

Mycotoxin Contamination of Foodstuffs and Feedstuffs in Iran

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Classification and Occurrence of Mycotoxins

Mycotoxins are organic and complex secondary metabolites produced by various fungi species. Aflatoxins (AF), aflatoxin M1 (AFM1), ochratoxin A (OTA), deoxynivalenol (DON), fumonisins (FM), zearalenone (ZEN) and patulin (PTN) are among the most important mycotoxins. They can contaminate human foods and animal feeds through fungal growth prior to and during harvest, or as result of improper storage. Mycotoxins are mainly produced by fungal genera including *Aspergillus*, *Fusarium*, *Penicillium*, *Claviceps* and *Alternaria* under proper conditions (1). Major food commodities affected are cereals, nuts, dried fruits, coffee, cocoa, spices, oil seeds, dried peas and beans and fruits, particularly apples (2).

There are a number of dimensions to the problems caused by mycotoxins. The first of these relates to their implications for both human and animal health. Mycotoxicosis is mainly acquired through ingestion of contaminated moldy food. Mycotoxins can induce a wide range of effects in animals such as immunosuppression, myelosuppression, hepatotoxicity, neurotoxicity, dermatotoxicity, estrogenicity, mutagenicity, carcinogenicity and teratogenicity. Even though mycotoxicoses, like ergotism, have been known for more than a thousand years, the causal relationship between exposure and human

diseases remains controversial. Experimental and epidemiological evidences suggest that certain mycotoxins are associated with different forms of human cancer. For certain types of mycotoxins, high doses can result in acute intoxication, and can be either sublethal or lethal, with numerous symptoms, including various organ pathologies or even cancer (1). Due to possible health risks to the consumers, many countries have set regulatory limits on the permissible levels of different mycotoxins in foods and feeds (3).

Mycotoxins also have a significant impact on economics, by causing losses in farm animals or giving rise to difficulties in their management, or by rendering commodities unacceptable for national or international trade (4).

In this article, contamination of Iranian foods and feeds with mycotoxins using published data are reviewed. Papers retrieved through searching databases including Entrez Pubmed, ScienceDirect, Chemical Abstract and IranDoc from 1960-2005.

Occurrence of mycotoxins in Iran

In 1997, Institute of Standard and Industrial Research of Iran (ISIRI) set maximum tolerated limit (MTL) for mycotoxins in foods and feeds (3). MTLs came into force in 2002, and currently some of the imported commodities are analyzed for mycotoxins.

1. Aflatoxins (B and G groups)

AF are known mutagenic, teratogenic,

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carcinogenic and immunosuppressive compounds. There are 4 main toxins namely aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). Different food commodities including cereals, nuts, dried fruit, cocoa, spices, oil seeds, pulses and beans are contaminated with AF. In Iran, most of the research regarding AF contamination of foods and feeds have been focused on cereals and nuts, especially pistachio. The history of AF contamination of foods in Iran dates back to 1967, where AF poisoning due to bread consumption was reported in Tehran (5).

1.1 Occurrence of AF in nuts

Pistachio nut, as an important member of tree nuts crop, is cultivated in several countries including Iran, USA, Turkey, Greece, Syria, Italy and other countries. Pistachio nuts are a rich source of fat and contain linoleic and linolenic fatty acids, essential for the human diet (6). However, they are very prone to AF contamination and may frequently contain an elevated levels of AF (7). *Aspergillus flavus* is dominant on tree nuts including pistachio, and usually produces only AFB1 and AFB2.

In 1971, for the first time, US Food and Drug Administration (FDA) reported AF contamination in pistachio nut consignments arriving into the USA. A more systematic testing on consignments arriving from Iran and Turkey led to rejection of 36 consignments out of total of 48 tested. For managing the problem, intensive research was initiated at mycotoxins research centers in Iran. Investigations revealed that AF contamination starts when pistachio nuts are still on the trees after ripening but before harvesting. It was also found that spraying trees with captan for a period of 30 days before harvesting could eliminate the problem (8). The fact that AF detected in pistachio nuts sampled from tree prior to harvest indicated that AF might not be solely from improper shipping and storage (8-9).

FDA inspection of imported pistachio nut consignments over a long period, revealed a wide yearly variation in the amount of AF contamination. Therefore, role of climate variations on the level of AF contamination was investigated. The results showed that yearly variation is directly related to the amount

of rainfall during the period of ripening to harvesting of the nuts. It is during this crucial period that AF contamination of the pistachio nuts occurs (10-11).

