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STUDYING THE PREVALENCE OF AFLATOXIN B_1 IN THE RICE OF THE STORES LOCATED IN WEST OF MAZANDARAN

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ABSTRACT

Aflatoxins are a group of secondary metabolites of fungi, the most toxic of which is aflatoxin B1 (AFB1). These compounds have carcinogenic and mutagenic properties and are often produced by Aspergillus flavus and Aspergillus parasiticus. Rice is the most consumed food type that is exposed to contamination with aflatoxins. In our country, due to different climatic conditions, the possible existence of toxin-producing fungi with a wide range of toxins in the environment is concerning. In this study, 40 samples of rice (Tarom and Hashemi varieties) were collected from shops in West Mazandaran towns (Marzanabad, Chaloos, Noor, Kelarabad and Tonekabon), and their aflatoxin contamination levels were measured by ELISA. The results showed that all samples were contaminated with AFB1. Total samples had AFB1 levels that were lower than the maximum tolerated level of AFB1 in Iran (5 ng/g). The analysis of variance revealed no significant differences among the mean AFB1 contaminations in all rice samples. Thus aflatoxin contamination is independent of type of rice and collection location.

Keywords: Aflatoxin B_1, Rice, Aspergillus, ELISA

INTRODUCTION

Rice (L. Oryza Sativa) is a plant with genetic diversity and high compatibility and it is one of the most important grains of the world in terms of providing the needed energy and calories of human societies and it has been cultivated in many parts of the world and especially in Iran from ancient times. Mazandaran Province is one of the most important areas of production of this product in the country. Due to having enzymes of amylase and protease and also minerals and compounds contributing to the growth, like other grains rice is also at the risk of fungal contamination and it can be a proper place for the growth of toxin fungi such as Aspergillus. aflatoxin B1 is also an important cause of fetal malformations (Teratogenic), genetic abnormalities, mutagenic and carcinogenic **[1]**. Although aflatoxin creates adverse effects in consumers of the foodstuffs that contain it but existence of a limited amount of it in foodstuffs is permissible. According to the Food and Agriculture Organization of the United Nations (FAO) the permissible amount of aflatoxin in most of foodstuffs except for milk is 20 ng /g. But the European Union has limited its amount to 5 ng/ g **[2]**. According to the standards of Iran the amount of aflatoxin B1 in rice should not be more than 5 ng/ g **[3].** Various researches and reports have been conducted about the contamination of rice to aflatoxin B1 in Sri Lanka **[4]**, Iran **[5, 6]**, China **[7]** and India **[8]**. A few studies have been conducted in Iran about the aflatoxin contamination rate in rice. Due to the importance of the issue, in the present study which is an applied research we have examined contamination of important varieties of rice of the west area of Mazandaran to aflatoxin B1 by using ELISA technique (Enzyme-linked immunosorbent assay).

MATERIALS AND METHODS

Sampling: In this study, 40 samples of rice (varieties of Hashemi and Tarom) which were harvested in 2012 were collected from the authentic stores of supply and sale of foodstuffs of 5 cities with different geographical location in the west of Mazandaran (Marzanabad, Chaloos, Noor, Kelarabad and Tonekabon). Sampling was performed according to sampling method of agricultural products for aflatoxin testing and it was done randomly **[9]**. The minimum amount of the purchased sample was 2 kilograms. After sampling, the samples were transferred to the laboratory in appropriate circumstances. Milled rice samples were then transferred to a nylon bag and were coded and until the time of testing they were kept in appropriate cryogenic conditions (temperature of -20°C).

Analysis of aflatoxin B1: Enzymatic kit of ELISA of the competitive immunoassay enzyme type was purchased from the Europroxima Company of Netherlands for determining the amount of toxin of aflatoxin B1 in foodstuffs (code AFB15121).

Extraction of aflatoxin B1: 3 grams of homogenized rice flour sample and 9 ml of 80% methanol were mixed for 5 minutes by using a mixer. Extract was centrifuged at 2000 rpm for 10 minutes. Then 50 µl of the filtered solution was diluted with 150 µl buffer. Fifty microliter of the diluted extract was used for each well in the test.

ELISA test: 50 µl of standard solutions of aflatoxin B1 (0.0313, 0.0625, 0.125, 0.25, 0.5 and 1 ng/ml) and 50 µl of the sample prepared for the test were poured separately into the wells of microtiter plate in two replications. After that, 25 µl of Conjugate enzyme (Aflatoxin-HRP) and 25 µl of aflatoxin antibody solution were added to each well and were incubated for 60 minutes at 37 ° C in darkness. Then the wells were washed three times with wash buffer. 100 µl of substrate solution was added to each well and was incubated for 30 min at room temperature. Then 100 µl of stop solution was added to each well and the absorbance was measured at 450 nm in an ELISA reader device.

Statistical analysis: In this study, analysis of the results was conducted by one way ANOVA ($p<0/05$) and comparison of the means by using Duncan test with the help of SPSS 16 software. Graphs were drawn by using Excel software.

