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SCREENING OF MYCOTOXINS PRODUCER FUNGAL AND AFLATOXINS LEVEL IN DRIED AND SMOKED FISH (*Clarias Sp.)* AND (*Oreochromis Sp.*) FROM LAKE FITRI - CHAD

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ABSTRACT

Fish is a protein source of high nourishing value consumed in the entire world. In Saharan countries it is consumed fresh, however as dried or smoked. However, fish is subject to both fungal and mycotoxins contamination. This study aims to identify fungal presumed producing mycotoxin and to evaluate the contamination level of aflatoxins B_1, B_2, and G_2. A total of 150 samples of dried and smoked fish (*Clarias* sp. and *Oreochromis* sp.) have been collected in different islands of the Fitri lake. Standard methods of microbiology have been used for fungal isolation and identification. Aflatoxins content in fish has been determined using HPLC associate to GCMS. Fifty samples (50) were contaminated by four major fungal kinds. Frequency of fungal was to 40 % (*Aspergillus niger*), 26 % (*Aspergillus fumigatus*), 20 % (*Mucor* sp), 8% (*Curvularia* spp) and 6% (*Scycadium*). Twenty (20) samples suspected to be contaminated and analyzed, only seven (7) was contaminated with aflatoxins (B1, B2 and G2). The aflatoxin contamination level vary according to the fish species. *Clarias* sp. samples are 50 % contaminated by aflatoxins when *Oreochromis* sp are 20 % contaminated. Aflatoxins rates ranged from 0.01 to 2.78 and 0.09 to 0.32 µg/Kg respectively for aflatoxin B_1 and aflatoxin B_2 for *Clarias* sp and from 0 to 0.4 µg/Kg of aflatoxin B_2 for *Oreochromis* sp. The mycotoxins level is in average high than the European Union recommendation for dried product. These results call for more sensitization and training for producer for safe dried and smoked fish.

Contribution/Originality: This study is one of the very few studies which have investigated the contamination level of aflatoxins B_1, B_2, and G_2 in dried and smoked fishes (*Clarias* sp and *Oreochromis* sp.) from Fitri lake in Chad.

1. INTRODUCTION

Fish is an important source of nutriments and animal proteins for a large part of the world population (Koranteng *et al.*, 2014). Many processes are used for fish transformation and cooking in several countries in the

Sahel. Fish is a protein source more than cow and pig meat and poultries. Fishing and aquaculture in the world provided about 167.2 million tons of fish in 2014. 93.4 % is provided by fishing. About 143 million tons of fish are destined to the human food. Other studies show that more than 90 % of the world production are destined to human consumption (Gumy *et al*., 2014; United Nations Food and Agriculture Organization, 2018). Fish production in Africa was estimated to more than 9.7 millions of tons in 2012 and is about 6 % of the world production (UNFAO, 2016). The contribution of Africa to the world production in increasing during the last 10 years because of the aquaculture in soft water in sub-Saharan Africa countries (UNFAO, 2018). In Chad, fishing is practiced in all type of surface waters including streams (Chari and Logone) and Lakes (lake Chad, lake Fitri, lake Toupouris and lake Iro) (CBLT, 2007). Fishing have an important place in the national economy and in communities' food security. Despite the fact that the national potential in fish production is estimate to about 186 500 tons, the production sometimes reaches 373 000 tons in a year of good raining. About 35% of this production is from Lake Chad and 65% from streams, flooded plains and secondary lakes Fish is a highly perishable food (Programme National de Sécurité Alimentaire PNSA, 2015). It can alter itself immediately after its capture more than any other food. At this step microbial proliferation, chemical compound change and endogenous enzymes deterioration make it quickly unfit to consumption and even dangerous for human health. There is a high percentage of losses and quality impairment after fishing with all the risks that ensue for the consumer's health (UNFAO, 2016). Dried and smoked fish may be contaminating by several pathogenic microorganisms as fungal flora susceptible of public health problem throughout their secondary metabolites (aflatoxins). Mycotoxins in found in a large skin of foodstuffs. According to Food and Agriculture Organization (FAO), about a quarter of the world production of different foodstuffs are contaminated which is responsible of significant economic loss. Cancer intoxications, immunotoxicity, nephrotoxicity, nephrotoxicity, hepatotoxicity and neurotoxicity are part of fungal toxins effects in human body (Gauthier, 2016). Mycotoxins are chemical compound mainly produced by fungal belonging to the *Aspergillus*, *Penicilliums* and *Fusariums* kinds (Khiat and Insaf, 2014). Food safety is nowadays an increasing preoccupation for consumers and of public utilities (Naima, 2017). The management of the risk passes therefore by the prevention of the contamination of the raw material, the respect of the good practices of hygiene's and a good knowledge on the storage. To avoid food contamination, there is a necessity of sensitization and training of associate human resources in fish production in good hygiene practice, good production and storage practices. An evaluation of the contamination level is then needed to take right decision. This study then aims to evaluate possible toxigenic fungal and aflatoxins level in dried and smoked fish from Chad.

