Toxin Reviews

ISSN: 1556-9543 (Print) 1556-9551 (Online) Journal homepage: http://www.tandfonline.com/loi/itxr20

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To cite this article: Mona Eslami, Zohreh Mashak, Ali Heshmati, Mohammad Shokrzadeh & Amir Sasan Mozaffari Nejad (2015) Determination of aflatoxin B_1 levels in Iranian rice by ELISA method, Toxin Reviews, 34:3, 125-128, DOI: 10.3109/15569543.2015.1074925

To link to this article: http://dx.doi.org/10.3109/15569543.2015.1074925

Published online: 23 Sep 2015.

http://informahealthcare.com/txr
ISSN: 1556-9543 (print), 1556-9551 (electronic)

Toxin Reviews

Toxin Rev, 2015; 34(3): 125–128
 DOI: 10.3109/15569543.2015.1074925

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ORIGINAL ARTICLE

Determination of aflatoxin B_1 levels in Iranian rice by ELISA method

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Abstract

This study was carried out to detect the presence of aflatoxin B_1 (AFB_1) in 40 samples of Tarom rice from Iran. Enzyme-linked immunosorbent assay (ELISA) was applied to analyze AFB_1 in the samples. All the analyses were conducted twice. Aflatoxin B_1 was found in all rice samples, the concentration of AFB_1 ranged from 0.29 to 2.92 μg/kg. The AFB_1 concentration mean in the rice samples produced in 2013 was higher ($P < 0.05$) than the findings in rice in 2012.. However, 25 of the 40 samples exceeded the maximum prescribed limit, i.e. 2 μg/kg of European Union Regulations and also none of the samples reached the maximum prescribed limit 5 μg/kg of the Institute of Standards and Industrial Research of Iran (ISIRI) for aflatoxin B_1. Although, rice is ranked the second among cereal staples consumed food in Iran and many countries, it can make a serious health problem for people even for a small amount of aflatoxin.

Keywords

Aflatoxin B_1, ELISA, Iran, rice

History

Received 24 May 2015
Revised 15 July 2015
Accepted 17 July 2015
Published online 8 September 2015

Introduction

Rice (*oryza sativa*) is one of the most consumed dominant grains after wheat and is the staple food crop of more than a half of world population (Elzupir et al., 2015). It is resistant to humid, desert, hot, flood, dry and cool conditions, and grows in saline, alkaline and acidic soils (FAO, 2004). The most important world rice producers in 2011 were China and India with 202.3 and 154.5 million metric tons, respectively (Suarez-Bonnet et al., 2013). In Iran, the average yield is almost 4.9 ton/ha, which makes the country the 11th rice producer in world (Eskandari et al., 2012). Mazandaran province is a one of the highest producers of rice in northern part of Iran. There are several varieties of rice such as Tarom, Khazar, Neda, Nemat, Fajr and Shiroudi in Mazandaran province.

Mycotoxins are serious problems for human and animal health; and they are natural contaminants of important plant products including oil seeds, dried fruits, nuts, spices and cereals (rice, corn, wheat, barley, etc) (Kamkar et al., 2014a; Mozaffari Nejad et al., 2014). Aflatoxins (Afs) are the most important mycotoxins for food contaminants worldwide especially for developing countries (Kamkar et al., 2014a). The main types of aflatoxins are Aflatoxin (AF) B_1, AFB_2, AFG_1, AFG_2, AFM_1 and AFM_2. AFB_1 is the most toxic type than other aflatoxins; and also it is the most potent human carcinogen. Hence, AFB_1 is classified into the primary group of carcinogenic compounds by the International Agency for Research on Cancer (IARC) of World Health Organization (WHO) (Elzupir et al., 2015; Mozaffari Nejad et al., 2013; Tavakoli et al., 2013). Aflatoxin B_1 is a stable compound which cannot be destroyed during most of the food processing operations. Also, several studies have been published about mycotoxins and especially aflatoxins contamination related with damages that lead to huge economic losses in some countries. Many different countries have set large regulations to control levels of mycotoxins in plant products and dairy products, moreover, this conditions vary from one country to another (Kamkar et al., 2014b; Mozaffari Nejad et al., 2013). European Union has established maximum tolerable limits (MTL) for AFs in rice as 4 μg/kg for total aflatoxins ($B_1 + B_2 + G_1 + G_2$) and 2 μg/kg for AFB_1 (Commission Regulation No. 1881/2006). The Institute of Standards and Industrial Research of Iran (ISIRI) has set a limit of 5 μg/kg for AFB_1 in rice (ISIRI No. 5925/2002).

