

Determination of Aflatoxin B₁ and G₁ in Fresh, Sun-dried, And Smoke-Dried Tilapia and African Catfish Marketed for Human Consumption in FCT-Abuja, Nigeria

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Abstract: Aflatoxins are fungal metabolites that contaminate food and have the potential of causing deleterious effects to humans and animals when consumed. This study determined and compared the occurrence of aflatoxins B₁ (AFB₁) and aflatoxins G₁ (AFG₁) in fresh, sun-dried and smoked-dried African catfish and Tilapia sold in major markets in the FCT. Two hundred and sixty (260) fishes were sampled from 6 major markets in four Area Councils of the Federal Capital Territory (FCT), for aflatoxin analysis using High Performance Liquid Chromatography (HPLC). Mycological examination for the presence of aflatoxigenic moulds was also determined. The mycological examination showed that 24.6% of the samples were contaminated with *Aspergillus flavus* and *A. parasiticus*. Of these, 15.77% were aflatoxigenic. The aflatoxin B₁ concentration ranged from 9.61 to 24.68 ng/g and that of AFG₁ ranged from 7.68 to 10.57 ng/g. Means of storage and processing played a major role in aflatoxin contamination as observed in the high concentration in sun-dried fish (AFB₁ - 24.68 ng/g; AFG₁ - 10.57 ng/g) as compared to fresh (AFB₁ - 9.61 ng/g; AFG₁ - 7.68 ng/g) and smoke-dried (AFB₁ - 13.75 ng/g; AFG₁ - 9.02 mg/g) fishes. This study has shown that African catfish and Tilapia sold in major markets within the FCT were contaminated with aflatoxins. The occurrences and levels of AFB₁ and AFG₁ in the fish samples indicated a major problem in controlling invasion of fish with *Aspergillus* fungi and aflatoxins. The mean concentration of aflatoxin B₁ in sun-dried fish observed in this study was higher than the maximum recommended level of 20 µg/kg, indicating a potential risk of aflatoxin poisoning for consumers. Strategies to control aflatoxin exposure and its effects need to be implemented to prevent potential aflatoxicosis. Necessary infrastructure for fish processing and storage needs to be utilized to prevent contamination and the process monitored to ensure that there is absence of aflatoxigenic moulds or aflatoxins higher than the maximum allowable levels.

In several African nations, about 17.5% of animal protein intake comes from fish [3]. Fish is an inexpensive source of animal protein with little to no religious scepticism attached, giving it an advantage over pork and beef [4]. Fish is a rich source of amino acids suitable to high carbohydrate diets [5]. It is also a significant source of essential minerals and oils including polyunsaturated fatty acids, monounsaturated fatty acids and saturated fatty acids [5] [6].

African catfish (*Clarias gariepinus*) and tilapia (*Oreochromis niloticus*) are known as one of the most widespread tropical species in western Africa [7]. These are also the most farmed species of fish in Nigeria. The aquaculture industry in Nigeria has seen an exponential growth over the years [8] and, as a result, the proliferation of various styles and brands of fish feed has become the reality of the industry. Due to inadequate infrastructural growth, the storing of imported and locally produced fish feed has become problematic and thus permits contamination of feed by aflatoxin [9].

Due to the high nutritious content of fish, it is easily contaminated and supports the growth of numerous microorganisms. The perishability of fish is heightened since the relative ease of preservation is not readily attainable. Preserving fish in Nigeria involves processes that should normally impede growth of microorganisms either by the addition of growth inhibiting ingredients or adjusting storage conditions by freezing or drying [10]. The different preservation methods utilized by locals in Nigeria such as smoke-drying, freezing, brining and sun drying have been reported to be inefficient in mitigating contamination and proliferation of microorganisms [11].

Aflatoxins are highly toxic, carcinogenic, fungal secondary metabolites produced primarily by the *Aspergillus* [12]. There are at least 13 different types of aflatoxins, but aflatoxins B₁, B₂, G₁, and G₂ are the most significant ones with B₁ known to be the most toxic and the most prevalent of the aflatoxins [13].

