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Short Communication

Natural Occurrence of T-2 Toxin in Domestic and Imported Rice

*M Riazipour [1, 2], AA Imani Fooladi [1], M Razzaghi-Abyaneh [3]

[1]Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
[2]Dept. of Parasitology and Mycology, Medical School, Baqiyatallah University of Medical Sciences, Tehran, Iran
[3]Dept. of Mycology, Pasteur Institute of Iran, Tehran, Iran

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Abstract

Background: Rice is one of the crops, which are prone to be contaminated with toxigenic fungi and their mycotoxins. This study aimed to investigate the natural occurrence of T-2 toxin in domestic and imported rice in Iran.

Methods: In a cross-sectional descriptive study in winter 2007, 140 samples of imported rice (125 samples of Thai and 25 samples of Pakistani rice) and 60 samples of Iranian rice were collected from warehouses of canteens of governmental offices in Tehran. After grinding and methanol extraction of the rice samples, the amount of T-2 toxin was measured using a sandwich ELISA. INSTATA statistical software was used for data analysis.

Results: All samples of rice were more or less contaminated with T-2 toxin but the amount did not exceed the permissible limit. Mean contamination of domestic and imported rice was 11.2±2.3 and 13±2.7 µg/kg, respectively. Regarding imported rice, mean of contamination was 14.5±4.6 µg/kg for the Pakistani rice and 12.6±2.2 µg/kg for the Thai rice. There was no significant difference between domestic and imported rice, nor did we find a meaningful difference among Iranian, Pakistani and Thai rice regarding the amount of contamination (P= 0.2).

Conclusion: Although the amount of contamination is less than the safe limit, the extent of natural occurrence of T-2 toxin in rice in Iran indicates that contamination occurs somewhere in the production process. This, in turn, necessitates screening of rice for contamination with mycotoxins from farm to table.

Keywords: *Natural occurrence, Mycotoxins, Rice, T-2 toxin*

Introduction

Trichothecenes are an important family of mycotoxins which can usually be found in grains and their products (1). These toxins are produced by different fungal genera but *Fusarium* spp. have the most important role in their production (2). So far more than 150 trichothecenes have been identified, including the most known of all, T-2 toxin, which is mainly produced by *F. sporotrichioides* (3). The most important effect of T-2 toxin and other trichothecenes is inhibition of protein synthesis, which may leads to secondary disruption in DNA and RNA synthesis. This toxin more severely affects cells which actively divide, such as gastrointestinal mucosa, skin, lymphoid and erythroid cells, and it also reduces immunoglobulins and the other humoral factors such as cytokines (3). Loss of appetite, vomiting, diarrhea, GI bleeding, dizziness and immune system deficiency are typical symptoms of trichothecenes toxicity (1).

Different crops have different tendencies to mycotoxin contamination. The contamination of rice with toxigenic fungi and mycotoxins has been proved in other studies (4-7) and there have been reports of acute poisoning through consuming rice contaminated with trichothecenes including T-2 toxin (8). In addition, rice is used as a common medium for production of most of the mycotoxins (4, 9-11), which shows the vulnerability of rice to mycotoxigenic fungi. Rice may be contaminated with field toxigenic fungi like *Fusarium* spp. during cultivation, and with warehouse toxigenic fungi such as *Aspergillus* and *Penicillium* spp. (4). Therefore, it is necessary to screen rice for mycotoxins at different stages before human consumption.

Like in many Asian countries (4), rice is a main food of people in Iran. Each Iranian consumes an average of 40 kg of rice every year. Two thirds of the consumed rice is produced in Iran, especially along the Caspian Sea strip, and the rest

*Corresponding author: Tel/Fax: +98 21 26127258, E-mail: mriazipour@yahoo.com

is imported from countries like Thailand and Pakistan. Several studies in Iran have shown significant contamination of crops with mycotoxins (12-15) but there are a few studies about *Fusarium* toxins (16-18), and there is not much information regarding contamination of rice with fungal toxins. This study aimed to find the natural occurrence of T-2 toxin in domestic and imported rice destined for human.

Materials and Methods

This cross-sectional descriptive study was done in Tehran in winter 2007.

Rice samples

Two hundred rice samples were taken from 9 warehouses of some canteens in governmental offices. There were 23 shipments of rice: 14 from Thailand, 6 from Iran and 3 from Pakistan in these warehouses, where125, 60 and 15 samples were obtained, respectively. The samples were ground using a household grinder. Five grams of each sample was extracted with 25 ml of methanol-water solvent (70:30) on shaker (150 rpm) for 10 min. After that it was centrifuged 10,000 rpm for 30 min and the upper layer was separated and kept at -20 ºC until next step.