Over the past years, the health authorities of European Union (EU) also reported a similar problem, regarding Iran's pistachio nuts arriving at EU entry ports, and in 1997, they imposed a temporary ban on importation of pistachio nuts from Iran for two months (12-13). This was a precautionary measure, following the reported findings of the Dutch authorities, indicating that a large number of pistachio nuts consignments from Iran were contaminated with unacceptable levels of AF. Subsequent analyses by other EU member states confirmed that up to 70% of the consignments contained AF, ranging from 11 to 400 ng/g (14). Although ban on Iran's pistachio nuts was removed later, based on the 1997 decision of European Commission (EC), a special condition was imposed on Iran's pistachio nuts entering EU (13). This decision was also re-stated again on 2000 (14) and 2003 (15).

Among the 47361 pistachio nut samples analysed during March 1998-March 2001 in Iran, 28227 samples were not contaminated (Lower than the limit of detection [LOD]), 7862 samples contained AFB1 between LOD-2 ng/g, 6583 samples contained AFB1 between 2-10 ng/g, and 4689 samples contained AFB1 higher than 10 ng/g. The maximum level detected was 1426 ng/g (16-17).

In the Food and Drug Control Labs of the Iran Ministry of Health, 7926 pistachio samples were analysed during March 2001-March 2002. The data indicated that 5390 samples were not contaminated (<LOD), 1324 samples contained AFB1 between LOD-2 ng/g, 451 samples contained AFB1 between 2-10 ng/g and 761 samples contained AFB1 higher than 10 ng/g. The maximum level detected was 261.8 ng/g (18).

During March 2001-March 2002, 3629 pistachio samples were also analysed by the ISIRI affiliated Food Quality Control Labs. The results showed that 2286 samples were not contaminated (<LOD), 201 samples contained AFB1 between LOD-2 ng/g, 689 samples contained AFB1 between 2-10 ng/g, and 453

samples contained AFB1 higher than 10 ng/g. The maximum level detected was 710 ng/g (19).

Pistachio nuts produced in Iran during March 2002 – February 2003 were analyzed for presence of AF (20). In this regard, 3356 pistachio nut samples were collected. After dividing samples to sub-samples, 10068 AF analyses were carried out. Among 10068 samples analyzed, AFB1 was detected in 3699 samples (36.7%), with the mean and median of 5.9 ng/g and 0.1 ng/g, respectively (Fig. 1). The total AF (AFT) was detected in 2852 samples (28.3%) with the mean and median of 7.3 ng/g and 0.4 ng/g, respectively (Fig. 2). AFB1 level in 1191 samples (11.8%) was above the MTL of AFB1 in the pistachio nut of Iran (5 ng/g) (3). Regarding AFT, the mean contamination level (7.3 ng/g) was lower than MTL of AFT in pistachio nut in Iran (3) as well as being lower than the proposed draft maximum level of the Codex Committee on Food Additives and Contaminants for AFT (15 ng/g), and only 7.5% of samples had levels above the MTL (20). In Iran, most of the contaminated pistachio nut samples contain only AFB1 and/or AFB2 (16-20).

In recent years, some measures have been taken by the Ministries of Health and Ministry of Jihad-e-Agriculture for the prevention and reduction of AF in pistachio. However, the results show that in spite of extensive efforts made by different parties in Iran, AF contamination in pistachio nuts is still substantial and a matter of concern for both producers and consumers. Despite the fact that Iran is the number one pistachio nut producing country in the world, very few research projects on different aspects of pistachio nuts contamination with AF and ways to control them have been performed. Therefore, the most resistant variety of pistachio tree to *Aspergillus* is not clearly known. There is no scientific evaluation of the implementation of GAP principles on the reduction of AF contamination. Even in cases that the present data proposed hull as a source of spore and mold contamination, no plan has been implemented yet to prevent returning hull to the orchard as fertilizer. No scientific published data presented to show the effect of shipping conditions on contamination and examined the

use of different conditions, such as packaging on preventing further contamination during shipment.

However, the results of an investigation on AF contamination of the Iranian peanuts showed that among 3 varieties, the Local Gilan-Iran variety, which resists seed infection by *Aspergillus*, was free from aflatoxin contamination (21).

1.2 Occurrence of AF in other commodities

Epidemiologic studies were undertaken in the Caspian Littoral of Iran to investigate the geographic distribution of factors (including AF) might underlie the signal differences in the incidence of esophagus cancer. In this regard, AF were analyzed in typical dietary items and the results showed no unusual levels of AF (22-23). In another study, AF contamination of cottonseed and cottonseed cake was measured (24). The results showed Iranian cottonseed cake and cottonseed were contaminated with AFB1 in 2 processes including before harvest and during storage (24).