Several methods have been used for aflatoxins analysis, including the thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) (Kamkar et al., 2014a; Tavakoli et al., 2012). ELISA is a simple method which enjoys some advantages as being rapid and selective and having routine diagnostic and reliability for the analysis of a large number of samples (Kamkar et al., 2014a; Mozaffari Nejad et al., 2014).

The aim of this study was to investigate the presence of AFB_1 in Tarom Rice samples including the two consecutive years yield in Qaemshahr, a northern city of Iran.

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Table 1. Occurrence and level of AFB_1 in rice in Iran.

Sample collected in	Analyzed samples	Positive samples (%)	Number of samples in concentration range, (μg/kg) <2[c]	2–5	>5[d]	Range[a] (μg/kg)	Average[b] (μg/kg)	SD (μg/kg)
2012	20	20 (100)	6 (30%)	14 (70%)	0	0.29–2.51	1.96	0.577
2013	20	20 (100)	9 (45%)	11 (55%)	0	0.31–2.92	2.22	0.744
Total	40	40	15 (37.5%)	25 (62.5%)	0	0.29– 2.92	2.09	0.669

[a]Min – Max.
[b]Mean of positive samples.
[c]Below than EU MTL (2 μg/kg).
[d]Higher than ISIRI and FDA (5 μg/kg).

Materials and methods

Samples

In December 2013, 40 samples of rice from local markets of Qaemshahr city in Mazandaran province, Iran, were randomly collected. The rice samples including the Tarom variety had been produced in 2012 (20 samples) and 2013 (20 samples). All samples were powdered with Moulinex blender (Black & Decker products Co., Towson, MD). Five hundred grams of each sample were stored at 4–6 °C in sealed plastic bags for the analysis (Mohamadi Sani et al., 2014). All samples were analyzed in duplicates.

Analysis of AFB_1 by ELISA

The quantitative analysis of AFB_1 in the samples was performed based on a competitive enzyme immunoassay using RIDASCREEN® Aflatoxin B_1 30/15 test kit (Art. No: R1211, R-Biopharm, Darmstadt, Germany). Preparation of the samples and ELISA test were performed according to the method described by R-Biopharm GmbH (2010).

Samples preparation

Samples preparation was preformed according to the instructions of the test kit manual of RIDASCREEN® Aflatoxin B_1 30/15 (R-Biopharm GmbH, 2010). Twenty-five milliliter methanol: water (70:30) was added to 5 g of rice after the samples were shaken vigorously for 3 min manually. The obtained extract was filtered through a filter paper and diluted with distilled water (1:1). At last, 50 μl of the diluted filtrate per well was used in the test.

ELISA test procedure

According to RIDASCREEN Aflatoxin B_1 30/15 (Art No.: 1211) test kit manual, 50 μl of the standard solution or prepared sample in duplicate was added to the wells of microtiter plate. Then, 50 μl of the enzyme conjugate and 50 μl of the anti-aflatoxin antibody solution were added to each well, mixed gently and incubated for 30 min at room temperature (20–25 °C). Liquid was removed from wells by tapping the wells upside down vigorously against the absorbent paper; the wells were then washed twice by a washing buffer (250 μl). After the washing step, 100 μl of substarte/chromogen solution was added to each well, mixed gently and incubated for 15 min at room temperature (20–25 °C) in a dark place. Finally, 100 μl of the stop solution (1 N H_2SO_4) was added to each well and the absorbance was measured at 450 nm in ELISA plate reader.