2. MATERIALS AND METHODES

Clarias sp and *Oreochromis* sp are the biological material. Acetonitrile, nitric acid, bromide of potassium, Methanol, Phosphate Salin Buffer, Standard B1 B2, G1, and G2 aflatoxins are the chemical reagent. Immuno Afinity Colums (ref PF-AFBG-3 Libios) was the specific column.

2.1. Methods

2.1.1. Sampling

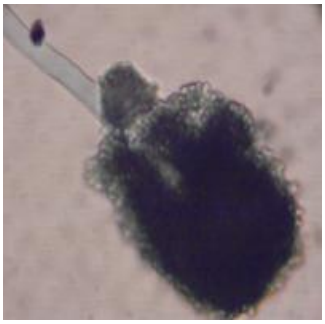
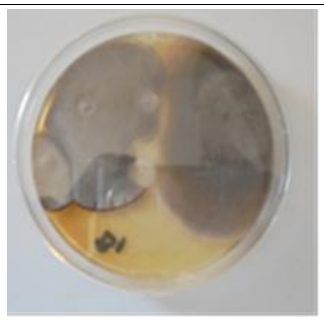
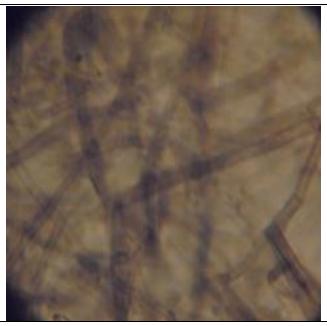
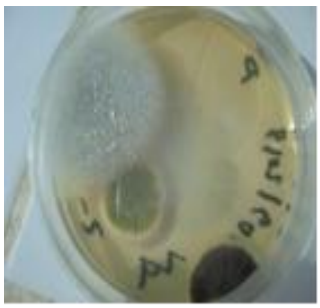
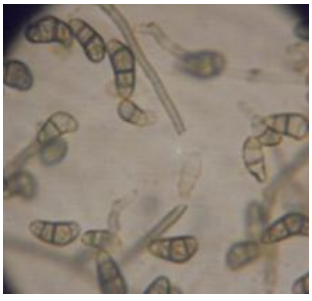
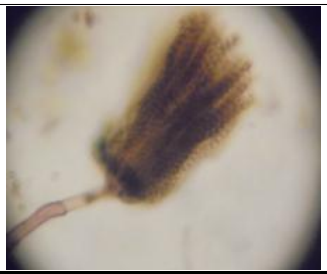
A total of 150 samples of dried and smoked fish including both *Clarias* sp and *Oreochromis sp* species have been collected in Birguimi, Maguiti, Doumrou, and Moudou, different islands of the Fitri Lake. The weight of each sample is about 300g. After identification of mycotoxin producer fungal, twenty (20) samples of contaminated samples have been selected for aflatoxins quantification.

2.1.2. Mycotoxin Producer Fungal Identification

2.1.2.1. Purification of Toxigenic Isolate Fungal

The preparation of samples and tenfold dilution for inoculation on Sabouraud containing chloramphenicol is carried out according to ISO 6887-1 (1999). Incubation is done under 30°C for 72 hours. Presumed toxigenic fungal are collected. These isolates are repiqued twice to get pure fungal cell which are kept in cryotubes containing Czapek Yeast Extratlted agar at 4°C (Botton *et al.*, 1999).

Table-1. Diversity of potential mycotoxins producer fungal.

Fungal species	Cultural aspect	Microscopic aspect (40X)
Mucor S1		
Aspergillus Niger S2		
Scycadium Dimidiatum S3		
Curvularia Spp S4		
Aspergillus Fumigatus S5		

2.1.3. Aflatoxins B_1, B_2, G_1 and G_2 Analyses

The aflatoxins have been quantify using Chromatography Liquid High Performance with derivation post column after a purification on column of immuno-affinity according to Norm European (pr EN 14123). Presumed toxigenic samples where was isolated presumed toxigenic fungal are concerned with the mycotoxins analyses using an extraction solution (Elalami and Abdelouahed, 2014).