In a 4-year study, regarding AF contamination of rice and wheat samples, negligible contamination was observed (25). Rasti et al. analyzed AFB1 in 70 imported and/or locally-produced corn samples collected from the central storage places in Isfahan. The results showed that almost all samples were contaminated in the range of LOD – 9.9 ng/g with a mean of 5.1 ng/g (26). In 1998, fourteen barley and nine corn samples, destined for animal feed, collected from the Golestan and Mazandaran provinces in the north of Iran, were analyzed for AF using high performance liquid chromatography (HPLC) (27). In corn samples, AFB1 and AFB2 were detected in 8 (88.8%) and 6 (66.6%) samples, at a mean level of 15.83 and 2.99 ng/g (median 1.72 and 1 ng/g), respectively (Fig. 3), and AFB1 level was above the Iranian MTL of AFB1 in corn for animal feed (5 ng/g) (3). None of the corn samples contained detectable amounts of AFG1 and AFG2 (27). In another study, Tayebi and Esavi reported that one out of twenty corn samples, collected from North of Iran, was contaminated with AFB1 and AFB2, at levels of 245 ng/g and 35 ng/g, respectively (28).

According to an unpublished data, among 44 corn samples collected from pre-harvest and

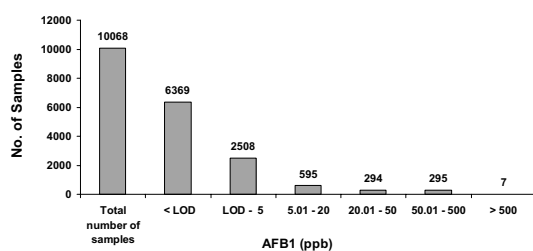


Figure 1. Incidence of AFB1 (ng/g) in Iran pistachio nut samples analyzed by food control labs during March 2002-February 2003.

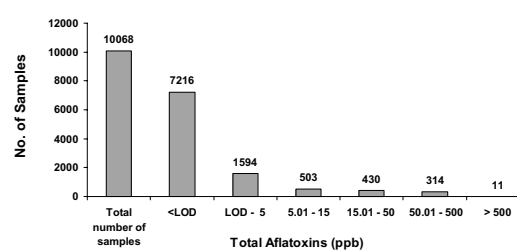


Figure 2. Incidence of AFT (ng/g) in Iran Pistachio nut samples analyzed by food control labs during March 2002-February 2003.

post-harvest stages as well as storage places in Golestan province, 79.5% were contaminated with AFT at mean and median levels of 52.8 ng/g and 10.2 ng/g, respectively. The AFT mean level was higher than the recommended Iranian MTL of 20 and 30 ng/g in corn intended for animal feed and human consumption, respectively. These data are in agreement with the other studies done in Iran and showed that corn produced in Northern Iran is susceptible to AF contamination. Therefore, there is an urgent need for intervention strategies to prevent and control AF contamination in corn in Golestan and Mazandaran provinces.

2. Aflatoxin M1

Dairy cattle fed rations containing AFB1 excrete, in their milk, a hydroxylated metabolite known as “milk toxin” or AFM1, which is specifically found in milk and milk products. There is a general consensus that approximately 1-3% of the AFB1 initially present in the animal feeds appears as AFM1 in milk (29). AFM1 is presumed to induce liver cancer in rodents, and its carcinogenic potency in sensitive species is about one order of magnitude less than that of AFB1 (30).

Occurrence of AFM1 in milk in Iran has been investigated (31-34). During 1973-1974, Suzangar et al. (31) analyzed AFM1 and aflatoxin M2 (AFM2) in 67 milk samples from village producers and in 28 milk samples from the commercial producers in Isfahan, Iran. Of the former, 50-56.7%, and of the latter, 0-2% were contaminated, respectively, with AFM1 and AFM2 (31). In another study, AFM1 was analyzed in 73 milk samples sold to Iran Pasteurization Milk Company (32). Sixty out of 73 samples were contaminated with AFM1 at

levels higher than 50 ng/l (32). Alborzi et al. (33) investigated AFM1 contamination in pasteurized milk samples in Shiraz city. During April to September 2003, 624 pasteurized milk samples were collected from different supermarkets. AFM1 was found in all milk samples, and 17.8% of the samples had levels higher than 50 ng/l (33).

In another study, among 111 raw milk samples obtained from dairy plants in Sarab city, 76.6% of the samples were contaminated with AFM1 in the range of 15 – 280 ng/l, and the levels in 40% of the samples was higher than 50 ng/l (34).