Statistical analysis

The data were analyzed using SPSS version 16 (IBM SPSS Inc., Chicago, IL). One-sample *t-test* was used for determination of the difference between levels of aflatoxin in rice samples and maximum limits levels permitted in Iran and Europe. Difference between aflatoxin mean in rice samples gathered in 2012 and 2013 was compared by independent t test. $P < 0.05$ was considered as a significant difference.

Results and Discussion

The occurrence and levels of AFB_1 in Tarom rice samples in 2012 and 2013 are presented in Table 1. Aflatoxin B_1 was found in all Tarom rice samples. The ranges of aflatoxin B_1contamination were from 0.298 to 2.511 μg/kg and 0.317 to 2.923 μg/kg for rice samples in 2012 and 2013, respectively. Also, none of the samples exceeded the ISIRI limit of 5 μg/kg for aflatoxin B_1. The AFB_1 concentration mean in the rice samples produced in 2013 was higher ($P < 0.05$) than the findings in rice 2012. However, no significant difference was observed in the AFB_1concentration mean in samples collected in 2012 and 2013.

Rice are produced and consumed in developing and developed countries. A number of surveys of aflatoxin B_1 contamination in rice are reported in the other studies. As shown in Table 2, several studies (Diaz et al., 2001; Feizy et al., 2011; Iqbal et al., 2012; Iqbal et al., 2014; Karajibani et al., 2013; Majeed et al., 2013; Mazaheri, 2009; Park et al., 2004; Rahmani et al., 2011; Reddy et al., 2009; Reddy et al., 2011; Reiter et al., 2010; Sales & Yoshizawa., 2005; Tansakul et al., 2013; Tri Nguyen et al., 2007; Wang & Liu, 2007; Yazdanpanah et al., 2013) reported the aflatoxin B_1 contamination in different varieties of rice. In a previous study by Lai et al. (2014) from China, they analyzed that the occurrence and concentration range of aflatoxin in 30 rice samples were investigated by HPLC-FLD. They reported that 24 (80%) of the samples were contaminated with aflatoxins. Also, AFB_1 and AFB_2 were found in 14 samples (46.7%) with a range of (0.03–2.08 μg/kg) and seven samples (23.4%) with a range of (0.02–0.48 μg/kg), respectively. Only one sample was found above the EU regulatory limit for AFB_1, also, no samples exceeded the maximum levels for total aflatoxins. However, our study results showed that all the samples were contaminated with AFB_1 and also 25 of 40 samples had

Table 2. Occurrence and levels of mycotoxins in rice reported in previous studies.

Country	No of samples	Positive *n* (%)	Method	Mycotoxin	Range (μg/kg)	Reference
Colombia	40	4 (10)	HPLC	AFB_1	1.0–103.3	Diaz et al. (2001)
Korea	88	5 (5.6)	HPLC	AFB_1	2.1–7.7	Park et al. (2004)
Philippines	78	74 (94.8)	HPLC	AFs	NR–8.66	Sales & Yoshizawa (2005)
Vietnam	100	51 (51)	HPLC	AFB_1	NR–29.82	Tri et al. (2007)
China	84	23 (27.3)	HPLC	AFs	0.15–3.88	Wang & Liu (2007)
Iran	71	59 (83)	HPLC	AFs	0.1–10	Mazaheri (2009)
India	1200	814 (68)	ELISA	AFB_1	0.1–308	Reddy et al. (2009)
Austria	81	24 (29.6)	HPLC	AFB_1	0.45–9.86	Reiter et al. (2010)
Iran	256	55 (21.5)	HPLC	AFB_1	0.0–5.8	Rahmani et al. (2011)
Malaysia	13	9 (69.2)	ELISA	AFB_1	0.68–3.79	Reddy et al. (2011)
Iran	182	11 (6)	HPLC	OTA	0.2–4.8	Feizy et al. (2011)
Pakistan	413	185 (44.8)	HPLC	AFs	NR–68.3	Iqbal et al. (2012)
Iran	18	9 (50)	HPLC	AFB_1	1.17–30.63	Yazdanpanah et al. (2013)
Pakistan	68	38 (56)	HPLC	AFB_1	0.05–0.15	Majeed et al. (2013)
Iran	100	47 (47)	HPLC	AFB_1	0.07–2.36	Karajibani et al. (2013)
Thailand	35	3 (9)	HPLC	AFB_1	0.06–36.64	Tansakul et al. (2013)
Pakistan	380	143 (37.6)	HPLC	AFB_1	LOD–96.6	Iqbal et al. (2014)

