determination of Total Viable Count (TVC) and Potato Dextrose agar plates at 28°C for 72 hrs for determination of fungal counts.

Fungal and bacteria colonies were counted visually and Colony Forming Unit calculated as :

$$CFU/g = \frac{\text{Number of colonies} \times \text{reciprocal of the diluting factor}}{\text{Volume plated}}$$

2.4.1 Phenotypic identification of fungal isolates and bacteria isolate

Mould isolates were carefully transferred and purified on PDA plates (supplemented with 0.01% chloramphenicol) and incubated at 25°C for 5 days. The purified isolates were maintained at 4°C as PDA plugs in 4 mL vials containing sterile water. Identification of all fungal isolates was based on the examination of characteristic morphological (macroscopic and microscopic) features such as colony color, texture and size, conidial features and size of sclerotia by the 5th day of incubation as previously described (Oyedele *et.al*., 2017; Adetunji *et al*., 2018).

The bacteria isolates were purified by subculturing and characterized using a standard biochemical test such as; colony morphology, cellular morphology, staining and biochemical reactions such as catalase test, oxidase test, Voges Proskauer test, Indole test (Cheesbrough, 2006; Oyeleke and Manga, 2008).

2.5 Quantitative analysis of aflatoxin by High Performance Liquid Chromatography

2.5.1 Chemicals and standards

Solvents such as acetonitrile, methylene chloride, and methanol were of HPLC grade. The standard aflatoxin was purchased from Chromogen International limited.

2.5.2 Extraction and quantification of aflatoxin in fish samples

Fish samples were prepared for analysis by the modified method of (Hassan *et al.,* 2011). The fish (25 g) was weighed and crushed out together with 5 g of sodium chloride and placed in a jar. Methanol: water (150 mL) in the ratio 80:20 was added to the jar and the sample was blended at a high speed for 3 mins to obtain a homogeneous mixture. The mixture was shaken for 30 mins on an orbital shaker (Lab-line® Orbit Shaker, Illinois) and filtered through a Whatman filter paper. Addition of 40 mL of 10% NaCl, 25 mL n-hexane, and finally 25 mL dichloromethane was done and the extracts were collected and evaporated to dryness in dark bottles for 30 mins. The fish sample (50 mL) was passed through an immune affinity cartridge/column. The column contains specific antibodies bound on to solid support materials. As the sample goes through the column, antibodies selectively bind with any aflatoxin (antigen) contained in the sample matrix. The column was prewashed with 10 mL of distilled water twice, using a disposable 25-mL syringe. The fish sample was loaded onto a cartridge and eluted at a flow rate of 10mL/min after which the eluate was discarded. The extracts were pooled and concentrated to about 0.5mL under a gentle stream of nitrogen gas. A volume of 200 µL was injected and analyzed for aflatoxin by HPLC. Extracted fish samples were analyzed by an HPLC system consisting of a Waters 6000A solvent delivery system and a WISP 710B sample processor for sample injections (Waters Associates India).

2.5.3 Method validation for HPLC analysis for aflatoxin determination

The operation conditions for the HPLC machine is shown in Table 1. Samples were eluted isocratically on a radically compressed 10 µm octadeclsilane cartridge (Waters Associates India) with a mobile phase of acetonitrile: methanol: water (15:15:70) at a flow rate of 0.8 mL/min, a prefilter was placed between the injector and the cartridge. The aflatoxin was detected fluorometrically (excitation wavelength, 365 nm; emission wavelength, 425 nm) with a fluorescence

detector (model 420C, Water Associates). The HPLC chromatograms were recorded on a Water Data Module (Waters Associates) at a chart speed of 1.0 cm/min. Matrix effect (ME) for each analyte was calculated by comparing the slope of the standard calibration curve with the matrix-matched calibration curve for the same concentration levels, while the sensitivity of the methodology or system used was evaluated by the limit of detection (LOD) and limit of quantification (LOQ), which were estimated for a signal-to-noise ratio (S/N) × 3 and × 10, respectively, from chromatograms of samples spiked at the lowest validation level. The accuracy of the analysis was evaluated through recovery studies of the spiked sample by calculating the ratio of the peak areas for each aflatoxin at four additional levels of 25, 50, and 100 and 200 µg/kg for all aflatoxins analyzed (AFB1, AFB2, AFG1, AFG2). The concentration of each aflatoxin type (AFB1, AFB2, AFG1 and AFG2) in the fish samples were determined using the peak area ratios and the retention time comparing them with the samples containing known concentrations of aflatoxin; spike sample and relevant standard calibration curves (AOAC, 2007).