Aflatoxins pose possible adverse threats to humans and farmed animals [14]. Aflatoxin reportedly causes immune suppression, growth disorder, changes in gene expression,

I. INTRODUCTION

Background

Traditional fishing is a large part of the overall catch of fish in most sub-African countries [1] and, due to the decline in wild fish population, aquaculture practices have frequently been promoted, but the decline in wildlife has encouraged aquaculture [2]. Fish is a key component of the diet and one of the few sources of animal protein available to many Nigerians [1] [2].

changes in biochemical parameters in fish, tissue damage in fish [15] [16] [17]. Aflatoxins are also capable of disrupting DNA replication, inhibiting RNA polymerase and disrupting mRNA transcription, inhibiting the transport of amino acids and protein synthesis, also cause poor growth rate, and inducing gross and microscopic lesions in fish [18].

Increasing evidence indicates that aflatoxin contamination is a major food safety issue in Nigeria due to inadequate storage, management and eventual food spoilage [19] [20]. The present study was conducted to determine and compare the occurrence of aflatoxins B₁ (AFB₁) and aflatoxins G₁ (AFG₁) in fresh, sun-dried and smoked-dried African catfish and Tilapia in the Federal Capital Territory.

II. MATERIALS AND METHODS

Study Area

The study area covered major markets in four Area Councils of the FCT; Abuja Municipal Area Council, Bwari, Kwali, and Gwagwalada Area Councils. The FCT has a landmass of about 8000km² and lies within latitude 9^o 25¹ N and 9^o20¹ N of the equator and longitude 5^o 45¹E and 39¹E.

Preparation of standard solution

Preparation of aflatoxin standard solution was carried out by using a method reported by Sailaja *et al.* [21]. Briefly, 100 µl from AFB₁ and AFG₁ were made up to 20 ml separately using methanol. Standard concentration having 10 ng/ml aflatoxin was obtained. From this, required dilutions were prepared accurately. Dilutions prepared ranged from 0.5 – 3.5 ng/ml.

Mycological Analysis

Isolation of fungal species was carried out using the spread-plate method after dilution [22]. Under aseptic conditions, 1 g from each stomached sample was homogenized in 9 ml of sterile distilled water followed by a serial dilution. One millilitre from each dilution was inoculated on isolation media and spread evenly using a sterile glass spreader and incubated at 30°C for 4 days. Primary isolation of fungi was carried out using Sabouraud Dextrose agar (SDA; Hi-Media) and suspected *A. flavus* and *A. parasiticus* were confirmed by inoculation on *Aspergillus flavus* and *parasiticus* agar (AFPA; Oxoid).

Identification of the *A. flavus* and *A. parasiticus*

Identification of *Aspergillus flavus* and *A. parasiticus* was carried out based on their phenotypic characteristics according to the method described by Bandh *et al.* [23]. Primary macroscopic morphological studies were carried out on AFPA. Microscopically, fungi were identified using lactophenol cotton blue.

Screening for Aflatoxin Production

Coconut Agar Medium (CAM) was used to screen for the fungi ability to produce aflatoxins. CAM was prepared following the method described by Davis *et al.* [24]. Ten

microliters of each of the isolate spore suspension was aseptically inoculated on CAM and then incubated at 27°C for seven days in the dark. Following incubation, the plates were then observed under UV light (365 nm) for fluorescence.

Quantification of Aflatoxin B₁ and G₁

Sample Extraction

Extraction was carried out using method described by Omeiza *et al.* [20] with some modifications. Briefly, 5 g of each of the pulverized fish muscle samples were extracted using acetonitrile, methanol and distilled water (3:1:1 v/v) respectively. The homogenate was left for extraction in the dark for 10 minutes. After which the homogenates were filtered using Whatman No. 1 filter paper and the filtrate was capped for HPLC analysis.

The method described by Herzallah *et al.* [25] was adopted for HPLC analysis. Chromatographic separation was carried on Inertsil ODS C-18 (250 × 4.6 mm; 5 µ id) column using water, acetonitrile, and methanol (60:20:20 v/v) as mobile phase at a flow rate of 1.0 ml/min and detection time set at 1 min. UV detection was carried at a wavelength of 365 nm. Sample volume of 20 µL was injected into the HPLC column and maintained at 40°C.

