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Assessing the Possible Effect of Gamma Irradiation on the Reduction of aflatoxin B1, and on the Moisture Content in Some Cereal Grains

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Abstract

Aflatoxin B_1 is the most potent hepatocarcinogen known in animals and it is classified by the International Agency of Research on Cancer (IARC) as Group I carcinogen meaning that it is a proven cancer-inducing agent. It also occurs in the environment contaminating a lot of different food and feed commodities. The aim of this study was to assess the possible effect of gamma irradiation on the reduction of aflatoxin B_1 in some cereal grains and the impact on nutritive values including, ash, &moisture. It was found that maize samples contain the highest level of aflatoxin B_1 than wheat and rice. Gamma irradiation is a suitable technique which reduces the levels of aflatoxin B_1 in cereal samples without affecting the nutritive values, at 4 KGy the reduction percents of aflatoxin B_1 were 15.54%, 22.25%, and 27.46% for maize, wheat, and rice respectively whereas at 6 KGy the reduction percents of aflatoxin B_1 were 32.39%, 43.84%, and 56.38% respectively and the 8 KGy radiation dose remove about 60.26% of the toxin in maize, 64.68% in rice and 69.29% in wheat samples. Higher radiation doses than 8 KGy are required to remove the toxin until it reaches the legal limit (5ppb) according to FAO.

Keywords: Gamma Irradiation; aflatoxin B1; Cereal Grains; radiation.

Introduction

Hepatocellular carcinoma constitute 80% of cancer cases in developing countries as Egypt. The incidence of HCC is increasing, ranging between 3% and 9% annually depending on the location, and several other etiologic factors. e.g. hepatitis B and C virus infections. While HBV and HCV may account for the majority of HCC in Egypt, there is another factor including (e.g. aflatoxins, AF). [1,2] Location and environmental condition in Egypt make it prone to aflatoxins. Studies suggest that aflatoxins are expected to spread and become more problematic with future climate conditions. There are many climatic reasons for an increase in aflatoxins, including an increase in temperature, humidity and moisture. [3]

Aflatoxins are a group of mycotoxins produced in tropical and sub-tropical regions.[4]. Aflatoxins are hepatic and carcinogenic secondary metabolities of moulds that produce mainly from *Aspergillus flavus* and *Aspergillus paraciticus*. Cereal grains are susceptible to aflatoxins. causing substantial losses of yield and frequently having a detrimental effect on grain quality.

This study aims to assess the possible effect of gamma irradiation on the reduction of aflatoxin B_1 in some cereal grains and the impact on nutritive values.

Materials and Methods

One group pre-post intervention design was conducted on 60 samples divided equally among the three different chosen cereals, wheat (*Triticum aestivum* L.), maize (*Zea mays*), and polished rice (Oryza sativa L). The minimal required sample units was calculated using STATA II software, precision= 10%, α=0.05, and was found to be 60 units.

Each sample unit was one kilogram; each kilogram was subdivided into equal four sub-groups. The first sub-group was used as a control subgroup. The second sub-group was subjected to a gamma radiation dose of Cobalt-60 of 4 KGy. The third sub-group was subjected to a gamma radiation dose of Cobalt- 60 of 6 KGy. The forth sub-group was subjected to a gamma radiation dose of Cobalt-60 of 8 KGy. All the sub-groups were stored in plastic bags at 4^{o} C until analysis. All samples were subjected to chemical analysis to ensure their quality and safety for human consumption.

Irradiation

Maize, wheat, and rice seeds (250 g) sub-groups were sealed in polyethylene plastic bags (0.1mm thickness) and irradiated at room temperature (25^{o} C) under ambient atmosphere. The irradiation of samples carried out by using ^{60}Co gamma ray (Indian irradiation unit) installed at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Egypt. All samples were exposed to a dose rate of 0.07 Gy/sec at the time of the experiment.

I Aflatoxin B_1 determination by High Performance Liquid Chromatography (HPLC).