NR: Not reported.

higher ranges than the tolerated limit by EU. The same study from Spain, by Suarez-Bonnet et al. (2013) analyzed the AFt values in the rice imported from France (AFt = 26.6 μg/kg) showed that imported rice samples were less contaminated than the previous ones. The rice originating from Mexico (AFt = 16.9 μg/kg) and imported from the United States (AFt = 14.4 μg/kg) or Uruguay (AFt = 15.6 μg/kg) revealed that the imported rice had better quality in terms of the presence of AFs. The previous study by Villa & Markaki (2009) reported a very high incidence of AFs (56%), with levels between 0.05 and 4.3 μg/kg in rice samples analyzed by HPLC method from Athens.

This study was the first report on Aflatoxin B_1 in Tarom rice samples in northern part of Iran while several reports have been presented by Iranian researchers about the imported rice in Iran. Mohammadi et al. (2012), using HPLC method, revealed that among 152 rice samples imported to Iran, 75% samples were contaminated with AFB_1 levels and also, no samples with AFB_1 above MTL of 5 μg/kg were confirmed by ISIRI, which is similar to our results. Although, the previous survey by Faraji et al. (2010) in Iran showed that 8.3% of the rice samples had more MTL ranges than that of ISIRI, but our results showed lower values. Moreover, in the similar study again from Iran which was performed by Karajibani et al. (2013) using HPLC method identified that 33 from 100 yellow rice samples contained AFB_1 but our results represent that all the 40 samples were contaminated with aflatoxin B_1. Asghar et al. (2014) reported that 250 (95.4%) from 262 rice samples from Pakistan were found to be contaminated with aflatoxin B_1. The aflatoxin of rice was in the concentration ranges from 1.07 to 24.65 μg/kg. The previous study by Bansal et al. (2011) surveyed some mycotoxins including AFB_1, OTA and FB_1 on rice samples from several different countries. They showed that the most contamination presence was related to AFB_1 that was higher than FB_1 and OTA. The content of AFB_1 and FB_1 and OTA was 56.90, 15.99 and 1.99 μg/kg, respectively. Also, the similar research done by Anthony Makun et al. (2011) from Nigeria reported that the total aflatoxins and specially AFB_1were the most observed toxins among other mycotoxins including OTA, zearalenone (ZEA), deoxynivalenol (DON). In previous studies conducted in Ivory Coast of West Africa by HPLC method, Sangare-Tigori et al. (2006) showed that 100% (10 of 10) rice samples were contaminated with AFB_1, which is similar to our results. They calculated AFB_1 ranging from >1.5 to 10 μg/kg. Furthermore, the results of some researches confirmed that the AFs and specially AFB_1 are the most current contamination of mycotoxins in rice. Hence, there has been a concern for consumer especially for children all over the world.

Besides, several studies showed that some processes are suitable for aflatoxin reduction. A recent study by Mohamadi et al. (2014) revealed that the highest aflatoxin reduction was observed when rice samples were cooked by rice cooker than traditional local method.

Conclusion

This study evaluated AFB_1 contamination in Tarom rice variety from Qaemshahr in Mazandaran Province, Iran. The present results showed that all the samples were contaminated with AFB_1, but all the samples MTL did not exceed the ISIRI limit. As rice consumption is high in Iran, we assume that the present status of this mycotoxin does pose a serious risk to public health; therefore, there is a need for routine monitoring of rice as a food quality-control measure. Initial approaches to control the occurrence of AFB_1 in rice have involved controlling contamination in the field; however, this is difficult as fungal growth is influenced primarily by climatic conditions, such as relative humidity and temperature.

Declaration of interest

The authors declare that there are no conflicts of interest.

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