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Research Article

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Assessment of Total Aflatoxin Contamination of Varieties of *Oryza sativa* and *Phaseolus vulgaris* in Anyigba Metropolis, Kogi State Nigeria

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Abstract Aflatoxin is a naturally occurring carcinogenic and immunosuppressive compound produced by *A. flavus*. This study was designed to assess the level of total aflatoxin (AFT) contamination in varieties *Oryza sativa* (rice) and varieties of *Phaseolus vulgaris* (beans). The AFT incidence and concentrations of one hundred samples of rice and beans samples, comprising of 20 samples each of local rice, foreign rice, brown beans, white beans (big seed) and white beans (small seed) were determined by Enzyme Linked Immnuno Sorbent Assay (ELISA) method. The results showed the ranges of AFT concentration for the groups of samples as 3.17±0.06–4.26±0.09µg/kg, for local rice, 3.63±0.06–6.42±0.04µg/kg for foreign rice, 3.35±0.06–6.92±0.04 µg/kg for brown beans, 3.23±0.11–4.81±0.06µg/kg for white beans (big seed) and 2.58±0.09–5.81±0.08µg/kg for white beans (small seed). The results also indicated the percentages of concentrations of aflatoxin contamination of the different groups of sample as 60% for local rice, 40% for foreign rice, 40% for brown bean, 40% for white bean (big seed) and 40% white bean (small seed). The results of this study showed generally that the levels of AFT were below the current maximum permissible limit by European Commission (EC) of 10µg/kg for total aflatoxins in foods. It is therefore safe to conclude that the post harvest practices of farmers and marketers in the study area are effective at controlling aflatoxins contamination.

Keywords Aflatoxin, *Oryza sativa*, *Phaseolus vulgaris*, ELISA

Introduction

Rice (*Oryza sativa*) is one of the most consumed staple food in the world. About 23 *Oryza species* are known which only *Oryza glaberrima* and *Oryza sativa* are widely cultivated [1]. Poor post harvest practices however leads to contamination of seed with fungi such as *fusarium spp, Aspergillus spp and Alternaria spp* among others [2]. This can lead to significant deterioration in quality of seeds leading to high economic loss [3]. In addition, fungi are capable of causing seed discoloration and reduce viability and germination of seeds [4], this is aside their ability to produce secondary metabolites on the foods and feeds they contaminate.

Humans are exposed to toxicfungi secondary metabolites such as aflatoxins by consuming foods currently or previously contaminated with *Aspergillus spp* of fungal. Evidence of acute aflatoxicosis in humans has been reported from many parts of the world, especially the under developed, and developing countries. Conditions increasing the likelihood of acute aflatoxicosis in humans include environmental conditions that favor fungal growth, Poverty, and lack or inadequate regulatory systems for aflatoxin monitoring and control. Severity of

aflatoxin-related diseases in humans may be influenced by factors such as age, nutritional status, sex and/or concurrent exposure to other disease causative agents such as viral hepatitis (HBV) or parasite infection [5-6].

The aflatoxins of interest in this study are produced by either *Aspergillus flavus* and *Aspergillus parasiticus*, which are common forms of weedy molds widespread in nature. The presence of these molds may not necessarily indicate that harmful levels of aflatoxin are present, but may indicate a significant risk [7].

Up to15% of the rice harvest is lost every year due to inappropriate storage conditions that results in fungal growth [8]. Rice is often contaminated with mycotoxins such as aflatoxins. The temperature and moisture conditions prevailing during storage promote aflatoxin production resulting in annual losses [9-11], although rice was not formally thought of as high risk commodity, in terms of aflatoxins contamination. There are substantial evidence indicating endemic low mg/kg occurrence of AFB_1 contamination in rice [12-13]. AFB_1 and other mycotoxins were detected in rice varieties in different countries including Nigeria, USA, UK, Malaysia, Egypt, Pakistan, India, Philippines, Iran Nepal and China [14-17].

No animal species is immune to the acute toxic effect of aflatoxins. Adult humans have relatively high tolerance to aflatoxin exposure and rarely succumb to acute aflatoxicosis, but children are mainly affected, and their exposure can lead to stunted growth and delayed development. High level aflatoxin exposure produced acute hepatic necrosis, carcinoma of the liver, acute liver failure (which is made manifest by bleeding, edema), alteration in digestion, changes to the absorption and/or metabolism of nutrients and coma [18].

The economic impact of aflatoxins derived directly from crop and livestock losses, lower yields for crops as well as indirectly from the cost of regulatory programs designed to reduce risks to animal and human health [6]. It is for the above reasons that this work was designed to determine the incidence and concentrations of AFS in rice and beans in Anyigba metropolis.