III. RESULT AND DISCUSSION

Distribution of Aflatoxigenic Fungi

In the current study, a total of 24.6% *A. flavus* and *A. parasiticus* contamination was observed. Of these, 15.0% occurred in African Catfish and 9.62% occurred in Tilapia. The occurrence of aflatoxigenic *A. flavus* in African catfish was 6.54% and 4.23% in Tilapia. Whereas, the occurrence of aflatoxigenic *A. parasiticus* in African Catfish was 3.08% and 1.92% in Tilapia. The occurrence of aflatoxigenic *A. parasiticus* in sun-dried fish was 3.85% and 1.15% in smoked-dried fish. In fresh fish, the occurrence of aflatoxigenic *A. flavus* was 0.77%, in sun-dried fish, the occurrence was 7.31% and 2.69% in smoke-dried fish (Table 1). The levels of aflatoxigenic moulds in smoke-dried fish observed in this study agrees with the submission of Sa'adatu *et al.* [26] who in their report stated that smoking if properly done is efficiently prevents microbial proliferation in fish.

The distribution of aflatoxigenic *A. flavus* in Gwagwalada was 3.46%, the occurrence of the fungus was 2.69% in Kwali similar occurrence was observed in Bwari, whereas an occurrence of 1.92 was observed in Abuja Municipal Area Council. The occurrence of *A. parasiticus* on the other hand was 3.08% in Gwagwalada and 1.92% in Kwali (Table 1). The higher occurrence in Gwagwalada could be attributed to the open display of fish in markets. This result corroborates the report of Sani *et al.* [27]. The presence of *Aspergillus* spp. in fresh *Clarias gariepinus* and *O. niloticus* could be attributed to the use of contaminated feed. This premise is supported by the observed correlation of fungal occurrence in fish and fish feed reported by Iqbal and Saleemi [28].

Table 1: Distribution of Aflatoxigenic Fungi

Fish types	No. of fish	<i>A. flavus</i> + <i>A. parasiticus</i>	<i>A. flavus</i>	<i>A. Parasiticus</i>	Aflatoxigenic +ve sample	<i>A. parasiticus</i>
	Samples tested	+ve samples	+ve samples	+ve samples	<i>A. flavus</i>	
African Catfish	130	39 (15.00)	21 (8.08)	18 (6.92)	17 (6.54)	8 (3.08)
Tilapia	130	25 (9.62)	16 (6.15)	9 (3.46)	11 (4.23)	5 (1.92)
TOTAL	260	64 (24.6 %)	37 (14.23%)	27 (10.38%)	28 (10.77%)	13 (5.00%)

Nature of sample	No. of fish	<i>A. flavus</i> + <i>A. parasiticus</i>	<i>A. flavus</i>	<i>A. Parasiticus</i>	Aflatoxigenic +ve sample	<i>A. parasiticus</i>
	Samples tested	+ve samples	+ve samples	+ve samples	<i>A. flavus</i>	<i>A. parasiticus</i>
Fresh	98	19 (7.31)	11 (4.23)	9 (3.46)	2 (0.77)	0 (0.0)
Sun-dried	96	34 (13.08)	19 (7.31)	12 (4.62)	19 (7.31)	10 (3.85)
Smoke-dried	33	11 (4.23)	7 (2.69)	5 (1.92)	7 (2.69)	3 (1.15)
TOTAL	260	64 (24.62%)	37 (14.23%)	27 (10.38%)	28 (10.77%)	13 (5.00%)

Nature Of sample	No. of fish	<i>A. flavus</i> + <i>A. parasiticus</i>	<i>A. flavus</i>	<i>A. Parasiticus</i>	Aflatoxigenic +ve sample	<i>A. parasiticus</i>
	Samples tested	+ve samples	+ve samples	+ve samples	<i>A. flavus</i>	<i>A. parasiticus</i>
AMAC	65	10 (3.85)	7 (2.69)	3 (1.15)	5 (1.92)	0 (0.0)
BWARI	65	17 (6.54)	12 (4.62)	5 (1.92)	7 (2.69)	0 (0.0)
KWALI	65	15 (5.77)	13 (5.00)	2 (0.77)	7 (2.69)	5 (1.92)
GWAGWALADA	65	22 (8.46)	5 (1.92)	17(6.54)	9 (3.46)	8 (3.08)
TOTAL	260	64 (24.62%)	37 (14.23%)	27 (10.38%)	28 (10.76%)	13 (5.00%)

Quantitative Aflatoxin

HPLC analysis indicated that sun-dried fish had a significantly higher ($p < 0.05$) AFB_1 (24.68 ± 2.57 ng/g) compared to fresh fish (9.61 ± 2.27 ng/g) and smoke-dried fish (13.75 ± 2.76 ng/g). There was no significant difference in the level of aflatoxin G1 contamination in fresh fish (7.68 ± 1.40 ng/g), sun-dried (10.57 ± 1.54 ng/g) and smoke-dried fish (9.02 ± 1.16 ng/g). The level of AFB_1 contamination was significantly higher than AFG_1 in all preservation methods (Table 2).

