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Quantitative Determination of Aflatoxin B1 Levels in Rice Grans Using Enzyme-Linked Immunosorbent Assay validated method in Kenya

Youmma Douksouna [1,*], Ronald Tonui [2], Andrew Nyerere [1,3], Steven Runo [1,4] and Zachée Ambang [5]

[1] Department of Molecular Biology and Biotechnoly, Pan African University Institute for Basic Sciences Technology and Innovation, P.O. Box 62000-00200 Nairobi, Kenya
[2] Department of Biochemistry and Microbiology, Rhodes University-South Africa
[3] Department of Medical Microbiology, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200 Nairobi, Kenya
[4] Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University, P.O. Box 43844-00100 Nairobi, Kenya
[5] Department of Plant Biology and Biotechnology, University of Yaounde1, P.O. Box 812 Yaoundé, Cameroon
* Correspondence: ydouksouna@gmail.com

Abstract: Aflatoxins are secondary metabolites produced by *Aspergillus* species distributed on three main sections of the genus namely section *A. Flavi*, section *A. Ochraceorosei*, and section *A. Nidulantes*. They are common contaminates of dietary staples worldwide, including cereals, oil seeds, nuts, spices, meats, dairy products, fruit juices, dried fruits, eggs, and feeds and foods derived from these products. Aflatoxins are unavoidable widespread natural contaminants of foodstuffs with serious impacts on food safety, health, agricultural and livestock productivity. Aflatoxin B_1 is the analyte with the highest toxic significance and the most potent hepatocarcinogenic among other aflatoxins, and humans may get exposed to it at any stage of life. Dietary exposure to aflatoxins is a public health concern due to their carcinogenic, acute aflatoxicosis and chronic effects, immunosuppression properties, among others. This study focused on aflatoxin B_1 in rice grains. Rice is important staple food consumed widely, and consists of a major part of the diets for half of the world population. In general, there have been few reports on the occurrence of the aflatoxin B_1 in rice grains compared to other cereals in Africa. However, love the occurrence of the aflatoxin B_1 levels compared to other crops, is of concern because of the high consumption of rice in several countries in Africa. This study assessed aflatoxin B_1 in rice grains, occurrence, control, socioeconomic and health implications. We quantitatively determine the levels of aflatoxin B_1 content using Enzyme Linked Immunosorbent Assay method. 43.1 % of examined samples were positive in which 15.9 % for local rice and 27.2 % of imported rice, respectively, and 11.3 % of examined samples are above the maximum limit of aflatoxin B_1 in rice established by European Union. According to the manufacturer instructions, the limit of detection is 1 µg/kg (ppb) in cereals. The concentration of aflatoxin B_1 in examined samples ranged from 0 µg/kg to 3.2 µg/kg. These results are indicative of exposure of the population to aflatoxin and possible health hazard. The procedure used in this study is suitable for detection of mycotoxins at a very low concentration.

Keywords: rice; contamination; aflatoxins; occurrence; incidence; exposure; food; safety

Key Contribution: This article provides insight into contamination of rice grains by aflatoxin B1, as rice grains is staple food in Africa. It assessed aflatoxin B1 in rice grains, occurrence, control, socioeconomic and health implications in order to ensure food safety.

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1. Introduction

Mycotoxins are among the fungal toxins of most concern to public health, associated with severe health problems when ingested, inhaled or absorbed. These severe health complications include acute toxic, carcinogenic, mutagenic teratogenic, and estrogenic effects [1].

The major factors that contribute to the significant incidence of mycotoxins in Africa have been identified as climate change [2], miss of awareness, bad of agricultural practices, and pre and postharvest management [3].

Among all mycotoxins, AFs are known as the most toxic with significant impact of economic burden to agriculture and consumers [4]. Rice is important staple food consumed widely, and consists of a major part of the diets for half of the world population. AFs are produced by several species of *Aspergillus* belonging to sections *A. flavi, A. ochraceorosei* and *A. nidulantes* [5, 6]. AFs are the most toxic mycotoxins eliciting acute and chronic toxicities, the most severe and notorious of which are genotoxicity, mutagenicity, and immunotoxicity. The major ones are aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2), named after the fluorescence they display in ultraviolet (UV) light (B for blue and G for green) [7].

In Sub-Saharan Africa (SSA), the mostly consumed food is rice grains among all food commodities, and who's its demand is rapidly growing, mainly driven by urbanization [8]. The problem of AFs is very important around the world and particularly in Africa, where aflatoxin contamination is reported in raw cereals with 50 % incidence with the infestation reaching 1642 μg/kg in rice [9]. According to Liu and Wu [10], distribution of hepatocellular carcinoma attributable to aflatoxin exposure is highest in Africa with 40 % of liver cancer incidences. Over 5.2 million cancer deaths happen every year, 55 % of which arise in developing countries. In SSA, 25.000 hepatocellular carcinoma related deaths happen frequently due to ingestion of aflatoxins through contaminated foods [11]. The largest outbreak of aflatoxicosis to date has been reported in Kenya, during incidence in 2004 where 317 Kenyans became severely ill when they ingested food with a high levels of aflatoxin infestation, 215 recognized deaths were reported [12]. More recently, acute aflatoxicosis due to ingestion of large quantities of aflatoxin was linked to 20 fatalities in the United Republic of Tanzania [13].