Table 1. Optimization condition for High Performance Liquid Chromatography analysis

Apparatus/activity	Condition
Derivatisation	KOBRA Cell at 100 Ma
Pump flow rate	1.0 mL/min
Column heater	Maintain guard and analytical columns at 40°C
Injector	Auto sampler
Injector Volume	200 µL
Elution order	G2, G1,B2,B1
Linear Curve	0.98 regression
LOD	B1=0.3, B2=0.03, G1=0.12, G2=0.12

2.6 Data analysis

Data were analysed using SPSS (package version 20.0) (SPSS Inc., Chicago, IL, USA). The mean bacterial and fungal CFU/g of the fishes were compared by one-way analysis of variance (ANOVA) and Statistical significance was set at probability, $P < 0.05$. Descriptive analysis was used to evaluate the effect of different concentration of MOM on aflatoxin contamination of fish during storage.

3. Results

3.1 Microbial load and diversity in fish samples

The colony forming unit (CFU/g) for bacteria and fungi ranged from 1.1-7.8 x 10^2 CFU/g and 1.1-3.9 x 10^2 CFU/g with mean colony forming unit of 2.8 x 10^2 CFU/g and 3.1 x 10^2 CFU/g for bacteria and fungi respectively (Table 2). A total of 4 different bacteria genera (*Bacilli*, *Staphylococcus*, *Serratia*, *Pseudomonas*) belonging to 5 different species were isolated and identified from the fish using the morphological and biochemical characteristics of the isolates (Table 3), while fungi isolates belonging to 4 different genera (*Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus*) of which *Aspergillus* spp. was the most predominant (57%) were recovered from the composited fish samples using the macroscopic and microscopic characterization of the fungal isolates (Table 4).

Table 2. Mean total microbial population of fresh fish

Fish Samples	Total Bacteria Count CFU/g	Total Fungal Count CFU/g
Retailer 1	$1.6\times10^2\pm 0.06^a$	$1.7\times10^2\pm 0.65^a$
Retailer 2	$4.7\times10^2\pm 0.17^b$	$3.6\times10^2\pm 0.09^b$
Retailer 3	$2.3\times10^2\pm 0.35^c$	$1.6\times10^2\pm 0.54^c$
Retailer 4	$2.9\times10^2\pm 0.43^c$	$1.6\times10^2\pm 0.71^c$
Retailer 5	$4.1\times10^2\pm 0.25^d$	$3.9\times10^2\pm 0.37^d$
Retailer 6	$1.2\times10^2\pm 0.08^a$	$3.0\times10^2\pm 0.06^a$
Retailer 7	$7.8\times10^2\pm 0.21^a$	$1.9\times10^2\pm 0.01^b$
Retailer 8	$3.5\times10^2\pm 0.42^a$	$1.4\times10^2\pm 0.02^b$
Retailer 9	$1.1\times10^2\pm 0.04^d$	$1.1\times10^2\pm 0.04^d$
Retailer 10	$2.8\times10^2\pm 0.32^d$	$3.1\times10^2\pm 0.51^d$
Mean Count	2.8 x 10^2	3.1 x 10^2

Values are expressed as mean±SD. Values with the same superscript within the column are not significantly different ($P>0.05$).

Table 3. Morphological and biochemical characterization of bacteria isolated in fish

S/N	Isolate Code	Shape	Colour	GR	CA	CI	CO	UR	IN	MO	GL	SU	LA	H_2S	GAS	Suspected organism
1	Control	Circular	Off-white	rod	+	+	-	+	-	+	+	-	-	-	+	*Bacillus cereus*
2		Spherical	Pink	cocci	+	+	+	+	-	+	+	+	-	-	+	*Serratia marcescens*
3	Treated Fish	Spherical	Golden yellow	cocci	+	+	+	+	-	+	+	-	+	-	-	*Staphylococcus aureus*
4		Spherical	White	rod	+	+	-	+	-	+	+	+	+	-	+	*Bacillus subtilis*
5		Irregular	White	rod	+	+	-	+	-	+	+	+	-	+	+	*Pseudomonas fluorescens*

+ = Positive, - =Negative, CS = Control sample, MS = Moringa sample, CI = Citrate, SU = Sucrose, IN = Indole, CO = Coagulase, LA = Lactose, UR = Urease, H_2S = Hydrogen sulphide, GR = Gram reaction, MO = Motility, GAS = Gas production, CA = Catalase test, GL = Glucose.

