Journal of Advances in Biology & Biotechnology
6(3): 1-6, 2016; Article no.JABB.24298
ISSN: 2394-1081

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Detection of *Aflatoxins* in Water, Sediment and Fishes of a Brackish Water Lake in the Nile Delta, Egypt

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2016/24298
Editor(s):
(1) Cosmas Nathanailides, Department Fisheries and Aquaculture Technology, Technological Educational Institute of West Greece, Greece.
(2) Rafael A. Canas, Department of Molecular Biology and Biochemistry, Málaga University, Spain.
Reviewers:
(1) Mary Augustina Egbuta, North-West University, South Africa.
(2) Shimaa R. Hameed, National Research Centre, Egypt.
Complete Peer review History: http://sciencedomain.org/review-history/14702

Original Research Article

Received 12th January 2016
Accepted 23rd April 2016
Published 20th May 2016

ABSTRACT

Regular samples of water, sediments and fresh fishes of *Nile tilapia* were collected from Burllus Lake for detection of fungi and evaluate the levels of aflatoxins (AFs) B_1, B_2, G_1 and G_2 contamination. The mean total length (TL) and body weight (BW) of fish (n=32) were 21.65 cm and 170.35 g, respectively. AFs are one of the most potent and dangerous groups of mycotoxins worldwide. Different species of fungi were isolated from water, sediments and fishes in the present study as: *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Aspergillus terreus, Aspergillus ocracheus, Aspergillus parasticus, Alternaria alternate, Cladosporium* spp., *Mucor* spp., *Candida* spp. and *Fusarium* spp. AFs producing fungi including *Aspergillus flavus, Aspergillus fumigatus, Aspergillus terreus, Aspergillus parasticus, Mucor spp.,* and *Fusarium.* The highest concentration of AFs was 244.526 ng/g in sediment and 1.758 ng/L in water samples, which was produced from *Aspergillus flavus isolation.* On the other hand, the isolate of *Mucor* spp. from fish samples has the ability to produce AFs with concentrations of 0.727 ng/g. High concentration of aflatoxin may affect negatively the economic value of fishes and the public A periodical examination of fishes grown in the lake is required in order to protect the public health.

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Keywords: Aflatoxin; Burllus Lake; Nile tilapia; fungi.

1. INTRODUCTION

Lake Burullus is brackish water Lake, located along the Mediterranean Sea in the Nile Delta of Egypt. It is about 460 km^2, and it is considered the second largest natural lake in Egypt [1]. Fish represent an important source of human dietary protein worldwide especially in Egypt. [2] who studied the contamination of fishes with aflatoxin and isolated some species of fungi from the body of the fish as *Aspergillus candidus, A. versicolor, A. oryza, Cladosporium spp.* and *Mucor spp*. Aflatoxins are naturally occurring mycotoxins that are produced by *Aspergillus flavus* and *Aspergillus parasiticus,* species of fungi. The major aflatoxins are B_1, B_2, G_1, and G_2 (based on their fluorescence under UV light (blue or green) and relative chromatographic mobility during thin-layer chromatography) [3]. Aflatoxin B_1 is the most potent natural carcinogen known and is usually the major aflatoxin produced by toxigenic strains [4]. [5] showed that aflatoxin contamination has been linked to increased mortality in farm animals and thus significantly lowers the value of grains as an animal feed and as an export commodity. Aflatoxin is associated with both toxicity and carcinogenicity in human and animal populations. The diseases caused by aflatoxin consumption are loosely called aflatoxicoses. Acute aflatoxicosis results in death; chronic aflatoxicosis results in cancer, immune suppression, and other "slow" pathological conditions. The liver is the primary target organ, with liver damage occurring when poultry, fish, rodents, and nonhuman primates are fed aflatoxin B_1. The aim of the present study was to detect unknown fungi and evaluate flatoxin types in sediment, water and fresh fishes of Burllus Lake to conserve public health and achieve high economic value of fishes in the lake.

2. MATERIALS AND METHODS

2.1 Samples Collection

Water, sediment and fishes (Nile *tilapia)* were collected from different sites from Burllus Lake. The fish samples were brought to laboratory in sterile polyethylene bags in aerated pond water and kept in glass aquaria with continuous air supply at ambient temperature. Total length (TL) and body weight (BW) of each fish was measured and health status of every specimen was observed. The glassware (containing media and distilled water covered with aluminum foil), vials and test tubes (cotton plugged) were autoclaved at 121℃ at 1.054 kg/cm^2 for 15 min.