Materials and Methods

Sample Collection

One hundred food Samples were randomly purchased from the local markets and vendors within Anyigba. Twenty samples each of brown beans, white beans (big seeds), white beans (small seeds) (*Phaseolus vulgaris L.*), foreign rice (*Oryza sativa*), and local/Africa rice (*Oryza glaberrima*) were used for this study. The samples labelled appropriately and aflatoxins were extracted immediately.

Extraction of Total Aflatoxins

Each of the samples were grinded using mixer grinder (CIPL MG 1, Wattage: 750 watts copper voltage for 220-240V, 50/60 Hz, R.P.M 18000 Approx) for 30mins. At the end of every round of grinding, the blender was often cleaned up using 70% methanol and wiped to dry to avoid cross contamination of the samples. 5g each of the ground sample was weighed using a Ja-P Series Lab electronic balance (model JA203P) into conical flasks.

Solvent solution (methanol:distilled water; 80:20) was prepared by v/v. 20ml of solvent solution was added to each sample in the conical flask. The samples were stirred thoroughly for 1 hour using a shaker (Orbital shaker model KJ-201BD). This was then followed by filtration using whatman No.1 filter paper and the filtrate was then collected in plane bottles and covered to avoid evaporation for analysis.Samples were tested according to the protocol of the Elisa kit as described below [19]

Determinationof total Aflatoxinsby Enzyme Linked Immuno Sobent Assay (ELISA)

All method adopted in this research is according to Donna *et al* [20] method with modification. The ELISA tube was coated with antibody of specific aflatoxin. It is usually coated with streptanden. Micropipette was used to measured standard (S0,S5) which range are in the orderof0.015, 0.25, 0.312, 0.625, 1.25, and 2.0µg/kg respectively, into each of the test tube kit. Each of the samples was then pipette using micropipette into the test tube kit with different micro pipette teeth. 20ml of the sample diluted was added to the sample in the test tube making it 25ml in order to conform

with the standard. HRP- conjugate reagent was added to all samples including the standard. The samples and standard were then incubated for 1hour using the dark method. This method involved putting the-test kits inside a lid. The plate is then washed using 20ml of distilled water and 1ml of wash buffer, this involved pipetteing them into each sample including the standard. It was then decanted into a plastic bowel these were done five (5) times. Chromogen solution A and B was mixed together to obtained 50ml. 25ml was pipette into each of the sample and standard in the test kit, there was changed in color from colorless to blue. The change sample was then incubated for 20mins using the dark method at room temperature. A stop solution was then added to end the reaction, at this point there was an immediate change of color from blue to yellow. The samples were then transferred to a Diatek microplate Reader (Model Number: DR-200Bs, High brightness and large size LCD, with highly sensitive touch screen input, Power supply: 100-240V, 50-60Hz). The intensity of the color is inversely proportional to the concentration of AFT in the samples or standard. Each sample was analyzed in triplicates, the absorbance (ABS) standard curve is plotted against the concentration (µg/kg) standard curve to obtain a straight line graph which was used to calculate the exact concentration of total AFT.

Statistical Analysis

Data obtained were subjected to statistical analyses using Microsoft Excel. Data were presented as Mean ± Standard error of mean (SEM).

Results

Table 1: Aflatoxins concentration in rice and bean (µg/kg)

Sample ID	Brown beans	White beans (Small seed)	white beans (Big seed)	Local rice	Foreign rice
1	ND	ND	ND	ND	ND
2	3.50±0.03	ND	3.81±0.05	ND	6.42±0.04
3	5.84±0.04	ND	ND	3.78±0.03	4.27±0.04
4	ND	2.78±0.04	ND	3.47±0.15	ND
5	6.92±0.04	5.70±0.05	ND	4.16±0.03	3.63±0.06
6	ND	ND	4.27±0.03	3.62±0.06	ND
7	ND	4.18±0.09	ND	3.21±0.04	ND
8	ND	ND	4.81±0.06	ND	ND
9	4.21±0.09	3.36±0.06	3.34±0.12	3.39±0.12	5.15±0.07
10	ND	ND	ND	ND	ND
11	ND	ND	ND	ND	ND
12	3.35±0.06	ND	3.65±0.09	ND	6.31±0.10
13	5.68±0.07	ND	ND	3.68±0.08	4.24±0.06
14	ND	2.58±0.09	ND	3.59±0.09	ND
15	6.85±0.09	5.81±0.08	ND	4.26±0.09	3.80±0.08
16	ND	ND	4.28±0.09	3.49±0.12	ND
17	ND	4.12±0.06	ND	3.17±0.06	ND
18	ND	ND	4.65±0.14	ND	ND
19	4.07±0.03	3.30±0.09	3.23±0.11	3.41±0.12	5.12±0.05
20	ND	ND	ND	ND	ND
Mean Concentration	5.78±0.41	3.98±0.07	4.00±0.07	3.60±0.08	4.87±0.06

Values are means ± SEM of triplicate determinations, Detection limit: 0.1 µg/kg,
ND = Not Detected