Table 4. Identification of fungi isolates found in the fish samples

S/N	Isolate Source	CS	TS	SEP	S	SO
1	Control sample	Deep brown-black	Conidia	Septate	Spherical, Globose and very rough	*Aspergillus niger*
2	Control Sample	Green/gray green	Conidia	Septate	Flask-shaped phialides and globose	*Penicillum chrysogenum*
3	Control Sample	White-pink	Microconida	Aseptate	Oval- club shape with a flat base	*Fusarium* sp
4	Treated Samples	Deep brown– black	Conidia	Septate	Spherical, globose and rough	*Aspergillus niger*
5	Treated Samples	Pure white	Conidia	Septate	Globose and smooth	*Aspergillus Candidus*
6	Treated Samples	Dark green colony	Conidia	Septate	Spherical, rough and thick	*Aspergillus parasiticus*
7	Samples	White-black	Sporangia	Aseptate	Ovoid	*Rhizopus* sp

TS = Type of spores, S = Shape, SEP = Septation, CS = Color of spores, SO = Suspected organism.

Table 5. Validation and repeatability of measurement using fish samples

Sample (ng/kg)	Retention time (min)	Area percent (%)	Recovery (%)
STDAFM 2.0	5.97	84.734	100
STDAFM 1.0	5.97	90.756	100
STDAFM 0.5	5.97	90.558	100
STDAFM 0.25	6.5	90.189	100
SPIKE 2.5	5.98	78.19	99.7
SPIKE2.5	5.99	77.13	100.13
SPIKE2.5	5.97	90.44	100

Table 6. Effect of *Moringa oleifera* Marinade concentration on aflatoxin contamination of stored smoked fish

Aflatoxin Concentration (µg/kg)										
Source	Fresh Fish	0% MOM (Control)			1% MOM			2% MOM		
	0 mth	1 mth	2 mth	3 mth	1 mth	2 mth	3 mth	1 mth	2 mth	3 mth
R1	12	12	18	19	12	12	13	12	12	11
R2	17	17	23	25	17	17	17	16	16	16
R3	3	4	4	4	3	3	4	3	3	3
R4	8	9	8	12	8	8	8	8	8	7
R5	12	12	13	12	12	13	13	12	12	12
R6	6	7	8	11	6	6	7	6	6	6
R7	0	0	1	1	0	0	1	0	0	0
R8	16	16	16	19	16	16	17	16	16	16
R9	9	9	9	9	9	9	9	9	9	9
R10	3	4	6	6	3	3	4	3	3	3
Mean Conc.	8.6	9	10.6	11.8	8.6	8.7	9.3	8.5	8.5	8.3

3.2 Quantification of aflatoxin in Moringa treated smoked fish

The operating conditions for the HPLC system are shown in Table 1. The standard calibration curves showed good linearity with R^2 values ranging from 0.98 to 1, the limit of detection (LOD) ranged from 0.03-0.3 µg/kg for each toxin with percentage recovery of between 99.7-100% and retention time of 5.97-6.50 mins for the aflatoxin standards and spiked samples (Table 5). The result showed the presence of aflatoxin in fresh catfish (Table 6). It was observed that aflatoxin concentration increased progressively (9.0-11.8 µg/kg) in smoked fish without moringa treatment (0% MOM) as storage period increased, treatment with 1% MOM prevented further increase in the aflatoxin concentration of the fish until the 3rd month, while 2% MOM treatment was able to suppress and further reduce the increase of aflatoxin concentration during the period of storage (Figure 1). On average, between 1-40% reduction in aflatoxin concentration was achieved after treatment while comparing samples treated with 0% and 2% MOM respectively at the end of the storage period.

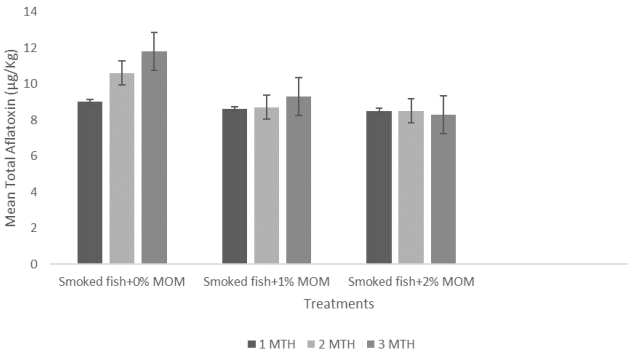

Figure 1. Effect of Moringa treatment and time of storage on aflatoxin content of smoked fish

