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A Study of Aflatoxins Production in Rice Bran from Mazandran Province, Northern Iran

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Abstract: Aflatoxins are produced by toxigenic *Aspergillus* species including *A. flavus* and *A. parasiticus*. They are secondary metabolites which considered as one of the threatening factors of food and feed consumer health. In this study the possible presence of aflatoxins (B_1, B_2, G_1, G2 and total aflatoxin) in rice bran were evaluated. In order to determine of aflatoxins and contamination of the samples with toxigenic fungi, especially A. *flavus* and *A. parasiticus*, 30 rice bran samples were collected from different factories in Mazandran province, northern Iran. Samples containing 15 specimens were conserved for one year in the storage and the other one was not subjected to storage. Aflatoxins were extracted, purified and finally quantified by high performance liquid Chromatography (HPLC). Results showed that almost all collected samples contained aflatoxins. The averages of total aflatoxins in new and old rice bran were found to be 18 and 17 µg/kg, respectively. No significant difference was obtained between total aflatoxin rate in new and old samples. The correlation between culture results and aflatoxins production were significantly observed only in old samples ($P<0.05$). The most common isolated fungi were *Aspergillus* spp, *Fusarium* spp, *Mucor* spp and *Rizopus* spp. These results confirmed that under study bran rice were contaminated with aflatoxins and different toxigenic fungi. So animals, especially ruminants, are in danger to be affected by contaminated feeds.

Key words: Aflatoxin · *Aspergillus* · Rice · Mazandaran province

INTRODUCTION

Aflatoxins are secondary metabolitis, produced by *A. flavus* link *A. parasiticus* [1,2]. These fungi survive in a wide range of environments and can be found in soil, plant and grains and their products [3]. Those fungi are responsible for spoilage of stirred grains and their products around the world [4]. *A. flavus* is the main fungus that causes preharvest aflatoxin contamination in field crops. The food and agriculture organization of the united nations (FAO) estimated that at least 25% of the worlds cereal grains are contaminated by mycotoxins including aflatoxins [5,6]. Because of the toxic and potent carcinogenic of aflatoxins, many developed countries have established very stringent regulations limiting the maximum allowable amount of aflatoxins in food and feed [7,8]. Some developing countries like Iran have set up regulations compatible with other progressive countries for human consumption [9,10]. In despite of above mention point, there are a little information about feed stuffy conservation.

Aflatoxicosis causes acute liver damage, liver cirrhosis, induction of tumors, impaired central nervous system, skin disorders and immune defects. The overall

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toxicity of aflatoxin in an animal appears to be determined by the rate of formation of the reactive intermediate, its binding to the largest macromolecules (DNA and RNA) [11,12]. Then for both food or feed safety and economic reasons, aflatoxin contamination is therefore a serious concern throughout the world. The northern region of Iran is essential producer rice bran. Almost there are equal types of climate with high level of rain and humidity, in which regulates fungal grows and mycotoxin expression. An investigation on aflatoxin contamination of agricultural commodities was carried out in different parts of Iran such as maize and rice grains, but a little date has been published regard the rice bran in Iran. In this study, the presence of aflatoxins and mold contamination in the samples (new and old) in Mazandran, one of the two major rice bran productions, provinces in northeastern Iran, has been exanimated.

MATERIALS AND METHODS

Rice Bran Samples: Thirty samples of rice bran were randomly collected from thirty factories of northern region of Iran, from between August and September 2009. Fifteen samples were storage for one year, while that the other ones were the fresh samples. To avoid sampling error due to the highly heterogeneous nature of fungal distribution, each 1 kg composite sample prepared by mixing of 4 sub a samples (25 g each).

