Aflatoxins and aflatoxigenic fungi in Iran: A systematic review of the past, present, and future

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Abstract: Aflatoxins are highly toxic compounds that are produced as secondary metabolites by some *Aspergillus* and *Emericella* species. Despite huge progress on aflatoxin research during the past 50 years, we have witnessed many cases of food contamination with aflatoxins all over the world. Aflatoxins have mutagenic and carcinogenic effects and they are capable of inducing hepatocellular carcinoma in human and animals. A wide array of substrates including nuts, oilseeds, cereals and dried fruits are susceptible to aflatoxin contamination which is a challenge for public health, especially in developing countries. Improper storage of food and feed provides conditions for aflatoxin production in crops, especially rice, wheat, pistachio, walnuts, and almonds which are the main sources of nutrients in the human food chain. Aflatoxins can be found in animal tissues and milk after ingestion of contaminated feed. Among nuts, pistachio is one of the most important exporting products of Iran which its contamination with aflatoxins is frequently reported. In general, despite frequent reports of aflatoxin–producing fungi and aflatoxin contamination of food and feed in Iran, limited data exist about human and animal exposure to aflatoxins and related consequences. This review highlights current status and future prospects of aflatoxin occurrence in food, feed and agricultural commodities in Iran, adverse effects on public health, advanced assay procedures and current control measures of aflatoxin contamination.

INTRODUCTION

In 1960, more than 100,000 young turkeys on poultry farms in England died in the course of a recently formed new disease that was termed "Turkey X ". A careful survey of the early outbreaks showed that they were all fedby Brazilian peanut meal. Investigation in 1960 revealed a possibility of fungal toxicity. In fact, the toxin–producing fungus was identified as *Aspergillus flavus* and the toxin was given the name "Aflatoxin" by virtue of its origin (FAO 1979, Abbas et al. 1984, Furlong et al. 1995). Aflatoxins are real public health hazards cause acute and chronic disorders. In western India in 1974, 108 persons among 397 people infected died from aflatoxin poisoning. Different symptoms such as anorexia, vomiting, severe jaundice, rapidly developing ascites, portal hypertension, and gastrointestinal bleeding were noticed. The number of deaths in men was twice than that of women. Anywhere, between two and six mg of aflatoxin seems to have been consumed daily by the affected people for many weeks (Krishnamachari et al. 1976, Jewers 1990, van Egmond et al. 2007, Saladino et al. 2016). A classic case occurred during a Chinese festival in Malaysia in which approximately 40 persons were affected and 13 children died after eating noodles highly contaminated with aflatoxin. Symptoms included vomiting, diarrhea, pyrexia and abdominal pain. A Reye–like symptom and coma occurred about eight hours after ingestion and death between two and nine days after onset. High level of aflatoxin was found on autopsy in liver, lung, kidney, heart, brain, and spleen (Kostarelou et al. 2014, Foerster et al. 2016). In a report from central and eastern Kenya in 2004, 317 and 125 patients died of poisoning and acute liver failure after consuming aflatoxin contaminated corn. Improper storage of fresh corn under hot and humid conditions was the main cause of contamination.

It has been estimated that about 25% of the world cereals are contaminated with known mycotoxins

produced by a variety of toxigenic fungi. Among naturally occurring mycotoxins, aflatoxins produced mainly by *Aspergillus* section *Flavi* have received major consideration because of their toxicity for biological systems and the ability for contaminating a wide range of substrates under suitable conditions (Fig. 1). Aflatoxins are chemically and biologically active secondary metabolites with an unexplored role in fungal development that produced by certain molds, which grow in soil, cereals, nuts, fruits, decaying vegetation, hay, and grains (Iram et al. 2016). In contrast to bacterial toxins which are usually macromolecules (polypeptides, proteins, lipo-polysaccharides), aflato–xins have lower molecular masses (Hosseininia et al. 2014, Groote et al. 2016). The disease caused by the use of aflatoxins, called "Aflatoxicosis" which usually occurs after eating foods contaminated with different types of aflatoxins (Yazdanpanah et al. 2012, Razzaghi –Abyaneh et al. 2014). The presence of fungi in food is not necessarily associated with aflatoxins and though the lack of it does not mean there are not toxins in food, because aflatoxins remain in food long after the disappearance of mold in food.

