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RESEARCH PAPER

Determination of the Aflatoxin B1 Level in Imported Milled Rice by ELISA in Duhok Province

[1]Jaafar A. Ali, [2]Nadhim Sulaiman A. Jakhsi

[1] Food Analysis laboratory, Directorate of Preventive Health Affairs, Duhok, Kurdistan Region, Iraq

[2] Department of Microbiology and Pathology, College of Veterinary Medicine, Duhok University, Kurdistan Region, Iraq

A B S T R A C T:
Aflatoxins are fungal metabolites, toxic and carcinogenic. Aflatoxin B1 is the most common toxic mycotoxin classified as Group I carcinogen by the International Agency for Cancer Research. The purpose of this study is to determine the level of Aflatoxin B1 in different varieties of milled rice imported from different origins to Kurdistan region of Iraq between October and December 2018 by using indirect competitive enzyme-linked Immunosorbent Assay (ELISA). A total of 150 rice samples (30 each group) were collected from different sources (Thailand, Russia, India, Kurdistan and different sources from Iraqi public food distribution system), to evaluate their Aflatoxin B1. Out of 150 samples 90 (60%) have been detected with Aflatoxin B1, while the others 60 (40%) samples were not detected (under the detection limit of 0.02µg/kg). The concentrations were ranged from (0.03 to 3.12) µg/kg with an average range 0.94 µg/kg. The highest percentage of detected samples were from Russia 25 (28%) followed by a group of origins distributed through national distribution system 22 (24%), Thailand 21 (23%), Kurdish 12 (13%) and the lowest percentage was from India 10 (11%). However, out of 90 positive samples, only 4 (4%) samples from Russia were exceeded the European Union regulations limit of 2µg/kg of Aflatoxin B1 and other 86 (96%) were within the permissible levels of international legislations, these concentrations can cause a serious health issues to human because of the large amount of rice consumption per capita per year in Kurdistan region where rice is included in the daily diet, as a result, accumulation of Aflatoxin B1 in a human body will raise.

KEY WORDS: Aflatoxin B1, ELISA, Rice.
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1. INTRODUCTION

Rice (*oryza sativa*) is the staple food grain of over 50 percent of the world population and is the second main grain consumed after wheat (Elzupir, Alamer and Dutton, 2015). Iraq ranked among top ten countries of rice importer in the world (Bedford et al., 2017). The largest amount of rice in the world in 2018 were produced by China with 208.1, followed by India with 169.5 million metric tons (FAO, 2018). It is flood resistant, dry, damp, cool and warm and grows in alkaline acid and saline soils (Eslami et al., 2015).

Mycotoxins are secondary fungal metabolites that can contaminate a broad range of foods (Ferre, 2016). In addition, the natural contaminants of important plant products (rice, wheat, barley, maize ... etc.). They created serious problems for human and animal health (Kamkar et al., 2014).

Aflatoxins are the world's leading food contaminant mycotoxins (Kamkar et al., 2014)

*** Corresponding Author:**
Jaafar Abdulmanaf Ali
E-mail: Email: jaferdhk73@gmail.com or jaferdhk73@duhokhealth.org
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which are a class of mycotoxins mainly produced by *Aspergillus flavus*, *A. parasiticus*, and seldom *A. nominus* (Alpsoy, 2010; Reddy et al., 2010). The main Aflatoxins are AFB1, AFB2, AFG1, AFG2, AFM1 and AFM2. They are harmful for human health because of their toxicity, carcinogenic, mutagenic, teratogenic and hepatotoxic characteristics. Amongst other Aflatoxins, AFB1 is the most toxic type; and also is the most powerful human cancer. AFB1 is therefore listed by the International Agency for Research Cancer (IARC) of the World Health Organization (WHO) as group I of carcinogenic chemicals compounds (Elzupir, Alamer and Dutton, 2015). There is a high risk of liver cancer for careers of hepatitis B or hepatitis C if Aflatoxin is exposed (Costanzo et al., 2015). The findings of epidemiological data indicate that liver cancer and exposure to AFB1 are in a positive correlation which may be increased by hepatitis B virus (Liu et al., 2012). The carcinogenicity of Aflatoxins is higher 30 times for populations infected by hepatitis B virus (Groopman, et al. 2008).