Extraction and Purification: Estimation of aflatoxins in the samples were performed one 25 gr a liquots of rice bran by solvent extraction followed by HPLC with fluorescence detection. Each 25 gr sub sample was added with 2.5 gr Nacl and 100 ml of 80 % methanol was prepared and added to it. This solution was mixed with hemogenazar for 3 min, then was filtered through filter paper and collected enter falcon tubes. Then, 7 ml of this solution that had been filtered with 35 ml phosphate buffer saline (PBS) was added and constant oscillation at 100 rpm for 30 min for the initial aflatoxins extraction. The extract was harvested by filtering out the in soluble debris with what man No 4 filter paper. The organic extract was future purified using a chromatography column. Then, 36 ml of extraction with speed one drop in second was passed of it. The column is then washed with 15 ml PBS, removing extraneous non-specific material. The bound toxin then eluted off the column, using 500 µl methanol HPLC the reaction was stopped after minutes, again 750 µl methanol HPLC was added with column and collected in a vial for HPLC analysis. Then, 1750 µl deionized water was added with vial and mixture. After filtration, 200µl of vial content injected into injector HPLC.

Detection: The standard of the five aflatoxins B_1, B_2, G_1, G_2 and total aflatoxin were a mixture of powder purchased from the Sigma Co. laboratory. The dry powder was dissolved in 5 ml acetonitrile and the diluted standards contained 4.2 µg B_1, B_2, G_1, G_2, total aflatoxin per ml according to the manufacture's manual. HPLC with fluorescence detection was used in determining the amount of aflatoxins in the samples. Chromatographi separations were performed using a stainless steel c18 reversed-phase column. Methanol- acetonitrile-water (20:20:60) was used as the mobile phase at a flow rate 1ml/ min. Excitation and emission wave lengths were 365 and 440 nm, respectively. Standard curves for aflatoxins B_1, B_2, G_1, G_2 and total toxin standard samples in a series of concentrations. Peak areas of the excitation curves were used for quantification.

Culture of Samples: The samples were cultured on plate containing sabouraud glucose agar (SGA) that previusly in order to identification of some fungi such as different *Aspergillus* species, *Fusarium* described. Special media such as potato dextrose agar and czapek-dox agar were used, as well.

Data Analysis: Students t test and chi-square test were used to asses of the results.

RESULTS

As shown in Tables 1 and 2 as well as Figs. 1 and 2, among the 30 samples, almost all them were found to content aflatoxins. Different aflatoxins B_1, B_2, G_1, G_2 and total toxin were detected in the samples. Only in two new samples aflatoxins B_2, G_1, G_2 had not been detected. Also, aflatoxin B_2 was not observed in old sample. The mean aflatoxin B_1, B_2, G_1, G_2 and total toxin in the old samples averaged 6.81, 4.04, 3.87, 3.53 and 17.53 µg/kg, respectively, while for new samples (without storage time) averages were 5.07, 4.09, 6.34, 3.43 and 18.7 µg/kg, respectively. The data indicated no significant linear relationship in whole rice bran between the amount of aflatoxins and the length of storage. However, in old samples the amount of aflatoxin B_1 is higher than in new

Table 1: Amount of aflatoxins in different new samples collected rice bran (µg/kg)

	Aflatoxins				
Sample	B_1	B_2	G_1	G_2	Total Toxin
1	1.25	1.25	1.71	0.8	10.80
2	1.48	2.2	10.3	1.08	11.83
3	1.57	2.25	11.7	1.12	12.33
4	1.58	3.11	14.7	1.25	12.69
5	12.8	3.2	2.3	1.87	14.32
6	2.81	3.3	2.41	2.32	15.59
7	3.2	3.66	3.22	2.65	16.25
8	3.4	4.25	3.77	3.48	17.49
9	5.32	4.35	5.1	4.25	18.98
10	5.71	4.48	5.48	5.11	21.23
11	6.32	6.48	6.32	6.23	27.50
12	6.33	6.9	7.29	6.95	29.62
13	6.75	7.8	8.13	7.48	29.69
14	7.6	nd	nd	nd	33.00
15	9.8	nd	nd	nd	9.15
Total	75.92	53.23	82.43	44.59	280.47

Table 2: Amount of aflatoxins in different old samples collected rice bran (µg/kg)