RESUILTS

In the present study the amount of aflatoxin B1 in two types of local and prominent rice samples (Hashemi and Tarom) of Mazandaran Province, which were collected from the wholesale centers of rice located in the west cities of Mazandaran (Marzanabad, Chaloos, Noor, Kelarabad and Tonekabon), was evaluated by ELISA method. Methods that are commonly used in the determination of mycotoxins are based on Thin Layer Chromatography (TLC), High-performance liquid chromatography (HPLC) and ELISA test **[10].** TLC method has a relatively high coefficient of variation, and it is only applicable in a place where mycotoxin contamination levels are higher than current control limits. Other chromatographic techniques such as HPLC techniques are very expensive and time consuming. On the other hand, TLC and HPLC methods require a solid-phase extraction process or the immunoaffinity column. Therefore, in routine studies and tests, present immunological methods are preferred over chromatographic methods **[11]**. Currently, the interest in using ELISA test kits is greater than conventional methods such as HPLC and TLC because of small sample size and simpler method of purification. These methods are fast, simple, specific, sensitive, and applicable in many cases and they are considered as the most common methods for detection of mycotoxins in foods **[12, 13]**. The obtained results show that all samples of Hashemi and Tarom rice were contaminated with aflatoxin B1 **(Figure 1 and 2)**. Comparison of the results of analysis of the variance of data obtained through the measurement of aflatoxin B1 in rice samples of Hashemi showed that there was no significant difference in the amount of aflatoxin of samples collected from the studied cities (p<0/05). The amount of aflatoxin B1 (range of 1.11 – 0/47 ng /g) in all rice samples of this type was less than standard limit of Iran (5 ng/ g) **(Figure 1)**.

In comparison of the amount of aflatoxin B1 in rice samples of Tarom there was also no significant difference in the amount of aflatoxin of samples collected from the studied cities (p<0/05). In this variety the amount of aflatoxin B1 was in range of 0/64-2/00 which was less than standard limit of Iran (5 ng/ g) **(Figure 2)**.

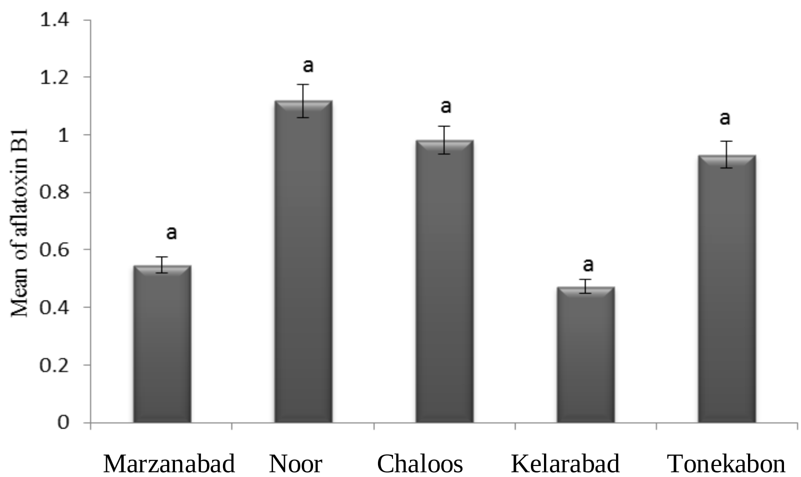

Figure 1: Mean of aflatoxin B1 contamination in rice samples of Hashemi variety in the studied cities

[a] Means of the column with the same letters do not have any significant differences at the 5% level.

- The results of the mean are four replications.

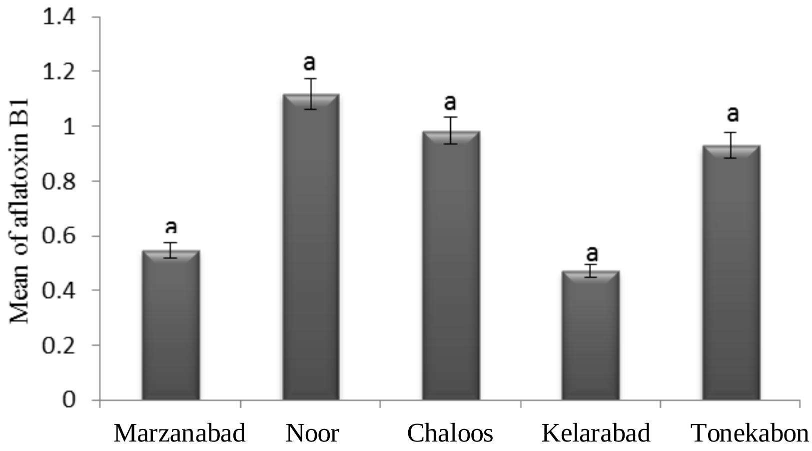

Figure 2: Mean of aflatoxin B1 contamination in rice samples of Tarom variety in the studied cities

Also analysis of variance showed that there is no significant difference between the average amount of aflatoxin B1 in two varieties (p<0/05).