The level of aflatoxin contamination based on fish species indicated that no significant difference existed in the levels of AFB_1 in African catfish (19.48 ± 3.31 ng/g) and Tilapia (16.42 ± 6.59 ng/g). There was also no significant difference in the level of AFG_1 in African catfish and Tilapia fish (Table 3). This result is in contrast to the suggestions of Santacroseet al. [29]. Santacroseet al. [29] reported that the differences in susceptibility to aflatoxins in aquatic organisms appeared to correlate with interspecies variations in the efficiency of AFB_1 biotransformation.

The levels of contamination of AFB_1 in sun-dried African Catfish and Tilapia was high, indicating public health concern for fish consumers. Contamination of fish with aflatoxigenic moulds cause varying public health concerns [27].

Table 2: Level of Aflatoxin Contamination Based on Method of Preservation

Preservation	Level of AFB_1 (ng/g)	Level of AFG_1 (ng/g)	Mean $AFB_1 \pm SD$ (ng/g)	Mean $AFG_1 \pm SD$ (ng/g)
Fresh	13.96	7.09	9.61 ± 2.27	7.68 ± 1.40
	13.00	7.00		
	9.60	6.00		
	1.10	13.10		
	10.45	5.20		
Sun-Dried	23.10	5.20	24.68 ± 2.57	10.57 ± 1.54
	17.00	15.50		
	18.20	13.10		
	22.70	6.00		
	24.10	8.00		
	23.05	13.56		
	40.30	12.60		
28.95				
Smoke-dried	22.50	8.00	13.75 ± 2.76	9.02 ± 1.16
	16.40	7.20		
	8.30	12.50		
	15.30	8.20		
	3.50	12.50		
	16.50	5.70		

Table 3: Level of Aflatoxin Contamination in Different Fish Type

Fish type	Level of AFB1 (ng/g)	Level of AFG1 (ng/g)	Mean AFB1 ± SD (ng/g)	Mean AFG1 ± SD (ng/g)
African Catfish Sun-Dried	10.45	5.20	19.48 ± 3.31*	8.45 ± 0.84
	24.10	8.00		
	15.30	8.20		
	1.10	13.10		
	23.05	13.56		
	8.30	12.50		
	9.60	6.00		
	41.30	6.10		
	16.40	7.20		
	13.00	7.00		
	28.95	6.50		
	22.50	8.00		
39.30				
Tilapia	6.00	8.00	16.42 ± 6.59	9.30 ± 1.61
	3.50	12.50		
	23.10	5.20		
	16.50	5.70		
	13.96	7.09		
	18.20	13.10		
	17.00	15.50		

* The mean difference is significant at the 0.05 level

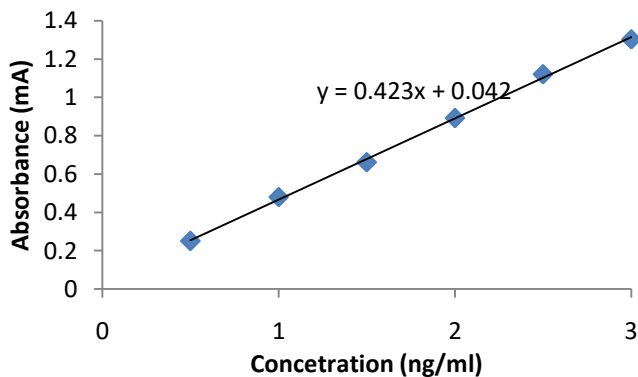


Figure 1: Standard Curve for AFB₁

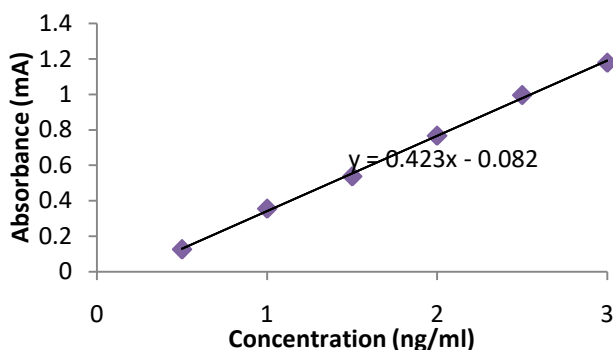


Figure 2: Standard Curve for AFG₁

III. CONCLUSION

The high incidence of *Aspergillusflavus* and *Aspergillus parasiticus* contamination in African Catfish and Tilapia indicates poor handling and storage. The presence of aflatoxigenic fungi in fresh fish can be attributed to the use of contaminated feed and environmental flora. The level of contamination of aflatoxin in sun-dried fish was higher than in fresh and smoke-dried fish. The mean concentration of aflatoxin B₁ in sun-dried fish observed in this study was higher than the maximum recommended level of 20 µg / kg, indicating a potential risk of aflatoxin poisoning for consumers.