3. Ochratoxin A

OTA is a nephrotoxic and nephrocarcinogenic mycotoxin. Although OTA has been found in many food commodities (such as dried vine fruits including raisins, currants and sultanas), most surveys indicate that the highest incidences and levels of OTA are found in cereals (2).

In one study in north eastern Iran, where esophageal cancer is common, grain was sampled from wheat and barley crops at harvest and after different periods of storage in underground pits or in buildings (35). OTA was detected in only one out of 141 samples and this had much *Penicillium* contamination. No other mycotoxins were detected, although occasional samples showed unidentified toxicity to brine shrimp larvae, and isolates of *A. flavus* and *A. ochraceus* produced AF and OTA, respectively, in culture. There was little evidence for mycotoxins being implicated in esophageal cancer in north-eastern Iran (35).

In 1998, Yazdanpanah et al. (27) analyzed OTA in fourteen barley and nine corn samples (destined for animal feed) collected from Golestan and Mazandaran provinces in the north of Iran. No contamination was observed in

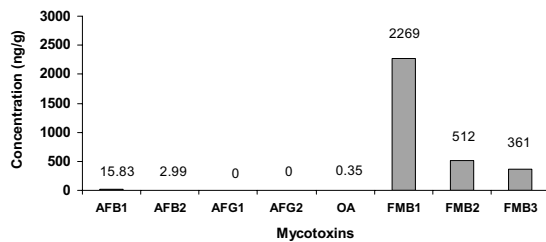


Figure 3. Mycotoxin Levels in corn samples from Mazandaran Province, Iran in 1998

barley samples. In corn samples, only one of the AF-contaminated samples was co-contaminated with OTA at a concentration of 0.35 ng/g (Fig. 3). This is the first report concerning natural occurrence of OTA and co-occurrence with AF in corn samples of northern Iran (27).

In the first phase of the Iran national project, concerning analysis of mycotoxins in foods in Tehran, OTA was analyzed in 18 rice (imported and locally produced) and 18 bread samples collected from retail market in 2005 (36). Seven rice samples were positive for OTA, at levels varying from 0.06 to 1.66 ng/g with a mean (\pm SD) of 0.38 (\pm 0.61) ng/g. None of the samples was above the Iranian MTL of 5 ng/g (3). Regarding bread, none of the samples was found to be contaminated with OTA. This is the first report of OTA contamination in rice in Iran (36).

According to the few published data, it seems that there is a low level of contamination with OTA in foods. But, further studies needed in order to confirm the findings. In addition, publishing the available data in Ministry of Health and ISIRI for raisins intended for export which have been analyzed in their laboratories may provide valuable information.

4. Zearalenone

ZEN is an estrogenic mycotoxin which frequently colonizes cereal crops worldwide. Despite its low acute toxicity, the presence of ZEN in feeds has long been a problem in animal husbandry, notably by causing infertility and related disorders in swine and sheep. Most surveys indicate that ZEN occurs primarily in corn, as well as in certain other grains and mixed feeds (37). There are a few reports dealing with ZEN contamination in Iranian foods.

During 1991-1993, 99 wheat samples,

infected with *Fusarium* species, were collected from the Mazandaran province (38). Analysis of ZEN in 37 samples showed ZEN contamination in the range of 3000-42600 ng/g. It should be noted that this study was performed on samples contaminated with *Fusarium* species, and, therefore, the result does not show a real picture of ZEN contamination in wheat samples of the Mazandaran province (38). In another study, in September 2000, 40 pre-harvest corn samples from farms of the Dashte-naz region in Mazandaran province were randomly collected and analyzed for ZEN (39). The results showed that 7.5% corn samples contained ZEA in the range of 100–212 ng/g, with a mean of 141 ng/g. This study, which is the first report of ZEA occurrence in the Iranian corn, showed that the ZEA level was lower than Iranian MTL for this mycotoxin (39).

Oveisi et al. analyzed ZEN in 38 samples of corn flour and a cheese snack derived from it. ZEN was detected in corn flour and cheese snack samples, with an average content of 377 ng/g (maximum, 889 ng/g) and 832 ng/g (maximum, 1471 ng/g), respectively (40).

5. Deoxynivalenol

The trichothecenes are a group of closely related secondary metabolites produced essentially by a broad range of *Fusarium* molds (41). One of the trichothecenes most often associated with *Fusarium* head blight is DON. It is mainly associated with cereals and can cause outbreaks of acute illness in humans (30).