Aflatoxin B1 are heat-stable chemical compounds that during most food processing are not totally destroyed. Several countries have therefore developed broad law to regulate mycotoxin levels in plant products, and each has its own different limits (Tavakoli et al., 2013; Kamkar et al., 2014).

The maximum tolerable limits for total Aflatoxins (B1+B2+G1+G2) and 2μg / kg for AFB1 in rice were established by the European Union as 4μg / kg for total Aflatoxins (B1+B2+G1+G2) (Commission, 2010). A joint FAO / WHO Expert Committee on food additives reviewed Afatoxins in 1987 (FAO/WHO, 1987) No daily intake was acceptable; individual intakes were advised to be reduced to the lowest executable.

Many methods, including thin-layer chromatographic (TLC) analysis, high-performance liquid chromatography (HPLC) and the enzyme-linked immunosorbent assay (ELISA), were used for analyzing Aflatoxins (Tavakoli et al., 2013; Sasan et al., 2014). Due to the adaptability, simplicity and sensitivity, ELISA is the most common method for the study of Aflatoxins (Magliulo et al., 2005), even accuracy of the study and routine diagnosis of a huge range of samples (Kamkar et al., 2014).

This work was determined the level of Aflatoxin B1 in most common consumed imported rice in Duhok province, Kurdistan region of Iraq.

2. MATERIALS AND METHODS

2.1. Sample Collection:

A total of (150) imported raw milled rice samples (250 g each) from five different sources (Thailand, India, Kurdistan, Russia and group origins distributed through national public distribution system of food) were collected randomly in Duhok governorate, Kurdistan region of Iraq during winter between October and December 2018. The samples were collected from local markets; retail stores and sampling unit of Ibrahim Khalil (border point between Iraq and Turkey). Long grain white rice (Basmati), white medium grain rice and round grain rice were evaluated for their Aflatoxins content. In addition, locally produced round grain rice (Kurdish) cultivated at a limited scale was also evaluated for Aflatoxins. The storage period of rice samples was ranged between 3-24 months. All samples were labeled then, placed in clean paper bags then, in zipped polyethylene bags. After that, the samples were shipped to the food analysis laboratory belong to directorate of preventive health affairs in Duhok governorate to be store at -20°C until analysis.

2.2. Detection of AFB1 by indirect competitive ELISA:

The quantitative detection of AFB1 and preparation of the samples were performed based on a competitive enzyme immunoassay using Helica Bio-systems, Aflatoxin B1, ELISA assay Low Matrix 69-1B10LFAB149 test kit, USA, 2018.

2.3. Extraction of AFB1 from rice samples:

The extraction procedure was carried out according to commercial ELISA kit of Helica Bio-systems, USA, 2018. The Samples (250g) were thoroughly mixed and 30g from a sub-sample were ground to powder. Twenty grams from powdered sub-sample were added to the 100ml of 80% methanol (20ml distilled water with 80ml of methanol v/v). The ratio of sample to extraction solvent is 1:5 (w/v). The samples were mixed by shaking in a sealed container for 10 minutes then, left for 30 minutes to allow the particulate matter to settle. Ten milliliter from aliquot was centrifuged 3500 RPM for 5 minutes and the extract were diluted 1:10 in phosphate buffer saline containing 0.05% Tween20 prior to ELISA test. (The final dilution was 1:50).