50g of each ground sample was placed into ultraturrax and then 100 ml of 60% acetonitrile/water (v/v) was added, and the mixture was stirred for 2 min. at high speed. The extract was filtered through a Whatman No 3 filter paper and then through a microfibre filter. 2 ml of the final extract, corresponding to 1 g of the original material was diluted with 48 ml of phosphate buffered saline (PBS, pH 7.4) to give a solvent concentration of 2.5% or less (in order to protect the antibodies in immunoaffinity columns). The mixture was allowed to pass through a column contains monoclonal antibodies to aflatoxin bound to a solid support. By passing the diluted extract through the column any aflatoxin exists in the sample will bound to the antibody within the column. The column was then washed with 20 ml of PBS. The elution of aflatoxin was done with 1.5 ml of methanol and 1.5 ml of pure water (it is a complete denaturation of monoclonal antibodies with the subsequent release of toxin into the solution). And samples were measured against standard at concentrations 0.05, 0.1, 1, 5, 10, 20, 50, and 100 ng mL^{-1} per injection to estimate the linearity of the instrument.

HPLC conditions

100 μl of the each extract was injected in triplicate using C18 ODS (Octa Dodecyl Sulphate) reversed phase column (250x4.6 mm, 5 μm), Japan, heated to 40°C. Mobile phase was water: methanol solution (60:40, v/v), Flow rate 1 ml/min and Ultra-violet detection of aflatoxin B_1 at wavelength of 265 nm in (SPD-6AV UV-Vis detector, Shimadzu).

II Determination of moisture content by method 44-15A of American Association of Cereal Chemists International (AACCI), one-stage procedure[5]

➢ **Statistical analysis**

Data analyzed in triplicate, fed to the computer and analyzed using IBM SPSS software package version 20.0. Quantitative data were described using mean and standard deviation. The given graphs were constructed using Microsoft excel software. Comparison between more than two populations was analyzed using F-test (ANOVA) and Post Hoc test (LSD). Significance of the obtained results was judged at the 5% level.

Results

Results expressed as mean ± standard deviation of triplicate observations for each sample.

In this work three types of seeds were employed, namely, Maize, Wheat and Rice. Twenty samples of each type were used each of one kilogram, to study the effects of gamma irradiation on the aflatoxin B_1 levels and the impact on nutritive values.

Aflatoxin B_1 levels before exposure to Gamma radiation

The distribution of aflatoxin B_1 in cereal samples (maize, wheat, and rice) before exposure to different doses of gamma radiation.

Figure 1 illustrates the levels of aflatoxin B_1 in maize, wheat, and rice before gamma-rays exposure.

Figure 2 represents a graphical comparison between aflatoxin B_1 levels in maize, wheat, and rice and the legal upper limit of aflatoxin B_1.

Aflatoxin B_1 levels after exposure to different doses of Gamma radiation

Table 1 describes the comparison between the alfatoxin B_1 levels in the different seeds samples before and after exposure to 4, 6, and 8 KGy gamma radiation.

Table 2 illustrates the reduction percents of aflatoxin B_1 in the different samples after exposure to different radiation doses namely; 4KGy, 6 KGy, and 8 KGy.