2.1.3.1. Extraction

Twenty-five gram (25 g) of every sample have been weighed and ground to obtain a fine powder which allow the release of toxins. Then 5 g of every obtained powder are introduced in a small plastic bottle containing 125 mL of the extraction solution previously prepared. The bottle is then agitated during 20 min. A Wattman filter paper is used to filter the previous solution in a tube containing Phosphate Buffer Saline.

2.1.3.2. Detection and Quantification

Immuno Afinity Colums (ref PF-AFBG-3 Libios) are used for purification. Respectively 15 mL of the extracted solution and 1.5 mLis introduced in every column following by 0.5 mL of distilled water. Standard of aflatoxins B1, B2, G1 and G2 are used. Detection and quantification are perfomed with fluorescence detector (HPLC/FLD) and a photochemical post-column reactor according to ISO 16050 (2003).

3. RESULTS AND DISCUSSION

3.1. Diversity of Toxicogenic Fungal

The cultural and microscopic observation showed five different fungal species in the Table 1. Identified species included *Aspergillus Niger*, *Aspergillus fumigatus*, *Mucor* spp, *Curvularia* spp and *Scycadium dimidiatum*.

A total of fifty species have been isolated in all the samples. Among these fungal species, *Aspergillus Niger* is the more abundant (40%) followed by *Aspergillus fumigatus* (26 %) and *Mucor* spp (20 %). *Scycadium dimidiatum* is the less representative fungal species. The frequency of each isolated fungal is as showed in Table 2.

Table-2. Frequency of isolated fungal species.

Species	Number	Frequency (%)
Aspergillus Niger	20	40
Aspergillus fumigatus	13	26
Mucor spp	10	20
Curvularia spp	4	8
Scycadium dimidiatum	3	6
Total	50	100

From the 150 collected samples, 50 of them are contaminated whith. The contamination rate is then about 30%. The percentage of contaminated samples is very high. Knowing that fungals growth in relative high humidity area, this importance of contamination reveal then an inappropriate process of dried and smoked fish or inappropriate storage conditions of fish. Dried and smoked fish are store around the lake where humidity is relatively higher. The following picture is an evidence of inappropriate storage conditions which is an important factor in fungal growth Figure 1.