DISCUSSION

In general, all samples of two varieties of local and prominent rice samples of Hashemi and Tarom of Mazandaran Province, which were collected from the wholesale centers of rice located in the west cities of Mazandaran (Marzanabad, Chaloos, Noor, Kelarabad and Tonekabon) were contaminated with aflatoxin B1 and the amount of aflatoxin B1 in all samples in the studied cities were less than standard limit of Iran (5 ng/ g). In comparison of the results of analysis of the variance of the obtained data, there was no significant relationship between aflatoxin B1 contamination mean in rice samples of cities and the studied verities(p<0/05). Therefore the obtained results showed that aflatoxin contamination levels are independent from the rice variety and the place of collection of the sample. About the aflatoxin contamination of the grains, initially, it must be considered that aflatoxin contamination is mainly caused by improper maintenance of products after harvest that allows storage molds such as Aspergillus to grow on products and produce mycotoxins. Now, it is clear that aflatoxin production is not simply caused by improper maintenance and these compounds are produced before harvest and in growing crops on the farm. Aflatoxin-producing Aspergillus species can make a parasitic relationship with the healthy plants and when the plants are under stress, such as drought, they can produce low but significant amounts of aflatoxin **[14]**. Contamination of rice to aflatoxin B1 has been studied and reported in numerous researches. Mazaheri (2009) in a study has measured the amount of aflatoxin B1, B2, G1 and G2 in 71 samples of rice which were collected from food supply stores in the cities of Tehran, Bushehr and Tabriz, by using high-performance liquid chromatography. The results showed that the average aflatoxin B1 in 83% of samples was 1/89 ng /g and in 8.2% of samples it was more than the maximum tolerance of aflatoxins in foods according to national standards of Iran (5 ng /g). Total aflatoxin average in 83% of samples was 2/09 ng /g which is less than the maximum of permitted level of aflatoxin in Iran and Europe (4 ng /g) **[6].** Reddy and colleagues (2009) have evaluated the amount of fungal contamination to Aspergillus mold species in 675 samples of husks and 525 samples of rice which were collected from the 20 states with different weather and storage conditions in India. The results showed that the species of Aspergillus flavus and Aspergillus niger have formed the dominant species in all samples. Alos their amount was reported to be high in rice samples with durability of a year. In this study the amount of aflatoxin B1 in the samples was measured by indirect competitive ELISA method. All samples were contaminated with aflatoxin B1 (average toxin of 0/5-3/5 micrograms per kilogram) and only in 2% of samples the amount of toxin exceeded the limit **[8].** Faraji and colleagues in a study have collected 60 samples of rice from food supply stores of 5 districts of Mashhad and they have measured aflatoxin contamination levels by using high performance liquid chromatography and fluorescence detection. The results showed that all samples were contaminated with aflatoxins and the amount of aflatoxin B1 and B2 was respectively 2/55 and 0/34 micrograms per kg, and the average of aflatoxin G1 and G2 are close to zero and negligible. Aflatoxin B1 contamination rate 8/3 percent of the samples was higher than the maximum tolerance of aflatoxins in foodstuffs according to national standards of Iran **[5].**

CONCLUSION

The overall results indicate that the amount of aflatoxin B1 contamination is acceptable according to standard level (5 ng/ g) in the rice samples collected from cities located in the west of Mazandaran Province (Marzanabad, Chaloos, Noor, Kelarabad and Tonekabon). In many Asian countries, rice is one of the major food groups in people's consumption patterns; in a way that more than 16% of annual energy is provided by rice and comprises more than 70% of its cereals and products in the daily diet. In our country, like many Asian countries, rice is one of the important food groups in consumption patterns of people. According to a consumer survey of Nutritional Food Research Institute in 2004 about 15% of annual energy is supplied by rice **[15].** Aflatoxin contamination of rice has been reported usually lower than other cereals, but considering the relative humidity of rice, it can be an ideal environment for mold growth. Due to the diverse climatic conditions of our country, there is the possibility of presence of a wide range of mycotoxin-producing fungi along with related toxins in the environment. It should be noted that the main factors that produce mycotoxins are related to farm that include droughts, rainfall, high humidity and the time of clustering of the product and insects contamination. The ways of dealing with these factors include reduction of contamination of product to insects (because the growth of mold is higher on damaged grain), using agricultural machinery to produce varieties resistant to fungi, observance of appropriate irrigation, weed control, crop frequency (transmission of diseases factors reduces from one year to another and contamination becomes limited) and balancing soil fertility to reduce the risk of stem rot **[16, 17]**. Correct and systematic storage, crop protection against moisture, insects and environmental factors, maintenance of product on dry and clean area and the use of additives which reduce mold growth are the solutions that must be implemented after the rice harvest so as to ensure foodstuffs safety. Preventing mold growth in post-harvest processes and proper storage conditions can control toxin production and its risks, while the reduction of aflatoxin levels is recommended to be done by using harmless natural compounds or treatment with ozone **[18]**. Suggestions may be submitted regarding the prevention of microbial contamination of food products which include following a strict hygienic control cycle of stages of production, transportation, storage of raw materials and product and packaging at temperatures less than 20 ° C to reduce fungal and microbial corruption.

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