In a global context, aflatoxin contamination is a constant concern between the 35 N and 35 S latitude where developing countries are mainly situated. With expanding boundaries of developing countries, aflato-xin contamination has become a persistent problem to those emerging areas. The continuing threat by aflatoxin contamination of food, feed and agricultural commodities to the world population hasmade aflatoxin research one of the most exciting and rapidly developing study areas of microbial toxins (Razzaghi–Abyaneh et al. 2014).

Aflatoxigenic fungi are able to grow and produce toxin in many foods and under different humidity, pH and temperature, however, they usually grow better in the foods that are kept under hot and humid conditions (Razzaghi–Abyaneh et al. 2014). The best conditions for mold to grow on organic materials are when the temperature is warm and when the material has a high level of moisture (7% or more).

Aspergillus flavus is the most important aflatoxin producing fungus, with increasing importance in food/feed poisoning and it is capable of producing at least 14 mycotoxins of which aflatoxins are the most important groups discussed in the present review (Goldblatt 2012).

GENERAL CHARACTERISTICS OF AFLATOXINS

Aflatoxins are defuranocoumarin crystalline compounds (Fig. 2). Although 20 compounds, all designated aflatoxins, have been isolated the term aflatoxins usually refers to 4 compounds of the group of bis–furanocoumarin metabolites produced by *A. flavus* and *A. parasiticus,* named B_1, B_2, G_1 and G_2 which occur naturally in agricultural products (Lillehoj 1970; Pitt and Tomaska 2001; Foerster et al. 2016). Aflatoxins M_1 and M_2 are hydroxylated metabolites of aflatoxins B_1 and B_2 (aflatoxin M_1 is 4–hydroxy derivative of AFB_1 and aflatoxin M_2 is 4–dihydroxy derivative of AFB_2.); it is also the metabolite present inthe milk of humans and animals who eat foods containing the toxin.
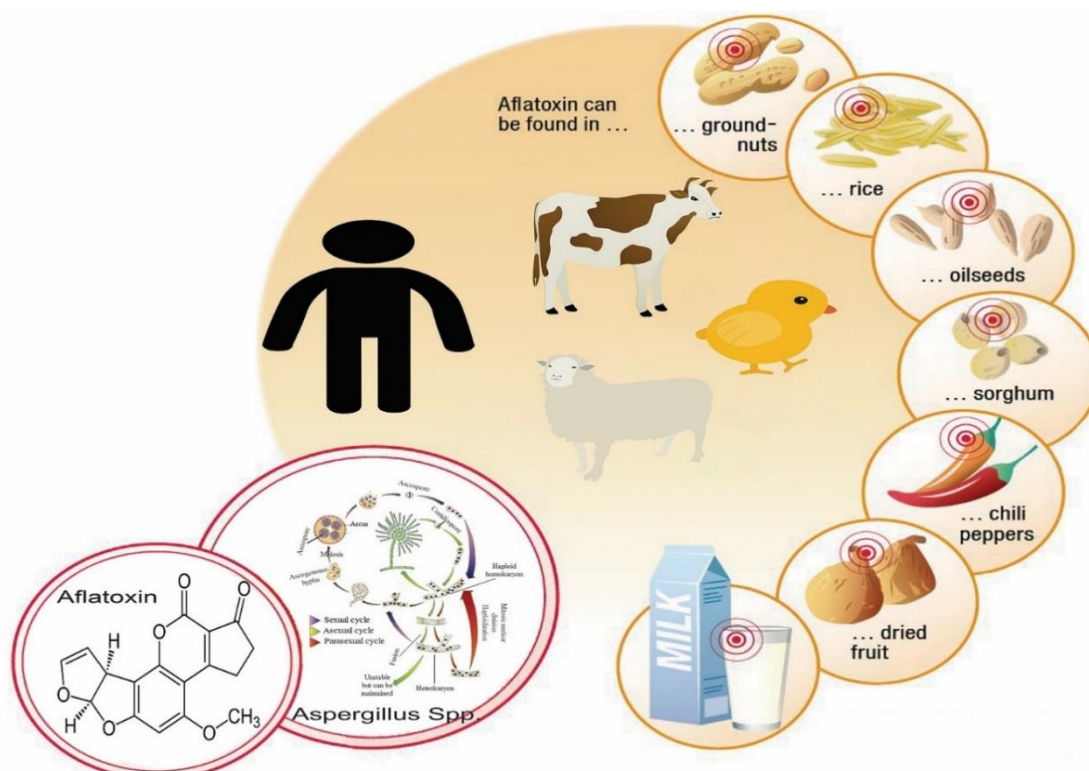