2.2 Isolation and Identification of Fungi

The fungi were isolated from infected organs of fish with sterile needle, inoculated on Czapek Dox Agar (DOX). Isolation was done in Laminar flow air cabinet to avoid contamination. Antibiotic streptomycin sulphate 250 mg was added to each preparation of media to reduce bacterial contamination. The agar plates were incubated at 28-30ºC and fungal growth was observed after 5-7 days. Pure fungal culture was done by picking a small portion of colony with the help of sterilized loop and poured it into distilled water to make spore suspension. Isolated fungi were identified morphologically using light microscope according to identification keys [6].

2.3 Preparation of Inocula

The fungal culture was grown on (PDA) slants at 28ºC for about 7 days or until good sporulation was observed. Spores were harvested by adding 10 ml of sterilized aqueous solution of Tween 80 (0.05% v/v) to cultures [7]. Spore suspensions were then centrifuged at 20,000 rcf for 5 min and the Supernatants discarded. The spore concentrations were adjusted to yield a final count of 105 spore/ml and the ensuing preparations were used as spore inocul.

2.4 Growth Media

The culture media used in this study was yeast extract sucrose (YES) to produce aflatoxins. The yeast extract sucrose culture was carried out according to the method of [8] at following (2% yeast extract and 15% sucrose / liter distilled water) were poured into 500 Erlenmeyer flask, and autoclaved at 121ºC for 15 min.

2.5 Preparation of Fungi for Production of Aflatoxins

Two hundred mL of yeast extract sucrose was transferred into 250 Erlenmyer flasks, and autoclaved at 121ºC for 15 min. The yeast extract sucrose medium was inoculated with fungal strain and incubated at 28ºC for 14 days.

2.6 Extraction of Aflatoxins from Liquid Media

Aflatoxins were extracted according to the method described by [9]. Extraction was carried out using 20 ml of chloroform (twice with 10 ml media), and homogenization for 3 min in a separation funnel. The chloroform phase was filtered through filter paper Whatman No. 3 and concentrated to dryness under a nitrogen stream.

2.7 Determination of Aflatoxins by HPLC

The derivatives of samples and standard were done as follow: 100 µl of trifluoroacetic acid was added to samples and mixed well for 30 s and the mixture stand for 15 min. 900 µl of water: acetonitrile (9:1 v/v) were added and mixed well by vortex for 30 s. and the mixture was used for HPLC. The HPLC system consisted of Waters Binary Pump Model 1525, a Model Waters 1500 Rheodyne manual injector, a Waters 2475 Multi-Wavelength Fluorescence Detector, and a data workstation with software Breeze. A phenomenex C18 (250 x 4.6 mm i.d.), 5 µm from Waters corporation (USA). An isocratic system with water: methanol: acetonitrile 240:120:40. The separation was performed at ambient temperature at a flow rate of 1.0 mL/min. The injection volume was 20 µl for both standard solutions and sample extracts. The fluorescence detector was operated at wavelength of 360 nm for excision and 440 nm for emission. Aflatoxins concentrations in samples were determined from the standard curve, using peak area for quantitation [10].