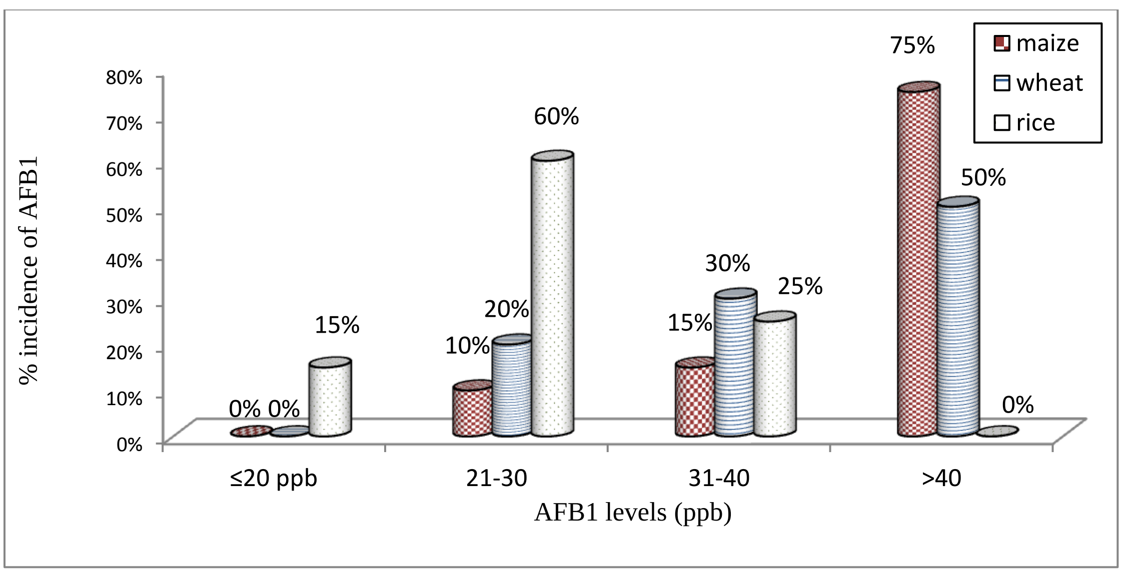

Figure 1. Distribution of aflatoxin B_1 in maize, wheat, and rice pre-irradiation

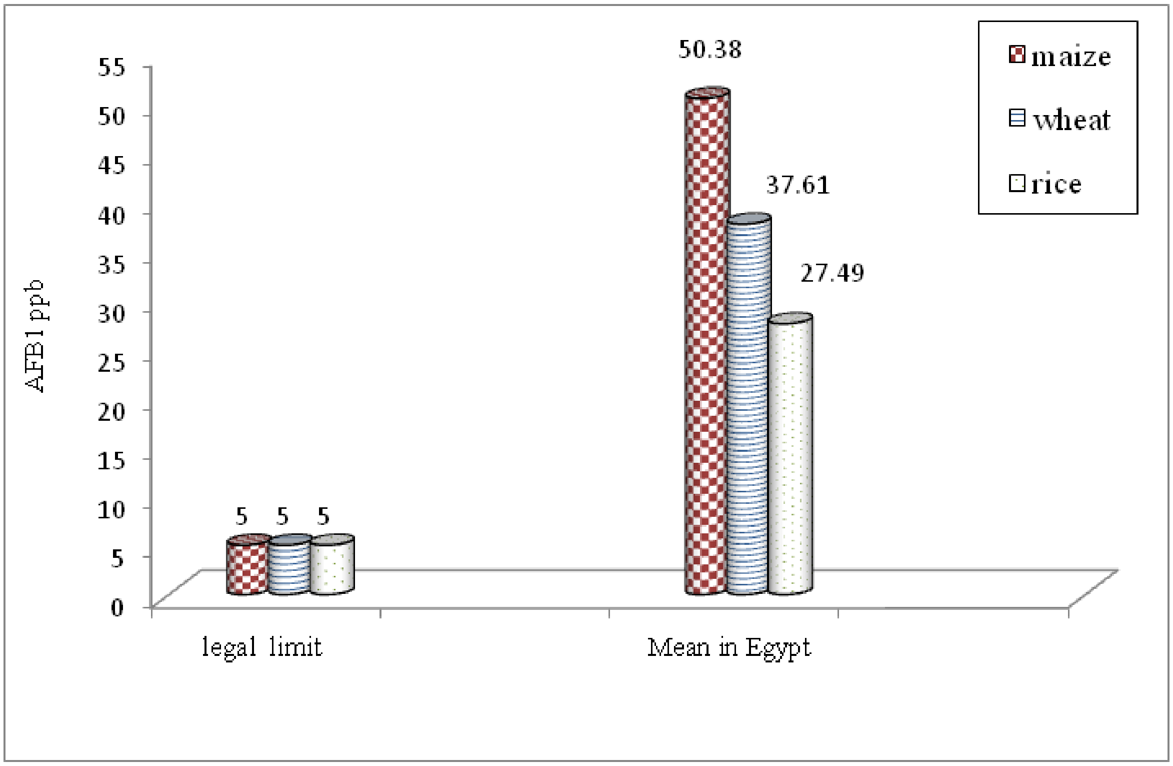

Figure 2. Aflatoxin B_1 legal limit and aflatoxin B_1 in Egypt.