4. Discussion

Among muscle food, fish is the most perishable and it easily loses freshness after death due to autolysis and microbial spoilage (Matak *et al*., 2005; Dehghani *et al.*, 2018). Contamination of smoked fish by Mesophilic bacteria and fungi are well documented in the literature (Dutta *et al*., 2018), but this work is reporting for the first time to the best of our knowledge the incidence of mesophilic bacteria and fungi in catfish in Ogun State. The fungal contamination level ($3.1x\ 10^3$) in the smoked fish reported here is however lower compared with previous report of higher fungal contamination in smoked dried fish in Nigeria and other countries (Olayemi *et al*., 2012; Saritha *et al*., 2012; Job *et al*., 2015). Job *et al*. (2015), reported a fungal load of 0.00 to 5.63×10^3 CFU/g in smoked fish sold at different locations in Jos with mean fungal load ranging from 2.00×10^3 to 3.09×10^4 for the various locations, furthermore load of 7.00×10^3 CFU/g was reported by Olayemi *et al.* (2012) previously in Kano. Similarly in India, a contamination level of 1.3×10^4 to 2.2×10^4 CFU/g had been reported for sun-dried fish (Saritha *et al*., 2012), likewise, 1.1×10^2 to 9.3×10^4 CFU/g in Ghana for smoke-dried fish and 5.22 $\log_{10}$ (CFU/g) for smoke-dried fish in Cameroon (Ahmed *et al*., 2011). The bacteria load in the fresh fish was within the permissible limit of 100×10^3 for aerobic bacteria in fish (Elliot and Michener, 1960). The lower microbial load recorded in this report might be due to the fact that the fishes were processed and cleaned at the laboratory scale level contrary to the previous report where most of the fish samples were bought at the open markets where they have been exposed to a lot of contamination from the environment and handlers.

Fish and fish products have been implicated as a source of 10%–20% of foodborne diseases (Pilet and Leroi, 2011) and the presence of pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella* spp., pathotypes of *Escherichia coli*, and *Listeria monocytogenes* have been reported in smoked fishes (Adeyeye *et al*., 2015; Ineyougha *et al*., 2015; Udochukwu *et al*., 2015; Ayeloja *et al*., 2018; Likongwe *et al*., 2018). In addition, *Bacillus cereus, Serratia marcescens, Staphylococcus aureus, Bacillus subtilis, Pseudomonas fluorescens* isolated from the MOM treated fish corroborates the findings of previous authors (Napoleon *et al*., 2009; Adeyemi *et al.,* 2013) who reported the incidence of similar bacteria in smoked fish.

The high load of mesophilic bacteria in the fish might be due to poor quality of raw material for compounding the fish feed, poor post smoking handling by vendors as well as environmental pollution at site of sales (Kpodekun *et al*., 2014). However, most of the identified bacteria are normal flora of fishponds and skin of fish processors (Fafioye, 2011). There is also the probability that the pathogenic microbes were not fully eliminated during smoking. Thermosensitive bacteria like *Enterobacteriaceae* which are used as indicators of hygiene conditions and contamination of food after cooking (Health Protection Agency, 2009) were not detected in the MOM treated fish. The presence of *B. cereus* and *S. aureus* in the fish calls for concern as the consumers' health is at risk of foodborne diseases (Dierick *et al*., 2005; Lindström *et al.*, 2011).

The incidence of mycotoxigenic fungi in the smoked fish reported in this work is an indication that the consumers of smoked fish might be at risk of mycotoxicosis as the isolated fungi; *Aspergillus niger, Aspergillus candidus, Aspergillus parasiticus, Penicillium chrysogenum,* and *Fusarium* sp. are potential mycotoxin producing fungi. The *aspergillus* sp. which were the most predominant (57%) in the fish are known aflatoxins producers especially *A. flavus*, *A. parasiticus and A.niger* which was recently reported as an aflatoxin producing fungus (Mircea *et al*., 2008) apart from OTA production which is a known toxin produced by *A.niger.* Consumption of fish contaminated with aflatoxins could lead to a medical condition called aflatoxicosis (Lewis *et al*., 2005; Wild and Gong, 2010). Various studies have reported the occurrence of aflatoxigenic fungi in smoked fish (Wogu and Iyayi, 2011; Ayeloja *et al*., 2018; Babalola, *et al*., 2018), which under certain conditions can produce mycotoxins. Most of the fungal agents associated with the contamination of smoked and smoke-dried fishes, such as *Aspergillus*, *Penicillium*, *Zygomycetes* and yeasts are opportunistic pathogens (Hungerford *et al*., 1998) causing varying degrees of health problems to both animals and man. A major challenge with some of the toxins produced by the toxigenic fungi is that most of the mycotoxins produced are heat stable and persist in the fish even after salting and smoking (Swaminathan and Sparling 1998; Tournas *et al*., 2001) which also continues throughout the storage period. These toxins cause serious systemic dysfunctions and public health hazards and several cases of human gastroenteritis, severe diarrhoea and food poisoning outbreaks have been recorded after smoked fish consumption.