Fig 1. The life cycle of *Aspergillus* and aflatoxins in food and feed.
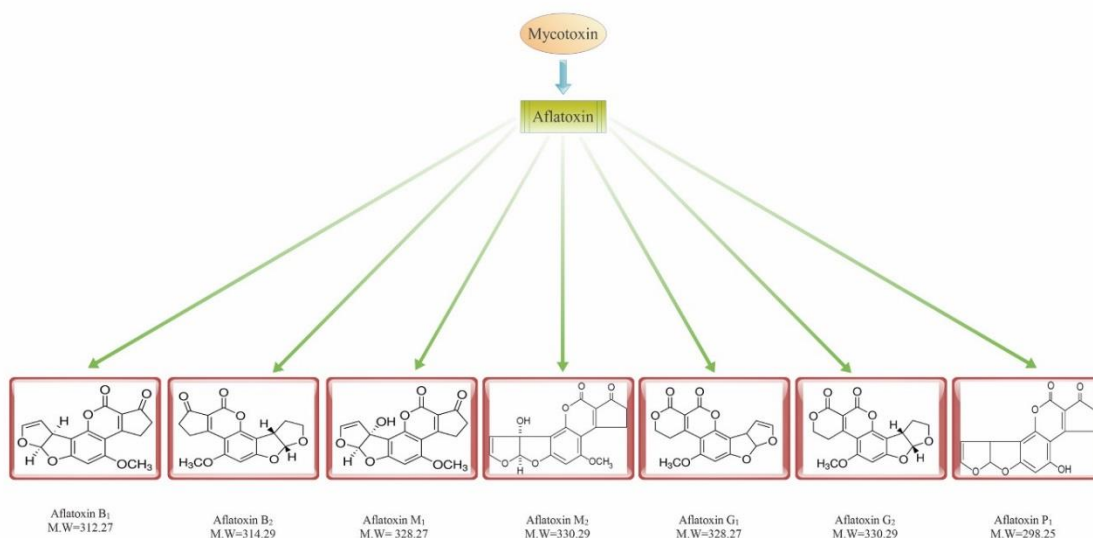
Fig. 2. Molecular formula and chemical structure of major aflatoxins.

It is estimated that about 1–2 % of aflatoxin B_1 is excreted in milk as the metabolite M_1 (Afsah-Hejri et al. 2013). AFB_1 has been classified as a Group 1 human carcinogen by the International Agency for Research on Cancer (IARC) which -possesses carcinogenicity in many animal species (Groote et al. 2016, Wang et al. 2016).

Aspergillus flavus, *A. pseudotamari*, and *A. pseudonomius* produce aflatoxins type B (B_1 and B_2). *Aspergillus nomius*, *A. bombysis*, *A. pseudocaelatus*, and *A. parasiticus* produce types B (B_1 and B_2) and G (G_1 and G_2) aflatoxins (Lillehoj et al. 1970).

AFLATOXIN PRODUCING FUNGI

Aflatoxin production has incorrectly been claimed for a long list of *Aspergillus* species and also for species assigned to the other fungal genera. Recent data indicate that aflatoxins are produced by thirteen species assigned to three sections of the genus *Aspergillus*: section *Flavi* (*A. flavus*, *A. pseudotamarii*, *A. parasiticus*, *A. nomius*, *A. bombycis*, *A. parvisclerotigenus*, *A. minisclerotigenes*, *A. arachidicola*), section *Nidulantes* (*Emericella astellata*, *E. venezuelensis*, *E. olivicola*) and section *Ochraceorosei* (*A. ochraceoroseus*, *A. rambellii*) (Varga et al. 2015). The species which produce aflatoxins have been synonymized with other aflatoxin producers, including *A. toxicarius* (=*A. parasiticus*), *A. flavus* var. *columnaris* (=*A. flavus*) or *A. zhaoqingensis* (=*A. nomius*).