REFERENCES

- [1] Ahmed, A., Dodo, A., Bouba, A.M., Clement, S. and Dzudie, T. (2011). Influence of Traditional Drying and Smoke-Drying on the Quality of Three Fish Species (*Tilapia nilotica*, *Silurusglanis* and *Arius parkii*) from Lagdo Lake, Cameroon. *Journal of Animal and Veterinary Advances*, 10 (3): 301-306.
- [2] Food and Agriculture Organization of the United Nations (FAO), (2015). Fisheries and Aquaculture. Fisheries and Aquaculture Country Profile, The Federal Republic of Nigeria. Available at www.fao.org/fishery/facp/NGA/en.
- [3] Abolagba, O. J. and Melle, O.O. (2008). Chemical composition and keeping qualities of a Scaly Fish Tilapia (*Oreochromis niloticus*) Smoked with two Energy Sources. *African Journal of Genetic Agriculture*, 4(2):113-117.
- [4] Adeyeye, S.A.O., Oyewole, O.B., Obadina, A. O., Omemu, A. M., Adeniran, O.E., Oyedele, H. A. and Abayomi, S.O. (2015). Quality and Safety Assessment of Traditional Smoked Fish from Lagos State, Nigeria. *International Journal of Aquaculture*, 5(15): 1-9.
- [5] Ibadon, S., Abdulsalami, M.S., Emere, M.C. and Yilwa, V. (2015). Comparative study of proximate, fatty and amino acids of wild and farm raised African catfish *Clariasgariepinus* in Kaduna, Nigeria. *Pakistan Journal of Nutrition*, 14(1): 56-61.
- [6] Adelakun, K.M., Mustapha, M.K., Amali, R.P. and Mohammed, N. (2017). Seasonal variation in nutritional quality of catfish (*Clariasgariepinus*) from upper Jebba Basin, Nigeria. *Journal of Nutrition and Food Science*, 7: 622.
- [7] Solomon, R.J. and Taruwa, S.M. (2011). The growth comparison of two catfishes (*C. gariepinus* and *Heteroclaris*). *Nature and Science*, 9(8):138-148.
- [8] Ayinla, O.A. (2007). Analysis of Feeds and Fertilizers for Sustainable Aquaculture Development in Nigeria." In *Study and Analysis of Feeds and Fertilizers for Sustainable Aquaculture Development*, edited by Hasan, M. R., Hecht, T., De Silva, S. S., and Tacon, A. G. J. Rome: FAO, 453-470.
- [9] Fallah, A. A., Rahnama M., Jafari, T. and Saei-Dehkordi, S.S. (2011). Seasonal variation of aflatoxin M1 contamination in industrial and traditional Iranian dairy products. *Food Control*, 22: 1653-1656.
- [10] Akise, O.G., Abolagba, O.J. and Eyong, M.M. (2013). Mycoflora of three fish species smoke-dried using rubber wood (*HeveaBrasiliensis*) in Nigeria. *Greener Journal of Agricultural Sciences*, 3 (5):396-402.
- [11] Russo, J.R. and Yanong, R.P.E. (2013). Molds in Fish Feeds and Aflatoxicosis1. Fact Sheet FA-95 School of Forest Resources and Conservation, Program in Fisheries and Aquatic Sciences, UF/IFAS Extension. Available from <http://edis.ifas.ufl.edu>
- [12] Khlangwiset, P., Shephard, G. S. and Wu, F. (2011). Aflatoxins and growth impairment: a review. *Critical Reviews in Toxicology*, 41 (9):740-755.
- [13] Alinezhad, S.M., Tolouee, M., Kamalzadeh, A., Motalebi, A.A., Nazeri, M., Yasemi, M., et al. (2011). Myobiota and aflatoxin B1 contamination of rainbow trout (*Oncorhynchus mykiss*) feed with