The moringa treatment (2% MOM) imposed a fungistatic effect on the aflatoxin producing fungi present in the fish from the first month of treatment to the third month, thereby reducing the concentration of aflatoxins in the fish as storage period increases, it also prevented further increase in the initial concentration of aflatoxin in the fish before storage. Treatment of fish with *Moringa oleifera* enhanced the shelf life of the

smoked catfish for three months and this corroborates the report of Adeyemi *et al*. (2013) who enhanced the shelf life of stored smoke-dried catfish by preventing it against microbial spoilage using different concentration of Brine and *Moringa oleifera* marinade. There were no significant differences observed in the efficacy of 2% and 1% MOM treatment against aflatoxins, however, 2% MOM treatment is recommended as it ensured constant inhibitory action against the increase of aflatoxin producing molds thereby reducing the aflatoxin content in the fishes throughout the experiment.

MOM antimicrobial activity could be traceable to the presence of some phytochemicals, the specific components of Moringa preparations that have been reported to have hypotensive, anticancer, and antibacterial activity include 4-(α-L-rhamnopyranosyloxy), benzyl isothiocyanate**,** niazimicin, pterygospermin**,** benzyl isothiocyanate**,** and 4-(α rhamnopyranosyloxy) benzyl glucosinolate. isothiocyanate (Guevara *et al*., 1999). It is also rich in vitamins and minerals as well as other more commonly recognized phytochemicals such as the carotenoids (including β-carotene or provitamin A). Lower Concentration of aflatoxins was reported in the MOM treated fish and fresh fish as compared to the report of Marijani *et al.,* 2017 who reported aflatoxin concentration as high as 90.1 µg/kg in his fishes. The aflatoxin content of the fresh fish was within the permissible level of 20 µg/kg recommended by NAFDAC in Nigeria for raw foods while 70% of the ready to eat smoked fish were contaminated with aflatoxins concentration higher than the EU permissible limit of 4 µg/kg permitted in ready to eat food product (EC, 2003).

The presence of aflatoxin in the catfish could be attributed to the use of moldy grains and unwholesome raw materials in preparation of fish feeds (Akwuobu *et al*., 2019). Also feeding fishes with a visibly moldy meal is a common practice among farmers and this would in return leads to aflatoxin-contaminated fishes. The climatic condition (32^{o}C and above) in the tropic region could also be an implicating factor to the high aflatoxin content of fish and other fish products (Fallah *et al.,* 2014). Human consumption of aflatoxin-contaminated fish could pose serious health challenge such as liver cancer, hemorrhages and could eventually lead to death (Spring *et al*., 2005). Furthermore, it was reported that long term exposure of fish to low aflatoxin concentration in feeds causes chronic aflatoxicosis and accumulation of aflatoxins in lambari (*Astyanax aitiparanae*) fish muscles and liver after 90 days of exposure (Michelin *et al*., 2017).

Further studies could be carried out to determine the effect of MOM on catfish for a longer period of storage.

5. Conclusion

Fresh catfish analysed in this report were contaminated with foodborne pathogens as well as aflatoxin producing fungi which might impose a health risk on its consumers. This study concludes that 2% MOM is suitable for enhancing the keeping quality of smoked dried catfish fresh for 3 months.

The green patches of *Moringa oleifera* leave seen on MOM treated fish could reduce the acceptability of such treated fishes by their consumers, hence it is recommended that the Moringa marinade be sieved with a fine mesh prior to its application on the fish. It is also recommended that Moringa Oleifera leave should be used as one of the raw materials for fish meal preparation. The role of good hygiene by fish handlers and vendors cannot be overemphasized. Relevant monitoring agencies that will enforce good hygiene practices on handlers should be empowered by the government. This would improve a great deal the quality of traditionally processed smoked fish.

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Conflicts of interest

The authors declare no conflicts of interest.

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