During 1991-1993, 99 wheat samples, infected with *Fusarium* species, were collected from the Mazandaran province (38). Analysis of DON in 37 samples showed contamination up to 10500 ng/g. Again, like ZEN, it should be noted that this study was conducted on samples contaminated with *Fusarium* species, and, therefore, the result does not show a real picture of DON contamination in wheat samples of Mazandaran province (38).

6. Fumonisin

FM are a group of mycotoxins produced by a limited number of *Fusarium* molds (42). According to the International Agency for Research on Cancer, 'toxins produced by

Fusarium moniliforme' (the old name for *F. verticillioides*) are possibly carcinogenic to humans (group 2B carcinogens) (43). Subsequently, this assessment has been refined and fumonisin B1 (FB1) itself was evaluated as a group 2B carcinogen (44). It has been shown that FM can be found almost wherever corn is grown.

During 1998-2000, the natural occurrence of FM in the Iranian corn was investigated (45-47). In 1998, corn collected in the Mazandaran and Isfahan provinces were analyzed for FB1, fumonisin B₂ (FB₂) and fumonisin B₃ (FB₃) (45). The samples from Mazandaran province were random samples obtained from farmers' corn lots collected in September 1998, whereas those from the Isfahan province, were bought as corn cobs in the local retail market during October 1998. All 11 samples from Mazandaran showed high levels of FM contamination, with FB1 levels between 1270 ng/g and 3980 ng/g, FB2 levels between 190 ng/g and 1175 ng/g and FB3 levels between 155 ng/g and 960 ng/g (Fig. 3). Samples from Isfahan showed lower levels of contamination with 100% samples had detectable FB1 (10 ng/g -590 ng/g), two of eight samples had detectable FB2 (50 ng/g -75 ng/g) and two of eight samples had detectable FB3 (50 ng/g -75 ng/g). This was the first report of FM contamination of corn from Iran (45). The overall results of FM analysis during 1998-2000 showed that all of the samples of Mazandaran province except one were positive for FB1. A comparison of the results indicated higher mean and maximum levels during 2000 (mean: 5670 ng/g, max: 12950 ng/g) than those found during 1999 (mean: 3180 ng/g, max: 7660 ng/g) and 1998 (mean: 2270 ng/g, max: 3980 ng/g), and the average concentration of FMB1 was higher than 2000 ng/g in all the years, which is higher than the recommended Iranian MTL of 1000 ng/g for FMB1 and FMB2 (45-47). However, the mean levels of FMB1 in samples collected from markets in the Isfahan province in 1998 and 1999 (170 ng/g and 220 ng/g, respectively) were significantly lower than the levels found in corn from Mazandaran (45-46).

To investigate the incidence of esophageal cancer in the Golestan province of North-East Iran, 1349 rural and urban inhabitants of Golestan

province aged between 35-80 years were invited to undergo extensive lifestyle interviews and to provide biological samples (48). In addition, samples of rice, wheat and sorghum were tested for FM contamination. However, no FM were detected in the samples analyzed (48).

For the control of FM contamination of corn, application of proper agricultural and sanitary measures and HACCP (hazard analysis and critical control point) principles will lead to the production of a better quality corn in Iran.

7. Patulin

PTN is a secondary metabolite produced mainly in rotten parts of apples by a wide range of fungi. Due to its mutagenic and teratogenic nature and possible health risks to consumers, many countries have regulations to reduce the level of PTN in apple products to as low as practically possible. A survey on the presence of PTN was conducted in 2002, on 65 locally produced commercial apple juice and apple juice concentrates purchased from retail outlets or producers in Iran (49). In this study, 42 apple juice and 23 apple juice concentrate samples surveyed for PTN contamination. Results of the study showed in 69% of apple juice and 78% of the apple juice concentrates, level of PTN was found to be higher than 15 µg/l. Overall, 33% of the apple juice samples had PTN levels higher than 50 µg/l, with a maximum level of 285 µg/l, and 56% of the apple juice concentrates had PTN levels of higher than 50 µg/l and a maximum level of 148 µg/l (49). Although the mean concentration of PTN in apple juice samples surveyed was slightly lower than Iran MTL, fairly high incidence of PTN in these samples indicates the need for improving production techniques by industry in order to reduce the incidence and level of PTN contamination in apple juices.

Conclusion

Considering suitable weather and temperature conditions for fungal growth in some parts of the country (such as the Northern Part), assessment of the occurrence of mycotoxins contamination of different kinds of commodities including nuts, cereals and foods should be a priority for

health policymakers. The main current concern is contamination of corn with AF and FM, and PTN contamination of apple juices, although the risk of contamination of foods and feeds with other mycotoxins should be considered.

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