Table 1: Comparison between different groups pre- and post-radiation according to aflatoxin B_1 levels (ng/g) in maize wheat and rice.

Cereal types	Aflatoxin B_1 Pre-radiation ng/g	Aflatoxin B_1 Post-radiation ng/g			F	P
	0 KGy	4 KGy	6 KGy	8 KGy		
Maize	$50.38^a \pm 14.46$	$42.55^b \pm 13.04$	$34.06^c \pm 12.08$	$20.02^d \pm 6.70$	23.702^*	$<0.001^*$
Wheat	$37.61^a \pm 6.85$	$29.24^b \pm 6.34$	$21.12^c \pm 6.54$	$11.55^d \pm 3.41$	70.182^*	$<0.001^*$
Rice	$27.49^a \pm 7.32$	$19.94^b \pm 7.87$	$11.99^c \pm 7.34$	$9.71^c \pm 2.43$	18.834^*	$<0.001^*$

Different superscripts are significant at $p \leq 0.05$
F: F test (ANOVA)
*: Statistically significant at $p \leq 0.05$

Results expressed as mean ± standard deviation of triplicate observations for each sample

Table 2: Mean %reduction of aflatoxin B_1 by different radiation doses at 4 KGy, 6 KGy, 8 KGy in maize, wheat, and rice

Cereal types	% Reduction of aflatoxin B_1 Post radiation		
	4 KGy	6 KGy	8 KGy
Maize	15.54	32.39	60.26
Wheat	22.25	43.84	69.29
Rice	27.46	56.38	64.68

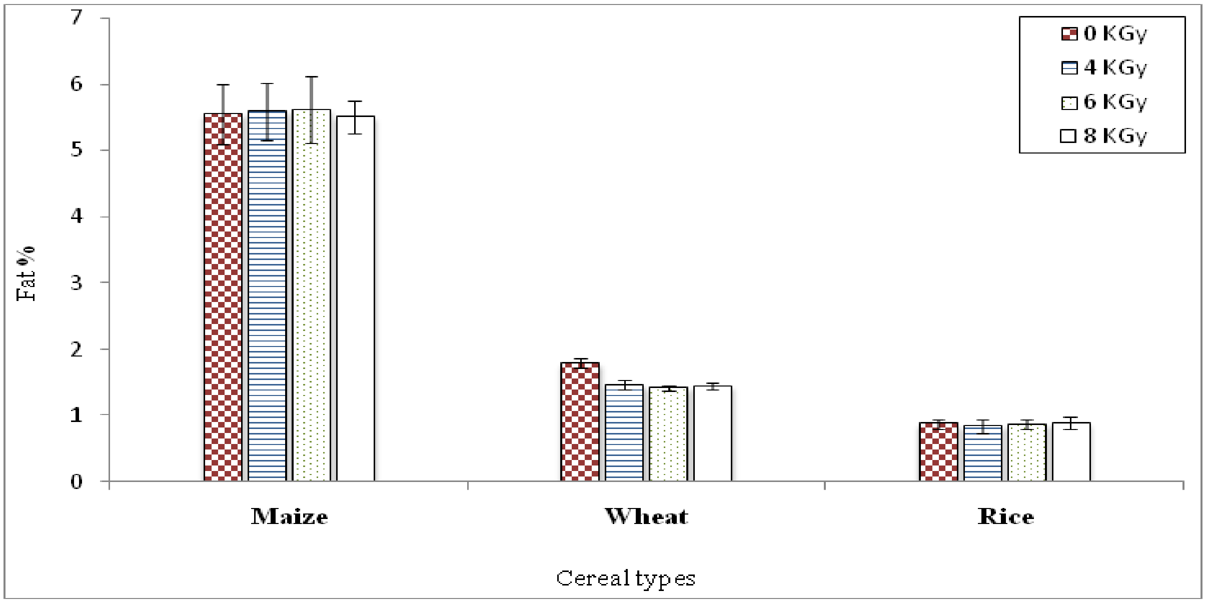

Figure 3. Comparison between the effects of different gamma irradiation doses on fat% as regard maize, wheat, and rice