2.4. ELISA test procedure:

According to manual instructions provided with Aflatoxin B1 (cat#981BAFL01LM-96) low matrix test kit, 100µl of each standard and prepared sample was gently mixed with 200µl of sample diluent in a mixing well. Then, 100µl from each mixing well was transferred to a corresponding antibody coated microtiter well, incubated at room temperature (20–25°C) for 30 minutes. Liquid was drained by firmly taping the wells against the absorbent paper; a washing buffer washing three times the wells (300 µl). After that, 100µl from horse-radish peroxidase (HRP) Aflatoxin enzyme conjugate was added to each well and incubated 30 minutes at room temperature. After a washing step repeated, 100µl of substrate solution added to each well, incubated 10 minutes at room temperature in a dark place. Finally, 100µl of the stop solution was added to each well and the optical density (OD) of the resulting color was measured at 450nm by using ELISA plate reader (BioTek-USA). To calculate the absorption percentage, the values obtained for the samples and standards were divided by the absorbance value of the 1st standard (zero standard) and then multiplied by 100 (percentage of maximal absorbance). The founded intensity of absorption in the samples was inversely proportional to AFB1 concentration. In order to create a generic curve and then measure the AFB1 concentration in every sample, Helica Bio-system was used.

2.5. Statistical Analysis:

Statistical analysis was applied using Minitab software version 17.1 (Minitab, LLC, USA, 2014). Results were given as mean ± SD to illustrate Aflatoxin B1 concentration ranges in each origin and study the differences.

3. RESULTS:

The Aflatoxin B1 contamination in different imported rice samples were presented in data sheet (Table 1). A total of 150 milled rice samples from 5 origins (Thailand, India, Russia, Kurdish and different origins from Iraqi public food distribution system), (Thirty samples each) were analyzed for Aflatoxin B1 contamination. Analytical results showed that 60% (90 out of 150) rice samples were detectable or positive (containing ≥ 0.03 µg/kg AFB1) ranged between 0.03-3.12 µg/kg of Aflatoxin B1 with an average 0.94 µg/kg , while the remaining 40% (60 out of 150) samples were undetectable or negative (containing less than 0.03µg/kg AFB1) to ELISA test (Figure 1).

Figure 1: Percentage of negative and positive samples.

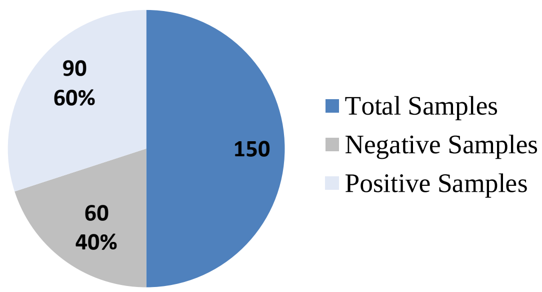

Table 1: Occurrence and level of Aflatoxin B1 in rice in different origins imported to Kurdistan region of Iraq.

			Number of samples in AFB1 concentration range, (µg/kg)				
Sample Origins	Analyzed Samples	Positive Samples	<1	1-2	>2	AFB1 ranges (µg/kg)	Average ±SD (µg/kg)
Russia	30	25 (83%)	11 (44%)	10 (40%)	4 (16%)	0.35-3.12	1.28©±0.70
Iraqi PDS*	30	22 (73%)	18 (82%)	4 (18%)	0	0.12-1.38	0.62±0.39
Thailand	30	21 (70%)	7 (33%)	14 (67%)	0	0.03-1.73	1.03±0.47
Kurdish	30	12 (40%)	9 (75%)	3 (25%)	0	0.06-1.51	0.77±0.41
India	30	10 (33%)	6 (60%)	4 (40%)	0	0.10-1.83	0.86±0.76
Total	150	90 (60%)	51 (57%)	35 (39%)	4 (4%)	0.03-3.12	0.94±0.60

* Origins selected from Iraqi Public Distribution System (Uruguay, USA, Argentina, Vietnam and Thailand).

The minimum limit of detection (LOD) for Aflatoxin B1 (low matrix) kit used was 0.02µg/kg. The (average ± SD µg/kg) ranges of Aflatoxin B1 contamination were 1.28±0.70 for Russia, 1.03±0.47 Thailand, 0.86±0.76 India, 0.77±0.41 Kurdish and 0.62±0.39 for origins (Uruguay, USA, Argentina, Vietnam and Thailand) distributed through Iraqi public food distribution system. The results showed that maximum level of AFB1 was found in rice samples originated by Russia and lowest from rice samples originated by India. Only 4 (4%) samples from all detectable (positive) samples were exceeded the European Union (EU) regulatory limit of AFB1 (2µg/kg); all of them were Russian origins while, none of the other 86 (96%) samples were passed over EU tolerance limits of AFB1.