It has been shown that production of aflatoxins as toxic secondary metabolites by producing fungi is closely related to fungal development (Jamali et al. 2012, Razzaghi–Abyaneh 2013, Jahanshiri et al. 2015, Varga et al. 2015). Members of the genus *Aspergillus* are able to grow at 6 to 54 °C. The

optimum growth temperatures are 35 to 37 °C. The proper temperature for the fungus toxin production is 28 to 33 °C. *Aspergillus* species can grow in water activity of 0.78 to 1 with an optimum of 0.95. Aflatoxigenic species are capable of producing toxins in the water activity at 0.83 to 0.97 and optimum water activity for toxin production is 0.90 to 0.95. *Aspergillus flavus* usually grows best in temperate and semi–warm and moist environments and *A. parasiticus* grows best in warm and moist environments. *Aspergillus flavus* is depen– dent more on the upper plants and *A. parasiticus* more in the soil (FAO 1979; Azarakhsh et al. 2011; Hosseininia et al. 2014; Foerster et al. 2016).

Although the members of *Aspergillus* section *Flavi* have been isolated from soil, air and other natural habitats in Iran, their taxonomic position and accurate identification have been a matter of controversy and little has been documented regard to their ability to produce aflatoxins (Razzaghi– Abyaneh et al. 2011).

Razzaghi–Abyaneh et al. (2006) studied soil isolates of *Aspergillus* section *Flavi* from Mazandaran and Semnan provinces with totally different climatic conditions for aflatoxins (AFs; B and G types), cyclopiazonic acid (CPA) and sclerotia production. A total of 66 *A. flavus* group strains were identified from three species *viz. A. flavus*, *A. parasiticus* and *A. nomius* in both locations. *A. flavus* (87.9 %) was found to be the prominent species followed by *A. nomius* (9.1 %) and *A. parasiticus* (3.0 %). Only 27.5 % of *A. flavus* isolates were aflatoxigenic (B_1 or B_1 and B_2), out of which approximately 75 % were capable to produce CPA. All the *A. parasiticus* and *A. nomius* isolates produced AFs of both B (B_1 and B_2) and G (G_1 and G_2) types but did not produce CPA. Sclerotia production was observed in only 4 isolates of *A. flavus* among all 66 isolates from three

identified species. *A. flavus* isolates were classified into various chemotypes based on the ability to produce aflatoxins and CPA.

Jamali et al. (2012) studied aflatoxigenicity of 193 *Aspergillus* strains isolated from a total of 100 soil samples of pistachio orchards, which all of them were identified as *A. flavus* as the most abundant species of *Aspergillus* section *Flavi* existing in the environment. Approximately 59%, 81%, and 61% of the isolates were capable of producing aflatoxins (AFs), cyclopiazonic acid (CPA), and sclerotia, respectively. The isolates were classified into four chemotypes (I to IV) based on the ability to produce AFs and CPA. The resulting dendrogram of random amplified polymorphic DNA (RAPD) analysis of twenty–four selected *A. flavus* isolates demonstrated the formation of two separate clusters. Cluster 1 contained both aflatoxigenic and non–aflatoxigenic isolates (seventeen isolates), whereas cluster 2 comprised only aflatoxigenic isolates (seven isolates). All the isolates of cluster 2 produced significantly higher levels of AFs than those of cluster 1 and the isolates that produced both AFB_1 and AFB_2 were found only in cluster 2. RAPD genotyping allowed the differentiation of *A. flavus* from *A. parasiticus* as a closely related species within section *Flavi*.

Sepahvand et al. (2011) studied genetic diversity and mycotoxin profiles of *A. flavus* isolated from air (indoors and outdoors), levels (surfaces), and soils of five hospitals in Southwest Iran had examined. From a total of 146 *Aspergillus* colonies, sixty-three isolates were finally identified as *A. flavus* by a combination of colony morphology, microscopic criteria, and mycotoxin profiles. No *A. parasiticus* was isolated from examined samples. Chromatographic analyses of *A. flavus* isolates cultured on yeast extract–sucrose broth by tip culture method showed that approximately 10% and 45% of the isolates were able to produce AFB_1 and cyclopiazonic acid (CPA), respectively. Around 40% of the isolates produced sclerotia on Czapek–Dox agar. The isolates were classified into four chemotypes based on the ability to produce AF and CPA that majority of them (55.5%) belonged to chemotype IV comprising non–mycotoxigenic isolates. Random Amplified Polymorphic DNA (RAPD) profiles generated by a combination of four selected primers were used to assess genetic relatedness of 16 selected toxigenic and non–toxigenic isolates. The resulting dendrogram demonstrated the formation of two separate clusters for the *A. flavus* comprised both mycotoxigenic and non–toxigenic isolates in a random distribution.