The European Union (EU) has established maximum tolerable limits (MTL) for AFs in rice specifically, 4 μg/kg for total aflatoxins (B_1+B_2+G_1+G_2) and 2 μg/kg for AFB_1 [14].

Hence, the aim of this study was to investigate with emphasis of aflatoxin B_1 levels occurrence in rice grains samples sold in markets in order to ensure safety of this product for human and animal consumption as the flagship aflatoxin for being the most toxic and widespread.

2. Materials and Methods

2.1. Samples

An overall of forty-four (44) samples (local rice produced in Mwea and imported rice originating from Biriyani, India, Pakistan, and Thailand) have been taken in the line with the alternative sampling plan for the Official Control of Mycotoxins in Food (OCMF) [15].

2.2. Quantitative Analysis of Aflatoxin B_1 levels by ELISA

Preparation of the samples and ELISA test were performed according to the method described by R-Biopharm GmbH [16].

2.3. Determination of the Absorbance

The absorbance values of the standards, and samples tested were calculated according to the manufacture's instruction.

3. Results and Discussion

3.1. Results

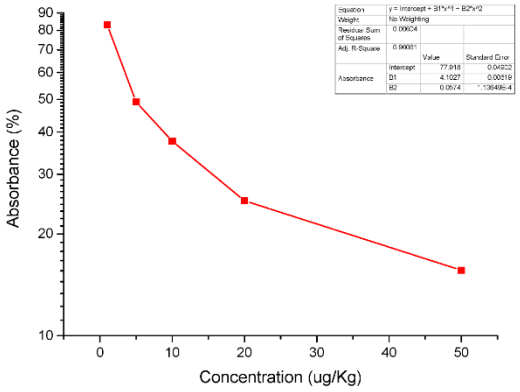

Figure 1. Standard curve of aflatoxin B_1 (R^2 = 0.96061).

The result revealed that 19 samples of rice examined were contaminated by aflatoxin B_1 as shown in table 1. Quantitative analysis reveals that 43.1 % of examined samples were positive in which 31.8 % are below accepted levels, however, 11.3 % are above accepted levels (table 1). The result of the present study shows that the concentration of aflatoxin B_1 in examined rice grains samples ranged from 0 µg/kg to 3.2 µg/kg using ELISA assay.

Table 1. Occurrence and level of aflatoxin B_1 in rice grains.

Source of Sample	№ of Samples	Sample Status		Concentration of Aflatoxin B_1(µg/Kg)			Accepted levels
		Positive	Negative	0	<2	>2	<2µg/Kg
Local	25 (56.8%)	07 (15.9%)	18 (40.9%)	17 (38.6%)	06 (13.6%)	02 (4.5%)	(Commission
Imported	19 (43.1%)	12 (27.2%)	07 (15.9%)	08 (18.2%)	08 (18.2%)	03 (6.8%)	Regulation No.
Total	44 (99.9%)	19 (43.1%)	25 (56.8%)	25 (56.8%)	14 (31.8%)	05 (11.3%)	1881/2006)

3.2. Discussion

More than 43.1 % of examined samples were positive contaminated by aflatoxin B_1 in which 15.9 % for local rice and 27.2 % of imported rice, respectively. Seven samples out of 25 rice samples homegrown from Mwea contained aflatoxin B_1. This is in right line with the study conducted in Turkey on level and incidence of mycotoxin in rice [17]. Quantification of aflatoxin B_1 by ELISA assay showed a higher significance incidence in imported rice samples; 27.2 % of imported rice samples being contaminated with aflatoxin B_1 in which 6.8 % of those samples have greater levels than 2.0 µg/kg limit established by EU. These findings are in accord with others survey conducted by several investigators [18] who assessed occurrence of aflatoxin B_1 in rice grains. The high percentage of contamination in imported rice could be explained by the conditions of storage, shipment and transport along the way up to the destination point. Our results agree with those reported by Nurshad [19] who reported aflatoxins in rice worldwide occurrence, and public health perspectives during the period from 1990 to 2015.

4. Conclusion

Aflatoxin B_1 levels was quantitatively determined by ELISA method in which 43.1% of examined samples were positive. The concentration of aflatoxin B_1 in examined samples ranged from 0 µg/kg to 3.2 µg/kg (43.1%) in which 11.3 % are above the maximum tolerable limits (MTLs) for AFs in rice established by European Union (EU) particularly 2 µg/kg for AFB_1.This is indicative the exposure of humans, animals, and livestock to aflatoxin B_1 with possible health hazard effects and indirectly with a significant economic impact on the society.

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