Table 2: Aflatoxins Analysis of rice and beans

Sample name	Sample size (N)	Number of contaminated sample (n)	Percentage (%) contamination	Range of Contamination (µg/kg)	Mean concentration (µg/kg)
Brown beans	20	8	40%	3.35±0.06–6.92±0.04	5.78±0.41
White beans (small seeds)	20	8	40%	2.58±0.09–5.81±0.08	3.98±0.07
white beans (big seeds)	20	8	40%	3.23±0.11–4.82±0.06	4.00±0.07
Local rice	20	12	60%	3.17±0.06–4.26±0.09	3.60±0.08
Foreign rice	20	8	40%	3.63±0.06–6.42±0.04	4.87±0.06

Values are means ± S.E of triplicate determinations, Detection limit: 0.1 µg/kg, ND = Not Detected

Discussion

The aflatoxin contents in rice and beans are presented in table 1. The result revealed that brown beans had the highest total aflatoxins concentration of 6.85±0.09 µg/kg compared to the two varieties of white beans which had the highest total aflatoxins concentration to be 5.81±0.08 µg/kg. It can therefore be deduced that brown beans are more susceptible to aflatoxins contamination than white beans. The result in table 1 also showed that total aflatoxins concentration was higher in foreign rice than the local rice considering the highest level of 6.42±0.04 µg/kg for foreign rice and 4.26±0.09 µg/kg for local rice. This may also be as a result of the possible longer storage time of the foreign rice, including transit time. Though, in most cases, the local rice are usually not stored for longer period of time due to local demand but may have been contaminated due to poor handling and poor processing techniques.

Table 2 shows that 8(40%) samples of Brown beans, 8(40%) of white beans (small seeds) and 8(40%) of white beans (big seeds), 8(40%) samples of foreign rice and 12(60%) samples of the local rice were found to be contaminated with AFT. Though, the local rice showed the highest incidence of contamination but the AFT contents is lower than that of foreign rice.

A qualitative study in Pakistan reported similar incidences of aflatoxin contamination in which 22.42% of brown rice samples, 33.13%of white rice samples, 39.39% of broken rice samples, 24.27% of Sella rice samples and 26.92% of parboiled rice samples were found to have been contaminated with AFB_1. AFB_2 was detected in 1.47% of brown rice samples, 3.20%of white rice samples and 3.03% of broken rice samples. While AFG_1 was found in 0.8% of white rice samples, 3.65% of brown rice samples, and 1.5 % of parboiled rice samples. AFG_2 was absent in all samples [21].

Of the 40 samples of rice and 60 samples of beans, 44 in all were found to be contaminated. 23 samples had aflatoxin level higher than the European Commission (EC) Regulations and Turkish Food Codex limit for total aflatoxin of 4 µg/kg [22]. The total aflatoxin concentrations of all samples of the current study were below the current regulation of EC of 10µg/kg for total aflatoxins in food [23].

The result of this study is similar to the work of Donna *et al* [20] in which the mean aflatoxin concentration of each of the fractions was below the European standard of 10 µg/kg total aflatoxins.

Similar reports also, have been recorded in Africa. 10 samples of rice in Ivory Coast and 21 samples in Nigeria have been found to be contaminated with aflatoxin in all analysed samples with an average level of 4.5 µg/kg of AFB_1 [24]. Onyedum *et al* [17] reported 100% incidence (range = 2.10 – 248.20µg/kg) of AFT in 58 samples of rice collected across Niger state, Nigeria.Austria and Spain have also, reported very low level of aflatoxin in rice. The results in Austria have shown that 15 out of 81 samples and only one sample from Spain have been contaminated with an average level 1.97µg/kg of AFT [1].

On the other hand, aflatoxin in rice in some countries in Europe have been reported to be higher than the EC of 10µg/kg for total aflatoxins in foods. In Scotland, the brown rice has shown contamination with an average 14.7 µg/kg of total aflatoxin [25]. In Spain and Mexico; the aflatoxin was detected in 66 out of 67 with an average concentration of 37.3 µg/kg [9]. Similar results were found in Sweden, in which the contamination ranged between 0.1-50.7 µg/kg of total aflatoxin [26]. And here in Nigeria, is an exceptional report of 82.5 µg/kg of total aflatoxin in rice [23].

Aspergillus flavus and *Aspergillus parasiticus* are the most important fungi species producing the secondary metabolite aflatoxins in rice [27]. This current study is expected to add to knowledge especially on aflatoxin incidences in beans since it appears that there are scanty reports regarding aflatoxins contamination in beans.

Conclusion

Total Aflatoxins content of various rice and beans examined were in compliance with EU and other international standards for aflatoxins. This compliance level may not necessarily mean safe limit because endemic low level consumption could constitute risk factor for humans. Therefore, the regulatory agencies should ensure the use of modern techniques in controlling food contamination in Nigeria especially with respect to aflatoxins.

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