Houshyarfard et al. (2014) examined soil samples from pistachio orchards in three major production regions of Iran. They reported that *A. flavus* (69.7, 65.3 and 57.9%), *A. parasiticus* (19.6, 25.4, and 29.3%), and *A. nomius* (10.7, 9.3, and 12.8%) were the predominant species in the regions of Rafsanjan, Damghan, and Feyz-Abad regions, respectively. Around 60 % of *A. flavus* isolates distributed in 16 vegetative compatibility groups (VCGs) were able to produce aflatoxins.

AFLATOXICOSIS AND AFLATOXIN–RELATED DISEASES

Aflatoxin poisoning can be diagnosed as either acute or chronic, but chronic disease is a common type (Khoshpey et al. 2011, Razzaghi–Abyaneh 2013). In cases of acute aflatoxicosis, an individual has been exposed to moderate to high levels of aflatoxins; which often occur in Africa and Southeast Asia. Acute aflatoxicosis is characterized by symptoms such as nausea, diarrhea, abdominal pain, fever, anorexia, drowsiness, acute liver damage, coma and finally death. A large amount of toxins are in the liver, kidneys, heart, lungs, spleen, and brain. In cases of chronic aflatoxicosis, an individual has been exposed to low to moderate levels of aflatoxins. Chronic aflatoxicosis is characterized by symptoms such as dysfunctional food conversion and slow growth rates. Symptoms include is liver cancer, chronic hepatitis, jaundice, hepatomegaly, gallbladder inflammation, cirrhosis and fatty liver (Fig. 3). Liver cancer in central Africa and parts of Southeast Asia is probably associated with this disease. Aflatoxin–induced immunosuppression may be manifested as depressed T or B lymphocyte activity, suppressed antibody production, and impaired macrophage–neutrophil–effector functions (Wang et al. 2016).

The liver is the main organ that affects aflatoxins and these toxins cause tissue damage and finally carcinogenesis (Eraslan et al. 2017). Aflatoxins after the entry into liver cells bind the nuclear and mitochondrial DNA. Aflatoxins induce mutations especially point mutation and change the message of the DNA. The genetic changes are leading to disturbances in the production of DNA and finally related protein. It has been shown that aflatoxins have immunosuppressive properties, probably related to their inhibitory effect on protein synthesis. AFB_1 metabolizes by oxidizing enzymes in cytochrome (CYP) P–450 in mitochondrial of the liver cell and converts to AFB_1–8,9–epoxides and AFB_1–2,3–epoxides or AFBO form, which is its active form. Aflatoxin epoxide has a very high ability to connect to DNA and proteins. In the codon 249 of p53 gene, AFBO bind to the guanine in 7 positions of DNA (AFB_1– N7– Gua) and by transforming it to thiamine or another alkaline, leading to the destruction of replication process and production mutagenic and carcinogenic substa–nces. In addition, the epoxide form of AFB_1 converts to dihydrodiol by hydrolysis that can bind to the protein and causes cytotoxicity (Karami–Osboo et al. 2012, Kostarelou et al. 2014). Aflatoxins in many animals is an active agent for liver cancer and even with the injection of a small amount of it, there is the possibility of a cancerous tumor. Aflatoxin B_1 in animals leads to slower growth
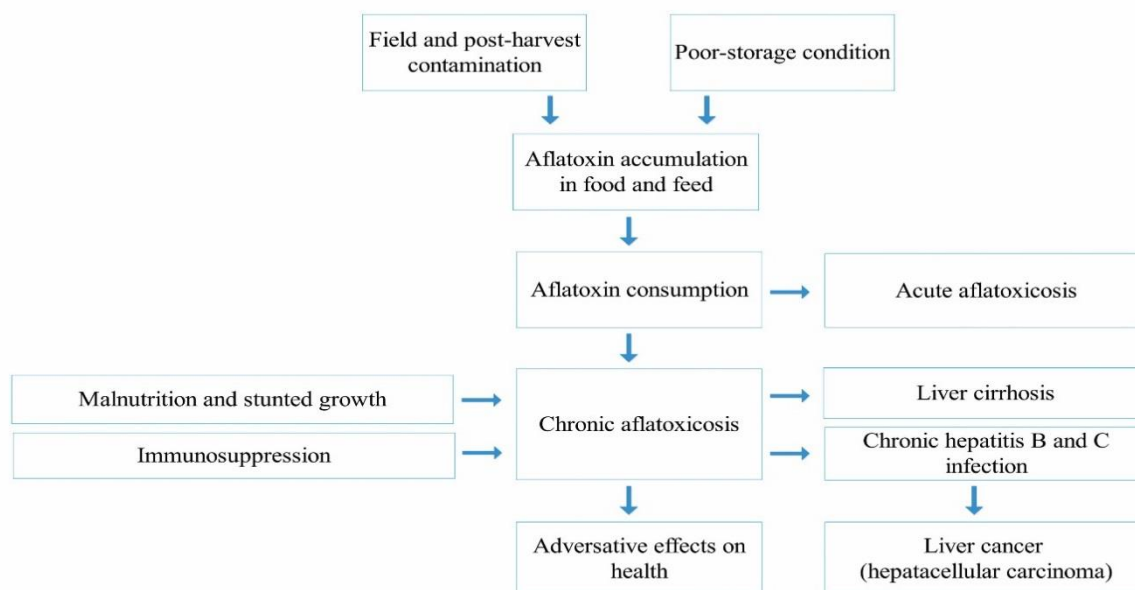
Fig. 3. Aflatoxins and implications in public health.