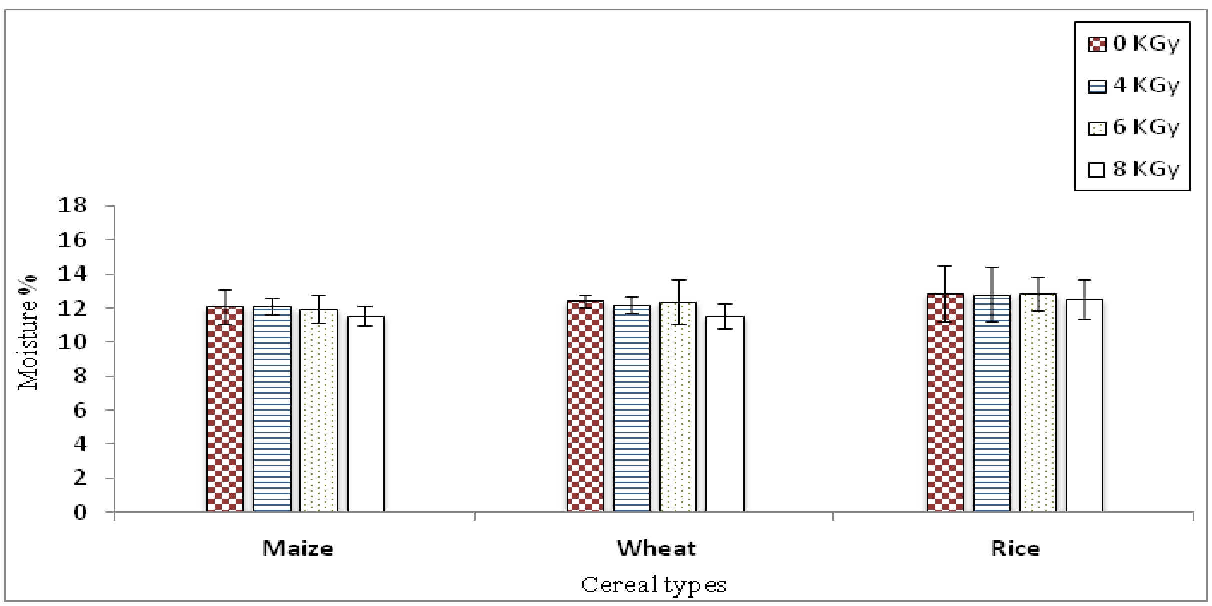

Figure 4. Comparison between the effects of different irradiation doses on mositure% as regard maize, wheat, and rice

Discussion

Aflatoxins are secondary metabolites of the common foodborne fungi *A. flavus* and *A. parasiticus*, which colonize crops in tropical and subtropical regions worldwide. These fungi can also produce aflatoxin during storage, transportation, and food processing. Aflatoxin contamination primarily occurs in cereals, spices, peanuts, tree nuts (almonds, pistachios, hazelnuts, pecans, and Brazil nuts), and milk. Aflatoxin B_1; the most toxic aflatoxin, is the most potent naturally occurring chemical liver carcinogen known.[6]

Distribution of aflatoxin B_1 in cereal samples (maize, wheat, and rice) was observed in terms of acceptable limit for aflatoxin B_1 according to Food and Agriculture Organization (FAO).[7]

From the data presented in Figure 1, it is evident that out of 20 samples of maize about 75% (15 samples) showed AFB_1 concentration greater than 40 ppb (part per billion), about 15% (3 samples) showed AFB_1 concentration in the range between 31 and 40 ppb, 10% (2 samples) in the range between 21 and 30 ppb, and none showed AFB_1 concentration less than or equal to 20 ppb.