ELISA

To measure T-2 toxin, a competitive method of ELISA based on monoclonal antibody was used according to manufacturer's guidelines (T-2 Toxin R-Biopharm RIDASCREEN®, Art. No.: R3801, Germany). In short, methanol extract of the samples were diluted in dilution buffer (1:7) and 50 µl of each unknown sample and also standard samples (6 standards) that come along with the kits were added to each well. Then 50 µl of conjugated enzyme and 50 µl of anti-T2 antibody was added to each well and left at room temperature (20-25 ºC). After one hour, the liquid in wells was emptied and rinsed for 3 times with distilled water. Then 50 µl of substrate and 50 µl of chromogen were added to each well and incubated for half an hour in darkness at room temperature. To stop the reaction, 100 µl of 0.1 M sulfuric acid was added and the amount of absorbance at wavelength of 450 nm was read against blank air. The absorbance values obtained for the standards and the samples were divided by the absorbance value of the first standard (0 standards) and multiplied by 100. Therefore, the zero standard is thus made equal to 100% and the absorbance values are quoted in percentages. The absorption is inversely proportional to the T-2 toxin (Fig.1).

Statistical analysis

INSTATA statistical software was used for data analysis. To measure the mean difference of contamination of different rice samples, *t*-student test was used. Correlation between the T-2 toxin level and storage time of rice (for the rice with production date) and the amount of T-2 toxin were analyzed by chi square test. $P< 0.05$ was considered significant.

Results

Mean contamination of all kinds of rice was 12.49±2.7 (mean±SD) µg/kg, which ranged from 7.9 to 19.7 (median= 12.1). Mean contamination of domestic rice was 11.1±2.3, (median= 10.9), and the mean contamination of imported rice was 13± 2.7, ranging from 9.1 to 19.7 with a median of 12.6 µg/kg (Fig. 2). Although the mean contamination of imported rice was a little more than that of domestic one, *t*- test analysis showed the difference was not significant($P= 0.2$). Mean contamination of Pakistani rice was 14.5±4.6, with a median of 12.6 and the mean contamination of Thai rice was 12.6±2.2, with a median of 13 µg/kg (Table 1). One-way analysis of variance and Kruskal-Wallis Non-parametric ANOVA test showed that there was no significant difference among these three kinds of rice ($P= 0.2$ and $P= 0.27$, respectively).

Among the studied rice, only the bags from Thai had printed date of production on them. Fig. 3 shows the relationship between the length of storage and the amount of contamination with T-2 toxin in this kind of rice. Chi square test shows there was no meaningful relationship between the length of storage of rice samples and the amount of contamination ($r^2= 0.77$).