and failure in food absorption and makes them susceptible to viral, bacterial, fungal and parasitic infections. In addition, it reduces the strength of fertility, laying eggs and lactation and creates severe liver toxicity. Young male animals are more sensitive to the toxins and the symptoms are more severe. The mice and guinea pigs are resistant to aflatoxicosis while the ducks, monkeys, rabbits, trout, and rats are sensitive. The fate of aflatoxin exposure in human and animals depends on the liver's detoxification system, genome structure, age and nutritional factors (Ghadarijani & Javanshah 2005, Chehri et al. 2015).

AFLATOXINS IN FOOD, FEED AND AGRICULTURAL COMMODITIES

Aspergillus species as the main producers of aflatoxins have a great tendency to food grains and oilseeds. Oilseeds, especially peanuts are highly susceptible to *Aspergillus* growth (Perrone & Gallo 2017). Corn, peanuts, and cotton are the most important nutrients associated with aflatoxicosis. The highest rates of infection to *Aspergillus* in these materials, occurs in pre–harvest, at the harvest, and during the storage. The risk of aflatoxin contamination in grains is high under water pressure, high ambient temperatures, mechanical damage during harvest, insect sting, rain during harvest, and storage under hot and humid conditions. The damaged grains (mechanical damage during harvesting or damage caused by insects) are easier to mold growth. Warm and moist environment accelerate the mold growth. Grains such as corn, barley, wheat, rice, oilseeds and oils including peanut, soybean, cotton and sunflower, spices, such as pepper

and turmeric seeds of trees, such as pistachios, almonds and walnuts, dried fruits such as figs and other materials food crops such as grains and milk are foodstuffs that aflatoxin is so far isolated from them (Razzaghi–Abyaneh et al. 2014).

Various studies in Iran have reported the presence of aflatoxins in foodstuffs. Moreover, the biomarkers of aflatoxins have also been reported recently in serum and urine samples, which can provide additional information for assessing aflatoxins exposure in Iran (Habibi et al. 2018). As shown in Fig. 4, aflatoxins are reported from nearly all parts of the country. Peanuts, cereals, spices, and their food products are the supplies most susceptible to aflatoxins contamination. Several studies have also identified aflatoxins in the milk and cheese. A general view on the occurrence of aflatoxins in food, feed, and food–and nut–based products is summarized in Table 1.

The first problem associated with aflatoxins was reported around the 1970s in Isfahan (Suzangar et al. 1976). Following the detection of aflatoxin M in cow's milk in the previous study in Isfahan, the contami–nation with aflatoxin of cotton–seed and cotton–seed cake which constitutes the principal feed of local animals was reported (Suzangar et al. 1976). The samples were taken from 2 factories. The grain from the 1st factory was grown in the center of the country in a dry climate, and those from the second source originated from the humid region of the North of Iran. The grain and cotton–seed cake from the first factory did not contain aflatoxin before storage. During storage, the contamination rate was increased with time in both regions, with the dry less than the humid. They concluded that cotton–seed and cotton–seed cake appear to be contaminated with aflatoxin B in two processes.