It was found that out of 20 samples of wheat 50% (10 samples) showed AFB_1 concentration more than 40 ppb, 30% (6 samples) showed AFB_1 concentration between 31 and 40 ppb, about 20% (4 samples) showed AFB_1 concentration between 21 and 30 ppb and none showed concentration less than or equal to 20 ppb.

In rice samples it was found that none showed AFB_1 concentration more than 40 ppb, 25% (5 samples) showed AFB_1 concentration between 31 and 40 ppb, 60% (12 samples) showed AFB_1 concentration between 21 and 30 ppb, and 15% (3 samples) showed aflatoxin concentration less than or equal to 20 ppb.

Accordingly, these results indicate that, aflatoxin B_1 levels in the collected samples of maize, wheat, and rice are exceeding the acceptable level (5ppb) defined by the FAO.[7]

Tables 1&2, indicate that before gamma irradiation the order of AFB_1 levels in the three grains is maize > wheat > rice and gamma irradiation reduced the toxin significantly with dose levels 8 KGy >6 KGy > 4 KGy. ANOVA test revealed that there is a direct proportionality between the gamma ray dose levels and AFB_1 reduction percents.

As indicated in (Figure 3), for **maize** samples, the initial level of aflatoxin B_1 before gamma irradiation is 50.38 ±14.46 ng/g and it reduced to 20.02±6.70 ng/g after irradiation (60.26 reduction percent) at a dose level of 8 KGy which is still above the legal limit which is 5 ng/g. For **wheat** samples the initial level of aflatoxin B_1 before irradiation was 37.61±6.85ng/g and reduced to 11.55±3.41 ng/g (69.29 reduction percent) after irradiation at the same dose level which is also still above the legal limit which is 5 ng/g and for **rice** the initial level of aflatoxin B_1 before irradiation was 27.49±7.32 ng/g it reduced to 9.71±2.43 ng/g (64.68 reduction percent) after irradiation at a dose level of 8 KGy and it is still above the legal limit which is 5 ng/g.

This means that the percentages of reduction in the levels of aflatoxins were greater in wheat than in rice and maize, respectively, using 8 kGy.

On the other hand the reduction percents at 6 KGy are 32.39%, 43.84%, and 56.38% for maize, wheat, and rice respectively, and at 4 KGy the reduction percents are 15.54%, 22.25%, and 27.46% for maize, wheat, and rice, respectively.

From the afford-said data, we concluded that the reduction percentages in aflatoxin B_1 in the maize, wheat and rice samples are directly proportional to the exposed radiation doses (dose dependent manner) with the highest reduction percent on using the 8 KGy gamma radiation,. Also the reduction percents are different with different cereal samples.

These results are in agreement with the data reported by Aziz and Youssef (2002)[8], who revealed that treatment of food and agricultural products with gamma irradiation at a dose level of 5 KGy destroy 44-48% of aflatoxin B_1 whereas application of 10 KGy dose resulted in reduction of aflatoxin B_1 by 82-88%, i.e., our results is nearly consistent with this study in that by increasing the radiation dose the percent of reduction of aflatoxin B_1 increases.

According to Ghanem et al., (2008)[9], at a dose level of 10 KGy the % reduction of aflatoxin B_1 degradation reached 81.1% for corn (maize), 87.8% for rice, and for feed samples aflatoxin reduction levels reached 31, 72, and 84% in corn at 4, 6, and 10 KGy, respectively.

According to Ghanem et al., (2008)[9] study, AFB_1 degradation is correlated negatively with oil content in irradiated samples and this is consistent with our study in that aflatoxin B_1 reduction level in maize is lower than wheat and rice as maize contains more oil content.

Also according to Ghanem et al., (2008)[9], there was a positive correlation between the increase in doses of gamma irradiation and the levels of breakdown of AFB_1 exist in the commodity and the maximum achievable level of AFB_1 break down varied between tested products and this consistent with our study.

Treatment of food with ionizing energy is a well-known method aimed at improving the safety of a wide range of food by reducing or eliminating foodborne pathogens. The results of our work indicated that gamma radiation affect on the moisture content in cereal samples which is in agreement with other investigators.[10,11]

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