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

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 B₁, B₂, and G₂. 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 B₁ and aflatoxin B₂ for Clarias sp and from 0 to 0.4 µg/Kg of aflatoxin B₂ for Oreochromis sp. The mycotoxins level is in average higher than the European Union recommendation for dried product. These results call for more sensitzation and training for producer for safe dried and smoked fish.

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 Columns (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 Extract 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<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> 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 Columns (ref PF-AFBG-3 Libios) are used for purification. Respectively 15 mL of the extracted solution and 1.5 mL is 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 performed with fluorescence detector (HPLC/FLD) and a photochemical post-column reactor according to ISO 16050 (2003).

3. RESULTS AND DISCUSSION
3.1. Diversity of Toxiogenic 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.

| 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 with. The contamination rate is then about 30%. The percentage of contaminated samples is very high. Knowing that fungi 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.
All the isolated fungal belong to four kinds of pathogenic fungal. It’s *Aspergillus*, *Mucor*, *Curvularia* and *Scyadum*. The frequency of each fungal is 40% for *Aspergillus Niger*, 26% for *Aspergillus fumigatus*, 20% *Mucor* spp 8% for *Curvularia* spp and 6% for *Scyadum 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; Mahibe, 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 seeral foods. *Aspergillus* have an important growth factor in an unappropriate storage conditions according to Hocking (2006). For Piliet 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 B₂ and aflatoxin B₁ are the more abundant 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 B₁ and aflatoxin B₂. Their is not a contamination in aflatoxin G₁ in all the analyzed samples. Two samples present a content in aflatoxin G₂ 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.
Among Oreochromis sp fish samples analyzed, two reveal the presence of aflatoxin B₂. None of the other mycotoxin is detected. The contamination in aflatoxin B₂ from 0 to 0.4 µg/Kg. The level in the mycotoxin content of each Oreochromis sp fish is as presented in Table 4.

In conclusion, this study reveals a high contamination of fish by potential toxigenic fungal. The representative fungal found are Aspergillus, Mucor, Curvularia and Scycharium. 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

### Table 3. Aflatoxins level in Clarias sp. Fish.

| Fish species | Samples | AFB1 | AFB2 | AFG1 | AFG2 |
|--------------|---------|------|------|------|------|
| 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     | -    | -    | -    | -    |

### Table 4. Aflatoxins level in Oreochromis sp fish.

| Fish species | Samples | AFB1 | AFB2 | AFG1 | AFG2 |
|--------------|---------|------|------|------|------|
| 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     | -    | -    | -    | -    |
presence of aflatoxins (B₁, B₂ and G₂) 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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