Fig 4. The natural occurrence of aflatoxins in food, feed and agricultural commodities in Iran

The 1st is aflatoxin contamination before harvest, in the humid region of Iran; the second is general storage aflatoxin contamination in both regions in which humidity and length of storage appear to be the principal factors (Emami et al. 1976).

Aflatoxin in nuts

In a study by Mirmomeni et al. (1979), aflatoxin B_1–induced liver damage in rats which were fed by different levels of dietary protein. AFB_1 was fed for 6 months to young rats in low or high protein diets. It was found that the high protein diet was associated with hyperplastic activity in the liver, of a type similar to that usually found in rats developing AFB_1–induced hepatoma. During the same period, negligible precancerous–like changes were seen in the rats fed the low protein diet with AFB_1. This model was used to test the sensitivity of various liver function tests to the dietary–induced difference in liver reaction to AFB_1 feeding. The serum enzymes lactic dehydrogenase and alkaline phosphatase were considerably elevated in rats with precancerous–like lesions, however, the former was the most sensitive enzyme.

Serum glutamate–oxalate transaminase and serum glutamate–pyruvate transaminase were both raised in the rats with precancerous–like lesions, however, they were much less than lactic dehydrogenase and alkaline phosphatase. Urinary aflatoxin metabolites were also measured. AFM_1 and AFP_1 were both found after feeding AFB_1. In the feeding period, AFP_1 excretion steadily increased, while AFM_1 only increased during the second and fourth months and then decreased. In the first 4 months, rats fed a low protein diet tended to produce a lower ratio of AFM_1 to AFP_1 compared to the rats fed a high protein diet. At 6 months, the ratio remarkably decreased, especially in the rats with the precancerous–like lesions. It was concluded that lactic dehydrogenase, alkaline phosphatase, and the ratio of urinary excretion of AFM_1 to AFP_1 could be useful for considering in a diagnostic procedure for AFB_1–induced precancerous liver changes that might be expected in human studies (Mirmomeni et al. 1979).

Mojtahedi et al. (1987) reported the presence of aflatoxins in Pistachio nut. Pistachio nut samples were taken during various stages of development from

Table 1 Natural occurrence of aflatoxins in foodstuffs in different parts of Iran.