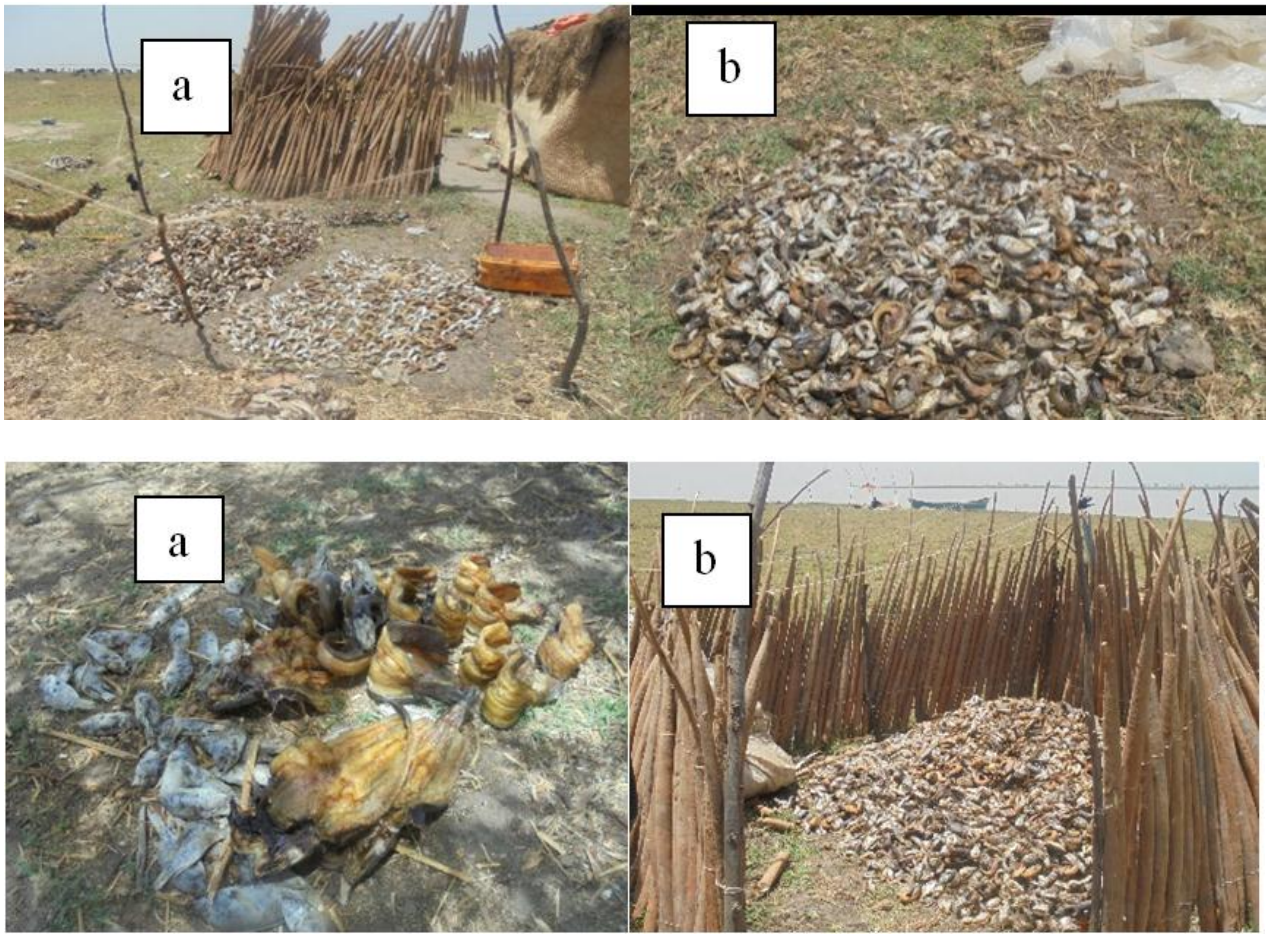


Figure-1. Dried procces (a) and storage environment (b).

All the isolated fungal belong to four kinds of pathogenic fungal. It's *Aspergillus*, *Mucor*, *Curvularia* and *Scycadium*. The frequency of each fungal is 40 % for *Aspergillus Niger*, 26 % for *Aspergillus fumigatus*, 20 % *Mucor* spp 8 % for *Curvularia spp* and 6 % for *Scycadium dimidiatum*. *Aspergillus Niger* is then the more frequent among the pathogenic fungal. Previous studies in Algeria, Burkina Faso and Chad also reveal *Curvularia*, *Aspergillus* and *Mucor* spp as the major pathogenic fungal in dried food (Tidjani *et al*., 2008; Mahibeb, 2015; Compaore *et al*., 2016). The frequency of fungal kinds here found is also similar to some previous studies Rebbouh (2016) and Laaid *et al*. (2009); Fatima *et al*. (2016) and Tidjani *et al*. (2007;2008). Other fungal species such as *Aspergillus falvus*, *Aspergillus aculeatus*, and *Aspergillus tubingenisis* have been isolated in dried fish by Ni *et al*. (2018). The predominance of *Aspergillus* kind among the contaminating flora of dried fish has been reported in several works (Le and Bars, 1987; Riba *et al*., 2005). *Aspergillus* kind protect itself throughout spores. Its spores is then spread in several areas and contaminate seral foods. *Aspergillus* have an important growth factor in an unappropriate storage conditions according to Hocking (2006). For Pihet *et al*. (2007) the contamination of dried and smoked fishes may occur during the process. For, the process is make on uncleaned green grass where previous fish have been dried. The perpetual contamination may then happen.

3.2. Aflatoxin Content of Fish

Aflatoxin B_2 and aflatoxin B_1 are the more abondant mycotoxins in *Clarias sp* fish. The content of these mycotoxins varie from 0.01 to 2.78 and 0.09 to 0.32 respectively for aflatoxin B_1 and aflatoxin B_2. Their is not a contamination in aflatoxin G_1 in all the analyzed samples. Two samples present a content in aflatoxin G_2 which level varie from 0.55 to 1.60. The level of aflatoxin in Carias sp. fish is as showed in the following Table 3.

Table-3. Aflatoxins level in *Clarias* sp. Fish.