- emphasis to *Aspergillus* section Flavi. *Iranian Journal of Fisheries Science*, 10:363–374.
- [14] Kensler, T.W., Roebuck, B.D., Wogan, G.N. and Groopman, J.D. (2011). Aflatoxin: a 50-year odyssey of mechanistic and translational toxicology. *Toxicological Sciences*, 120(1): 28-48.
- [15] Almeida, I.F.M., Martins, H.M.L., Santos, S.M.O., Freitas, M.S., da Costa, J.M.G.N. and d'Almeida Bernardo F.M. (2011). Mycobiota and aflatoxin B1 in feed for farmed sea bass (*Dicentrarchus labrax*). *Toxins*, 3: 163-171.
- [16] Vaziryan, M., Banaee, M., Nematdoost H.B. and Mohiseni, M. (2017). Effects of dietary exposure to aflatoxins on some plasma biochemical parameters of common carp (*Cyprinus carpio*). *Iranian Journal of Fisheries Sciences*, In Press.
- [17] Mohapatra, S., Sahu, N.P., Pal, A.K., Prusty, A.K., Kumar, V. and Kumar, S. (2011). Haemato-immunology and histo-architectural changes in Labeorhita fingerlings: effect of dietary aflatoxin and mould inhibitor. *Fish Physiology and Biochemistry*, 37(1): 177-186.
- [18] Mahfouz M.E. and Sherif A.H. (2015). A multiparameter investigation into adverse effects of aflatoxin on *Oreochromis niloticus* health status. *Journal of Basic and Applied Zoology*, 71:48–59.
- [19] Salau, I.A., Shehu, K., Muhammad, S. and Umar, R.A. (2017). Determination of Aflatoxin levels in groundnut oils marketed in Sokoto State, Nigeria. *International Research Journal of Biochemistry and Biotechnology*, 3(1): 051-054.
- [20] Omeiza, G.K., Kabir, J., Kwaga, J.K.P., Kwanashie, C.N., Mwanza, M. and Ngoma, L. (2019). Aflatoxin risk in dairy production; assessment of dairy cattle feed contamination by *Aspergillus flavus* and *A. parasiticus* in both conventional and traditional dairies. *Global Journal of Medical Research: Veterinary Science and Veterinary Medicine*, 19(2):
- [21] Sailaja, O., Krishnaveni, G. and Manoranjani, M. (2017). Identification and High-performance Liquid Chromatography Quantification of Aflatoxins in Red Chili (*Capsicum annum L.*) in Guntur, Andhra Pradesh, India. *Asian Journal of Pharmaceutics*, 11 (4): S933- S97.
- [22] Sanders, E.R. (2012). Aseptic laboratory techniques: Plating methods. *Journal of Visualized Experiments*, 2012(63): 3064.
- [23] Bandh, S., Kamili, A. and Ganai, B.A. (2012). Identification of some *Aspergillus* species isolated from Dal Lake, Kashmir by traditional approach of morphological observation and culture. *African Journal of Microbiology Research*, 629(29):5824-5827
- [24] Davis, N.D., Iyer, S.K. and Diener, U.L. (1987). Improved method of screening for aflatoxin with a coconut agar medium. *Applied and Environmental Microbiology*, 53: 1593 - 1595.
- [25] Herzallah, M.S. (2009). Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UV detectors. *Food Chemistry*, 114:1141-1146.
- [26] Sa'adatu, A. J., Nura, S. and Muhammad, A. (2019). Assessment of Mycological Quality of Smoked African Catfish (*Clarias gariepinus*) Sold at Sabon-Gari Market, Kano Nigeria. *Annals of Microbiology and Infectious Diseases*, 2(1): 13-18.
- [27] Sani, F.M., Nasir, I.A. and Torhile, G. (2016). Mycological Evaluation of Smoked-Dried Fish Sold at Maiduguri Metropolis, Nigeria: Preliminary Findings and Potential Health Implications. *European Journal of Health Sciences*, 2: 5-10.
- [28] Iqbal, Z. and Saleemi, S. (2013). Isolation of pathogenic fungi from fresh water and commercial fish catlacatla (Hamilton). *Science International*, 25:851-855.
- [29] Santacrose, M.P., Conversano, M.C., Casalino, E., Lai, O., Zizzadoro, C. and Centoducati, G. (2008). Aflatoxins in aquatic species: metabolism, toxicity and perspectives. *Review of Fish Biology and Fisheries*, 18:99–130.