4. DISCUSSION

AFB1 is one of the most toxic mycotoxins in various studies examined in rice samples. In this study 150 imported milled rice samples from different sources were analyzed for Aflatoxin B1 content. A total of 60% (90) samples were detected, 25 (28%) samples from Russia, 22 (24%) from sources distributed through Iraqi public food system (Uruguay, USA, Argentina, Vietnam and Thailand), 21 (23%) from Thailand, 12 (13%) from Kurdish (locally cultivated) and 10 (11%) from India. The contamination range of AFB1 in rice samples cultivated in Russia 4(4%) were out of normal ranges comparing with European regulations limit, while all other samples were in the normal ranges. The finding of highest percentage of contaminated samples in rice originated by Russia may be because of inappropriate harvesting, transporting and storage conditions. Moreover, the variety of rice cultivating in Russia (round rice) has more ability to affected by Aflatoxigenic fungi as it contain more starch and produced more broken seeds through milling process. On the other hand, the appropriate harvesting, good conditions of storage, pigmentation and flavoring, as well as quality improvement may be result from smallest percentage of contaminated samples from India because all of these processes and conditions play an important role in propagation of Aflatoxigenic fungi. Regarding in rice cultivated locally (Kurdish), although it has low level of harvesting, milling, storage and packing, it registered a small portion of positive samples in our study due to its freshness and a short time of both transporting and storage. This finding is strongly significant therefore the author strongly recommend further studies on different varieties of locally cultivated rice should be conducted.

Aflatoxin B1 contaminations in rice are reported in previous studies most of them analyzed different varieties of rice. In a study by Park et al., 2005 (Je, Lee and Kim, 2005) in Korea, they analyzed the concentration range of Aflatoxin B1 in 88 samples of polished rice and reported that 5 (5.6%) samples with mean 4.3 µg/kg were contaminated with Aflatoxin B1. Also, AFB1 found in 21 (11.2%) samples from 187 rice samples analyzed with a maximum range of (63.32µg/kg) in Brazil by Katsurayama et al., 2018. In Canada, Bansal et al., 2011 Investigated 99 different varieties of rice from retail markets and registered (56.1%) as incidence of Aflatoxin B1 and they discovered that the most contamination presence was related to AFB1 which was higher than other mycotoxins. In another study carried out by Wang and Liu, 2007 they detected AFB1 in 16 out of 84 rice samples with a range of (0.15–3.22µg/kg) which is like our study ranges. Similar incidence of AFB1 in rice samples (67.8%) to our study were reported in a survey by Reddy et al. 2009, when they found the AFB1 in 814 (68%) out of 1200 rice samples of Basmati variety collected from field and where-houses in India. In (2011), Makun et al. published a maximum incidence (100%) in Nigeria from 21 different rice samples, Also, in (2012) and (2013) a similar incidence of AFB1 in rice samples showed by (Eslami et al., 2015) in Iran.

Such concentrations of AFB1 are high and are most likely to cause acute human symptoms and are therefore disturbing and essential for experts and government bodies, producers and consumers. They would play an important role in the propagation of hepatocellular carcinoma (HCC) in rice in countries with a high intake of rice in AFB1. As a result, the outcome of these investigations places a responsibility on the member states of such populations to ensure food safety for this basic food commodity.

5. CONCLUSIONS AND RECOMENDATIONS:

To conclude, the level of Aflatoxin B1 in most varieties of consumable imported, and some locally cultivated rice are in the allowed ranges while, excessive observation should be taken by continuous screening of AFB1 in imported rice in all custom points of Iraqi borders because Aflatoxins have a negative impact on consumer health especially in rice-consumer countries such as Iraq where, rice consider as a main part of the daily food system.

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Conflicts of interest Statement

The authors declare that they have no conflict of interest.

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