	Aflatoxins				
Sample	B_1	B_2	G_1	G_2	Total Toxin
1	1.2	1.4	1.12	1.12	4.39
2	1.25	1.8	1.23	1.12	7.49
3	2.1	1.8	1.32	1.12	9.08
4	2.1	2.32	1.44	1.78	9.23
5	3.32	3.11	2.85	1.83	9.71
6	4.48	3.12	3.21	2.83	11.05
7	5.75	3.25	3.25	2.9	12.40
8	6.48	3.92	3.25	4.25	13.28
9	7.4	4.43	3.28	4.55	17.32
10	9.32	4.48	4.73	4.7	23.01
11	9.32	5.4	5.12	5.29	23.82
12	9.93	6.08	6.25	5.33	25.42
13	11.87	7.2	7.8	5.77	28.43
14	13.1	8.25	9.4	6.78	33.49
15	14.5	nd	nd	nd	34.85
Total	102.12	56.56	54.25	49.37	262.97

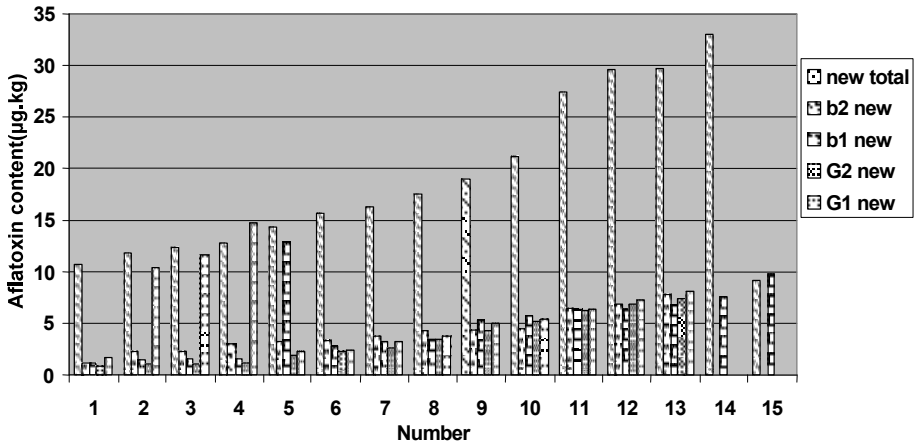

Fig. 1: Amount of aflatoxins in different new samples collected rice bran (µg/kg)

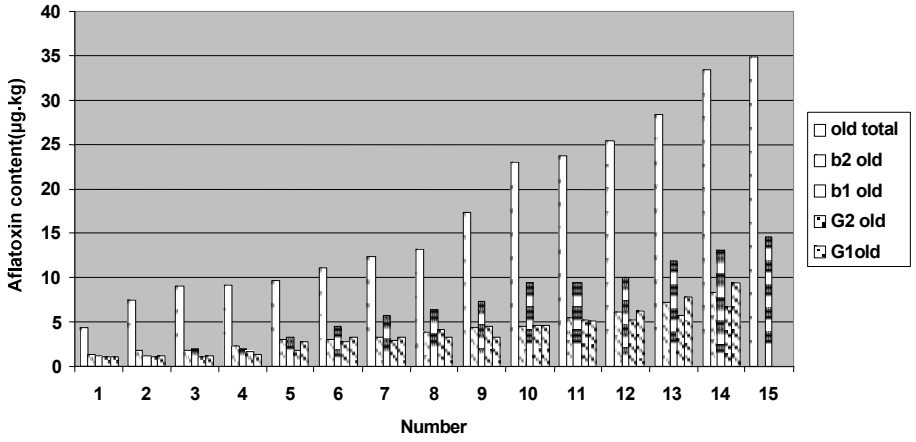

Fig. 2: Amount of aflatoxins in different old samples collected rice bran (µg/kg)