Fish species	Samples	AFB1	AFB2	AFG1	AFG2
		Quantity (µg/ g)			
Clarias sp	E 1	-	-	-	-
Clarias sp	E 2	-	-	-	-
Clarias sp	E 3	0.01	0.09	-	-
Clarias sp	E 4	-	0.14	-	1.60
Clarias sp	E 5	0.02	0.03	-	-
Clarias sp	E 6	0.13	0.01	-	-
Clarias sp	E 7	0.86	0.32	-	-
Clarias sp	E 8	-	0.30	-	-
Clarias sp	E 9	2.78	-	-	0.55
Clarias sp	E10	-	-	-	-

Among *Oreochromis* sp fish samples analyzed, two reveal the presence of aflatoxin B_2. None of the other mycotoxin is detected. The contamination in aflatoxin B_2 from 0 to 0.4 µg/Kg. The level in the mycotoxin content of each *Oreochromis* sp fish is as presented in Table 4.

Table-4. Aflatoxins level in *Oreochromis* sp fish.

Fish species	Samples	AFB1	AFB2	AFG1	AFG2
		Quantity (µg/ g)			
Oreochromis sp	E 1	-	0.04	-	-
Oreochromis sp	E 2	-	0.40	-	-
Oreochromis sp	E 3	-	-	-	-
Oreochromis sp	E 4	-	-	-	-
Oreochromis sp	E 5	-		-	-
Oreochromis sp	E 6	-	-	-	-
Oreochromis sp	E 7	-	-	-	-
Oreochromis sp	E 8	-	-	-	-
Oreochromis sp	E 9	-	-	-	-
Oreochromis sp	E10	-	-	-	-

Nine of the twenty fish samples have been contaminated by aflatoxin. The results also showed a contamination of aflatoxins B2, B1 and G2 in fish samples. *Clarias* sp samples (50 %) are more contaminated by aflatoxins than *Oreochromis* sp (20 %). The high contamination level is also founded among *Clarias* sp samples (2.78 µg/Kg).

The drying and smoking process of *Oreochromis* last more than *Clarias* sp one. It may have dried or smoke less well than *Clarias* sp. As moisture, content in very important. Eventual residual water allowed mycotoxins contamination. The mycotoxin level here found is less than those of Olajuyigbe *et al.* (2014). He works reveal of aflatoxins level range between 1.05 and 25.00 µg/kg for aflatoxin B_1 and from 10 µg/kg to 20 µg/kg for aflatoxin B_2. Josefa *et al.* (2018) and Sa'adatu *et al.* (2019) also noted a signifiquant level of aflatoxin B_1, B_2, and G_2 in the dried fish and other food products. Atef *et al.* (2011) also found in Egyptian fish, a level of aflatoxin varying 32.0 to 96.0 µg/kg for aflatoxin B_1 and from 22.0 to 70.5 µg/kg for aflatoxin B_2. In Burkina Faso Pane *et al.* (2012) found in cereals a contamination level of 7.367 to 16.573 µg/kg for aflatoxin B_1 and 0.595 to 1.736 µg/kg for aflatoxin B_2. Despite of the relative contamination level, le quantity found is high than the European norm (2010). To this recommandation the acceptable level of aflatoxin in cereals is of 2 µg/kg for aflatoxin B_1 and aflatoxin 4 µg/kg for both aflatoxin B_2, G_1 and G_2. Aflatoxin B_1 is more toxic than aflatoxin B_2, G_1 and G_2. Cole and Cox (1981) demonstrate that the toxicity aflatoxin G_1, B_2 and G_2 are respectively 50, 80 and 90 % than the aflatoxin B_1 ones. The toxic effects of aflatoxins have been largely proved (Pier *et al.*, 1980; Pier *et al.*, 1986; Mahibeb, 2015; Ninoek *et al.*, 2015).

In conclusion, this study reveals a high contamination of fish by potential toxigenic fungal. The representative fungal found are *Aspergillus*, *Mucor*, *Curvularia* and *Scycadium*. The high contamination level reveal lake in good hygiene practice during the process and the storage and call for more sensitization and training for producers. The

presence of aflatoxins (B_1, B_2 and G_2) contamination level is an evidence of probable human toxicity in large level. The contamination of *Clarias* sp samples (50%) more than *Oreochromis sp* also put in evidence lake in the process mastery. There is a need in followings study to evaluate factors that have an incidence on fungal contamination and their mastery and emergency for public leaders to take right decision for appear to be a public health problem.

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