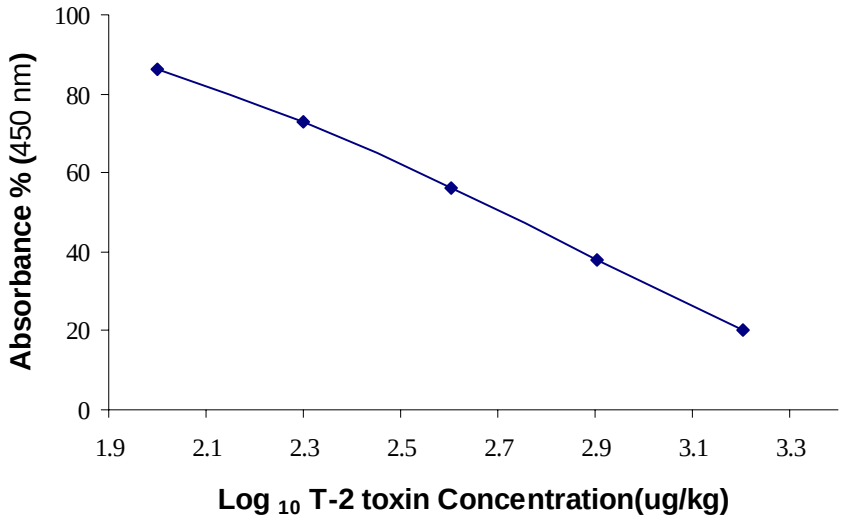

Fig. 1: Calibration curve of T-2 toxin

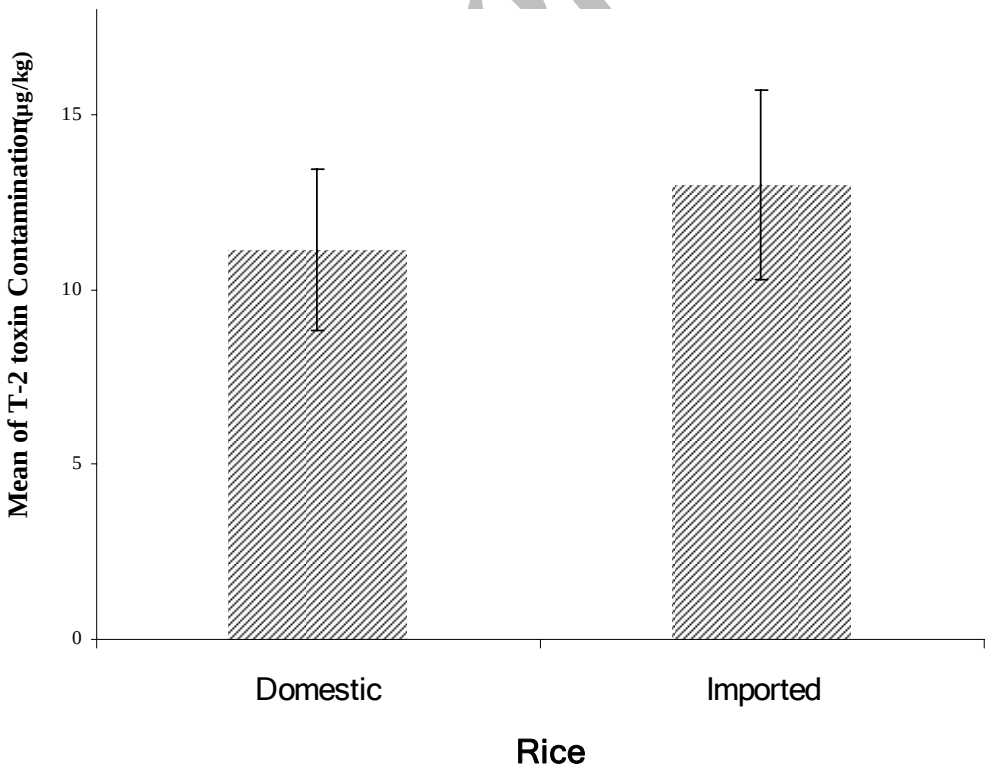

Fig. 2: T-2 toxin contamination in Iran domestic and imported rice shipments

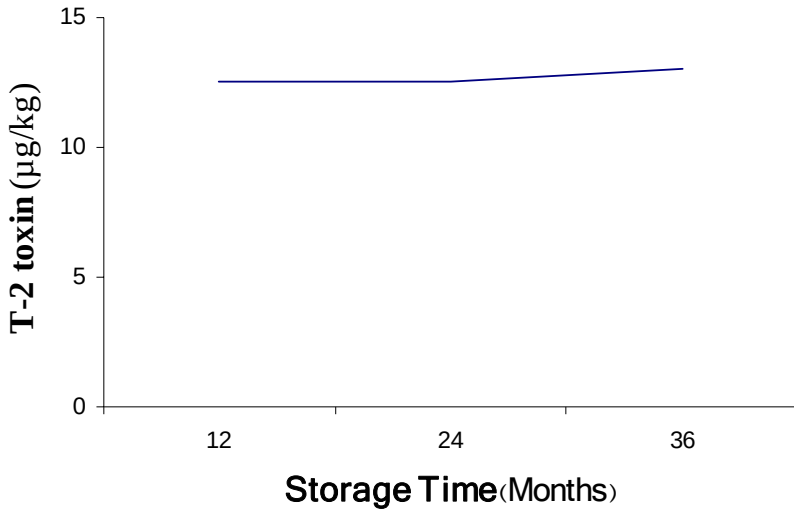

Fig. 3: Correlation between storage length and T-2 toxin contamination of rice shipments (Only samples with production date are included)

Table 1: T-2 toxin contamination in rice samples with different origin

Rice origin	n	Mean of contamination (±SD)
Iran	60	11.1(±2.3)
Thai	125	12.6(±2.2)
Pakistan	15	14.5(±4.6)

Discussion

Rice is grown in areas where necessary humidity and temperature are available. These conditions increase contamination of rice with fungi and mycotoxin (7). In Iran, most of the rice is grown in north of the country, where the climate is appropriate for the spread of field fungi. Studies show that the farm soil in this area is more vulnerable to potentially toxigenic fungi than that of other parts of the country (19).