3. RESULTS AND DISCUSSION

As shown in Table 1, seven genera of *Aspergillus* were isolated from water, sediment and fishes samples*: A. flavus, A. niger, A. fumigates, A. terreus, A. ocracheus, A. parasticus, A. candidus* in addition to *Alternaria alternate, Cladosporium spp., Mucor spp*., *Candida, Penicillium spp.* and *Fusarium spp.* It was clear that the most common of *Aspergillus* species that isolates from *Tilapia nilotica* was *A. flavus, A. terreus, A. fumigates, A. niger* in addition to *Alternaria alternate,* and *Fusarium spp.*, while the least common recovered in fish samples is *Candida* and *Penicillium spp*. The current results were in agreement with those recorded by [11] and [12] who isolated *A. flavus* from most of all samples of fish examined in their studies. Many members of the isolated fungi were incriminated in cases of pulmonary infections, urinary tract infection, arthritis, osteomylitis, dermatitis, endocarditic, meningitis and eye infection [13]. The occurrence of these species in the ecosystem of Burllus Lake may affect on the health status of fish. As shown in Table 2, several strains of moulds especially *Aspergillus flavus, A. parasticus, A. fumigates, A. terreus* in addition to *mucor spp*. that isolated from fish, water and sediments were able to produce aflatoxins on YES media, under the ideal experiment condition. The highest level of total aflatoxin in the present study was produced by *Aspergillus flavus* with value of244.526 ng/g in sediment samples. It was worth that the present results that the most of the isolated moulds are toxigenic types and have the ability to produce mycotoxins whenever the condition are right and become of public health hazards previously reported by [14]. Most of detected levels of aflatoxins were over the permissible limits in food reported by [15] who stated that the aflatoxins must be not more than 15 ppb and [16] and [17] who stated that the levels of aflatoxins must be not more than 20 ppb in food. Hence, most of detected levels were health hazard for consumers where, cases of carcinogenic effects for internal vital organs are resulted particularly for liver and kidney addition the consumption of food contaminated with mould and their toxins induced food poisoning, hemorrhages, hepatotoxicity, nephrotoxicity, neurotoxicity, dermatitis, carcinogenic, hormonal and immunosuppression effects [18]. Table 3 showed AFs production by some toxigenic fungi isolated from water samples. In the present study, *Aspergillus flavus* produce aflatoxin B1 and G2 from water samples with a value of 1.652 and 0.106 ng/mL, respectively. *Aspergillus fumigates* produce $AFG_1$with value of 0.20 ng/ml in addition to *mucor spp.* produce $AFG_2$with value of 0.162 ng/mL. The obtained results showed the toxicity of AFG_2 which produced from *Aspergillus flavus* isolate causing harmful effects on public health and reduce the quality of water for microorganisms living. Table 4 showed AFs production by some toxigenic fungi isolated from fish (liver and gill) samples. It is showed that *Aspergillus parasiticus* produce AFG_2 from gill with value of 0.529 ng/g, *Mucor spp.* produce aflatoxin B_1 and G_2 with values of 0.522 and 0.205 ng/g, respectively, from the liver. The presence of *Aspergill*us in fish organs does not always indicate that harmful levels of aflatoxin also are present; it does imply a significant risk in consumption and economic value. The infection of fishes by AFs may be due to moldy feed was caused by high moisture content and improper

storage of their feeds, Feeds were left on the road near the farm for the farmers to pick up but due to unavailability of transport, feeds were left for days and they got wet by the rain [19]. Table 5 showed that AFs production by some toxigenic fungi isolated from sediment samples. The present results revealed that *A. fumigatus* produce AFB_1, $AFG_1$and AFG_2 with values of12.17, 0.50 and 0.847 ng/g, respectively. It is showed that *A. flavus* produce AFB_1, AFG1 and AFG_2 with values of 242.6, 0.166 and 1.76 ng/g, respectively. *Aspergillus terreus* isolates produce AFG_1 and AFG_2 with values of 0.433 and 0.145 ng/g, respectively.

Table 1. Incidence of fungal species in examined samples

Identified mould species.	*Tilapia nilotica* (n=15)		Water (n=15)		Sediment (n=15)	
	No of +ve samples	%	No of +ve samples	%	No of +ve samples	%
Aspergillus flavus	15	8.8	29	15.3	14	15.7
Aspergillus fumigatus	21	12.4	2	1.05	6	6.7
Aspergillus terreus	5	2.9	5	2.6	4	4.4
Aspergillus parasticus	10	5.9	0	0	8	8.9
Aspergillus niger	12	7.1	1	0.52	4	4.4
Aspergillus ocracheus	0	0	4	2.1	0	0
Cladosporium spp.	10	5.9	0	0	25	28.08
Mucor spp.	5	2.9	24	12.6	0	0
Fusarium spp.	48	28.4	6	3.1	26	29.2
Pencillium spp.	5	2.9	17	8.9	0	0
Candida spp.	0	0	80	42.3	0	0
Alternaria alternate	38	22.4	21	11.1	2	2.2

+ve: Present

Table 2. Aflatoxins production by some toxigenic fungi isolated from water, fish and sediments samples