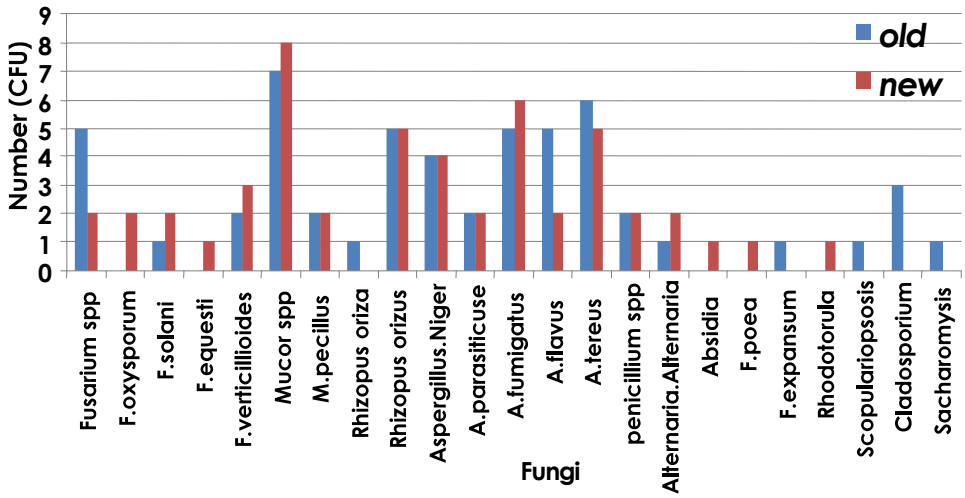

Fig. 3: Comparative frequency of fungi isolated from old and new samples

samples, but aflatoxin G_1 and total toxin increased in the new samples, ranged from 17.53 to18.70 and G_1 from 3.87 to 6.34 µg/kg received, respectively. Based on statistic-test the correlation between aflatoxin B_1/B_2, in old samples, were significant with G_2 and total aflatoxin ($P<0/05$). Also, the correlation between aflatoxin B_1 in new samples with aflatoxin G_1 in the same sample was significant ($P<0/01$). The correlation between aflatoxin G_1 in new samples with both G_2 and total toxin in the same sample were significant ($P<0/01$). The fungi recovered from these samples are given in Fig. 3. The more presence of *Aspergillus* spp was correlated with increasing aflatoxin of whole bran rice ($P<0/05$). Also, the correlation between culture results and aflatoxins were significant only in old samples ($P<0/05$).

DISCUSSION

Aflatoxin contamination and *A. Flavus* infection are often associated with drought and temperature [13,14]. Mazandran province is located northern Iran. It covers area with moderate weather conditions. The temperature is relatively warm and humid all year around. All of the areas have a similar weather condition. The aflatoxins content in these samples were less than the minimum standard institute regulated level. Since rice bran are main diet of the local animals and consumed in large quantities and added with diet of them either directly or in directly. So, aflatoxin contamination could be a serious even at low levels [15]. Improvement in storage conditions to prevent of spoilage and reduce aflatoxin contamination is recommended [16]. The aflatoxin content in the samples stored for 1 year was less than the non stored samples. The reason for the decline on storage is unclear. Some researchers found that the aflatoxigenic fungi and aflatoxin formation might be inhibited by some synthetic pesticides or natural protectants during storage [15]. Also, it was states that low aflatoxins content could be due to good storage conditions [17]. Usually rice bran was kept in old fashioned store houses with poor ventilation and high temperature and humidity. In general, the toxigenic *A. flavus* strains express aflatoxins B_1 and B_2 [18], while *A. prarasiticus* strains produce B_1, B_2, G_1 and G_2 [19]. Aflatoxins B_1 and B_2 are the most potent and the most common toxins in contaminated grain and their products and are the focus of research in most countries [20-22]. According to the data presented, types of aflatoxins detected in the present samples were common in the old samples. Aflatoxin B_1 was higher than the new one. It is necessary to mention that *A. flavus* was isolated from old samples rather than new samples. Also, it is possible the fungi grown on bran rice might be a new toxigenic species other than the two aflatoxingenic *A. flavus* and *A. parasiticus*. So other aflatoxingenic fungi may exist in rice bran samples and need to identify. Even it the rice plant might have altered the aflatoxin producing pattern by plant-fungus interaction previous reports [23,24] whereas certain volatile compound generated by plants inhibited aflatoxin formation or were shown to interfere with aflatoxin formation [25,26]. So the rice bran might contain or have produced certain enzymes or chemicals that inhibited the production of aflatoxin or a type of aflatoxin [27]. The correlations between toxins in this study are not fully understood. But, it might be in correlation with gene clusters, chemical pathway and contamination of rice bran with aflatoxins [28-30].

In conclusion, investigation of aflatoxins in Mazandaran province demonstrated that feed in northern Iran could be contaminated with mycotoxins. Also, further studies are recommended.

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