There is little information regarding contamination of rice with mycotoxins compared with other crops (10) and a few studies have focused on the occurrence of trichothecene contamination of rice (20). Fifteen percent of rice samples in stores of Rabat (Morocco) had ochratoxins higher than the permissible limit (6) and 30% of organic rice cultivated in Spain was contaminated with ochratoxin (7). It is said that mycotoxin contamination in rice is lower than in other crops (20), but studies have proved this claim wrong; for instance, Pande showed that rice samples were contaminated with aflatoxin more than wheat and maize (21).

Although rice is a main food for most Iranians, who consume 40 kilograms per capita, mycotoxin identification tests are limited to imported rice, which comprises just one third of the consumed rice in Iran. In addition, tests are taken only once-before importation- and like some other countries (4), out of all mycotoxins, only aflatoxins are checked. Considering the vulnerability of rice to contamination with many fungi and their toxins, screening of rice for important mycotoxins including T-2 toxin is essential in different stages from production to consumption.

There are no previous data about the occurrence of trichothecenes in rice in Iran. Our study showed that all samples of imported and domestic rice are somewhat contaminated with T-2 toxin. Natural contamination of rice with trichothecenes has been revealed in several studies (20). It is reported that the typhoon struck rice was contaminated with trichothecenes (22). Also some cases of acute and extensive poisoning with trichothecenes in human communities have been reported (8). The extent

of contamination in our study is alarming because it shows that conditions from farm to store were ready for fungal growth and mycotoxin production. Despite the high prevalence of contamination, none of the samples had more than 20 microgram per kilogram of the toxin. Controversy over the health hazards of mycotoxins has caused different standards in different countries. These standards are somewhat related to economic situation and the sensitivity of agricultural products in each country (23). In Iran, the only standard for T-2 toxin is in animal feed (25 microgram per kilogram), but there are no regulations for human food. In many other countries, there is no permissible upper limit for the toxin. In countries with standards for T-2 toxin, the permissible limit varies from 20 to 300 µg/kg, but most countries have set 100 µg/kg as safe limit (24). If we consider the standards of most countries, none of our samples were contaminated more than the permissible upper limit.

The little contamination of our domestic rice might be because wheat or barley is not grown in rice farms of North of Iran. Studies show that rice is more contaminated in fields that are cultivated rotationally with wheat or barley (4).

In Iran, all imported crops including rice are subject to mycotoxin measurement and rice with contamination beyond the upper limit is rejected. Therefore, the amount of contamination should be in an acceptable range at the time of importation, but keeping rice in warehouses for a long time may leads to growth of fungi and production of mycotoxins. In our study, some imported rice had been kept in warehouse for more than 3 yr after production. Such a long time of storage may leads to an increase in probability of contamination with toxigenic store fungi. Although there was no significant relationship between duration of being kept at warehouse and the quantity of toxin (Fig. 3), among the rice that had production date, the most amount of contamination (16.2 µg/kg) belonged to the rice whose production date was over 36 months old. Unfortunately, most of the rice bags in Iran do not have registered date and place of production, which makes studying the relationship of production time and the amount of toxin impossible.

Our study showed that most of the domestic rice which is grown in Northern provinces of Iran- Mazandaran, Golestan, and Guilan- had less contamination than the permissible maximum limit. However, studies show that other agricultural products in this region have higher contamination rate than the safe limits. Shephard et al. showed that all samples of maize in Mazandaran province were contaminated with fumonisins and the amount and prevalence of fumonisin in Mazandaran were much higher than those in Isfahan, a province in central Iran (14, 15). In addition, aflatoxin was found (B1 and B2) in 88.8% and 66.6% of maize samples in Golestan and Mazandaran provinces, respectively (12, 13). Hedayati reported 80.5% of the wheat samples in Mazandaran silos contaminated with zearalenone and 64.4% with contamination beyond the safe limit according to Iranian standard (13).

In conclusion, the low rate of T-2 toxin contamination in our study should not lead to the presumption that rice consumers are not at risk of mycotoxin intoxications. Screening domestic and imported rice in different stages is vital for identifying the possible contamination with mycotoxins and for removing the rice that are contaminated higher than the safe limits from food chain.

Acknowledgments

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