Fungal species	Concentrations of aflatoxins (ng/mL and ng/g)				
	AFG_1	AFB_1	AFG_2	AFB_2	Total AFs
Fusarium (liver)	ND	ND	ND	ND	ND
Fusarium (Sediment)	ND	ND	ND	ND	ND
Fusarium (water)	ND	ND	ND	ND	ND
Fusarium (Liver)	ND	ND	ND	ND	ND
Aspergillus terreus (Sediment)	0.433	ND	0.145	ND	0.578
Aspergillus fumigates (Sediment)	0.50	12.17	0.847	ND	13.517
Aspergillus flavus (Sediment)	0.166	242.6	1.76	ND	244.526
Aspergillus flavus (water)	ND	1.652	0.106	ND	1.758
Mucor spp. (Liver)	ND	0.522	0.205	ND	0.727
Mucor spp. (water)	ND	ND	0.162	ND	0.162
Mucor spp. (Sediment)	ND	ND	ND	ND	ND
Fumigatus (water)	0.20	ND	ND	ND	0.20
Parasticus (Gill)	ND	ND	0.529	ND	0.529

ND= Not detected, ng/mL for water samples and ng/g for sediment or fish samples the same in all tables

Table 3. AFs production by some toxigenic fungi isolated from water samples

Fungi producing toxins	Concentration of AFs (ng/ mL)				
	AFB_1	AFB_2	AFG_1	AFG_2	Total aflatoxin
Aspergillus flavus	1.652	ND	ND	0.106	1.758
Aspergillus fumigatus	ND	ND	0.20	ND	0.20
Mucor sp.	ND	ND	ND	0.162	0.162
Fusarium equiseti	ND	ND	ND	ND	ND

Table 4. AFs production by some toxigenic fungi isolated from fish (liver and gill) samples

Fungi producing toxins	Region	Concentration of AFs (ng/ g)				
		AFB_1	AFB_2	AFG_1	AFG_2	Total aflatoxin
Aspergillus parasiticus	Gill	ND	ND	ND	0.529	0.529
Mucor sp.	liver	0.522	ND	ND	0.205	0.727
Fusarium equiseti	liver	ND	ND	ND	ND	ND

Table 5. AFs production by some toxigenic fungi isolated from sediment samples

Fungi producing toxins	Concentration of AFs (ng/ g)				
	AFB_1	AFB_2	AFG_1	AFG_2	Total aflatoxin
Aspergillus fumigatus	12.17	ND	0.50	0.847	13.517
Aspergillus flavus	242.6	ND	0.166	1.76	244.526
Aspergillus terreus	ND	ND	0.433	0.145	0.578
Fusarium equiseti	ND	ND	ND	ND	ND
Mucor sp.	ND	ND	ND	ND	ND

Our results in agreement with [20] who found that *A. flavus* produce AFB_1 in soil with value of 272ng/g that may be attributed to that soil is considering the natural habitat of this fungs. Also soil serves as a reservoir for primary inoculum for the infection of susceptible crops. The population size of *A. flavus* has been correlated with soil organic matter and nutritional status, with the most fertile soils containing the greatest concentration of aspergilla. It is showed that aflatoxin B_1 can permeate through the skin. Dermal exposure to these aflatoxin in particular environmental conditions may lead to serious health risks [21]. Because aflatoxin B_1 can cause immune suppression, exposure is associated with an increased viral load in HIV positive individuals [22]. Finally, a new approach to the detoxification of aflatoxins is the addition of inorganic sorbent materials, known as chemisorbents, such as hydrated sodium calcium alumino silicate (HSCAS) to the diet of animals. HSCAS possesses the ability to tightly bind and immobilize aflatoxins in the gastrointestinal tract of animals, resulting in a major reduction in aflatoxin bioavailability.

4. CONCLUSION

The results of the present work demonstrated that, sediments and fishes were contaminated with *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Aspergillus terreus, Aspergillus ocracheus, Aspergillus parasticus, Alternaria alternate, Cladosporium* spp., *Mucor* spp., *Candida* spp. and *Fusarium* spp. Some of them produced some toxins as B_1, B_2, G_1 and G_2. High levels of AFs contamination may be related to high temperatures and moderate humidity. High concentration of aflatoxin may affect negatively on economic value of fishes and the public. A periodical examination of fishes grown in the lake is required in order to protect the public health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14702