Food products	Samples	Province	Important results	Reference
Rice	65	Guilan, Mazandaran	46% of the samples were contaminated with at least one of the emerging Fusarium mycotoxins. Beauvericin was the most prevalent mycotoxin, which was found in 26 out of 65 rice samples at the concentrations up to 0.47 µg/kg. Enniatin A1 which was the only member of Enniatin was detected in the samples, occurred in 7.7% of samples with an average level of 0.06 μg/Kg. No detectable level of Fusaproliferin and moniliformin was found.	Nazari et al. 2015
Rice	90	Khuzestan	Highest concentration of AFB_1 and total AFs in the rice samples were 2.3500 and 2.7040 ng/g, respectively. The different mean concentration of AFB_1 and total AFs in three brands of the rice samples was significantly lower than the maximum tolerable level (MTL) of AFB_1 (5 ng/g) and total AFs (30 ng/g) set by the Institute of Standards and Industrial Research of Iran.	Nazari et al. 2014
Poultry feed	89	Azerbaijan	Overall incidence of AF was 42 percent. The average contamination levels of AF were 2.83ppb (µg/kg), with minimum and maximum level of 0.1 and 43.8ppb, respectively. The FDA's Aflatoxin Regulations Policy can support enforcement action if aflatoxin levels exceed for Feed of chicks (such as broiler chickens) 20 ppb and for Mature poultry (such as laying hens) is 100 ppb.	Taifebagerlu et al. 2015
Corn	100	Kermanshah	One–hundred *Aspergillus* isolates were collected and identified into six species i.e. *A. niger* (40%) followed by *A. flavus* (27%), *A. ochraceus* (15%), *A. fumigatus* (10%), *A. japonicus* (5%) and *A. sclerotiorum* (3%). We also determined the Aflatoxin B (AFB) contamination 11 statuses in the samples by enzyme–linked immune sorbent assay (ELIZA).	Chehri et al. 2015
Cheese	102	Khorasan	AFM1 was detected in 14 (32.55%) traditional and 21 (35.59%) pasteurized cheese samples. In total, 25.42% of pasteurized and 27.90% of traditional cheese samples were contaminated at above level of the Iranian standard limits (50 ng/kg).	Khodadadi et al. 2014
Raw milk, meat, and liver	210	Tabriz, Urmia, Ardabil	Samples were analyzed by ELISA method. The zearalenone was found in 92 of the 210 samples (43.80%). Significant differences in the mean values of zearalenone were observed between milk, meat, and liver samples (p<0.05). The highest mean level of zearalenone was observed in liver samples (2.37 ± 1.18 ng/g), followed by milk (1.34 ± 1.42 ng/ml) and meat (0.79 ± 1.27 ng/g) samples. The overall contamination rate during autumn was significantly more than the summer (p<0.05).	Mahmoudi 2014
Rice	80	Tehran	Ochratoxin A and zearalenone were found in none of the samples. However, 54.8% samples of imported rice and 22.2% samples of Iranian rice were contaminated with aflatoxin. The highest and the lowest amounts of aflatoxin were 2.46 and 0.34 ng/g in imported rice and 1.09 and 0.79 ng/g in the Iranian rice, respectively. The most abundant aflatoxin in both imported and Iranian samples was aflatoxin B_1.	Tavakoli et al. 2014
Sesame	296	Khorasan	Aflatoxins at >1 µg/kg were found in 50 % of all samples, but at low levels in most cases, which is illustrated by mean AFB_1 and total AF levels of 1.25±3.70 and 1.43 ± 4.38 µg/kg, respectively. A few (1.9 %) samples exceeded the National Iranian Standard maximum accepted level for AFB_1 (5 µg/kg) or total AF (15 µg/kg); the maximum total AF level found in one sample was 48 µg/kg.	Hosseininia et al. 2014
Milk	45	Khorasan	AFM1 contamination was detected in all of the milk samples. The mean concentration of aflatoxin M_1 was 27.2 ng/l. The range of AFM1 content was 8.8–64ng/l. Thirteen (28.8%) of the samples had AFM1 levels exceeding the maximum levels (50 ng/l) accepted by the European Union.	Riahi–Zanjani andBalali–Mood 2013
Egg	36	Khoramabad	Results revealed that 500 µg/kg AFB_1 significantly (P< 0.05) reduced feed consumption, feed efficiency, egg production as well as egg weight. Supplementation of HMB partially restored feed consumption and egg production alleviating some side effects of AFB_1.	Manafi and Khosravinia 2012
Lighvan cheese	82	Rafsanjan	Aflatoxin M1 was detected in 39 (47.6%) samples, consisting of 29 (64.4%) white cheese (mean: 135 ng/kg; range: 93.3–309 ng/kg) and 10 (27%) Lighvan cheese samples (mean: 90.8 ng/kg; range: 70.5–203 ng/kg). According to Iranian national standard limit for AFM1 in cheese (200 ng/kg), 9 samples (20%) of white cheese and 1 sample (2.70%) of Lighvan cheese had levels above the limit.	Mohajeri et al. 2013
Rice	65	Tehran	The most prevalent fungal metabolites were brevianamide F (81.5%), emodin (46.1%) and tryptophol (43.1%). The occurrence of aflatoxin B_1 (AFB_1), ochratoxin A (OTA), zearalenone (ZEN) and fumonisin B_1 (FB_1) was 21.5%, 4.6%, 29.2% and 9.2%, respectively.	Nazari et al. 2014
Wheat, Corn, Plant residues, and Animal feeds	–	Zanjan	The dominant recognized isolates were *Fusarium solani*, *F. oxysporum*, *F. graminearum*, *F. moniliformei*, *F. sambutinum*, *F. culmorum*, and *F. equiseti* as the main mycotoxin producers. The common *Fusarium* mycotoxins such as zearalenone, moniliformin and fusaric acid can be discovered from these species.	Saremi and Okhovvat 2013
Dried fruits	15	Mashhad	Around 30% and 3.33% of examined apricot samples and 13.33% and 20% of examined prunes samples contained aflatoxin B_1 and ochratoxin A more than 0.2 ng/g. The average recoveries were found to be 91.1% and 98.5% for aflatoxin B_1 and ochratoxin A, respectively, while the detection limit was 0.2 ng/g for both mycotoxins.	Janati et al. 2012

Table 1. Continued

Food products	Samples	Province	Important results	Reference
Rice, Bread	72	Tehran	The average recovery and coefficient of variation in different foods ranged 92.7–107.1 and 4.9–13.8%, respectively. The amount of ZEA in corn CRM was in the acceptable range of FAPAS. The limit of quantification was 3 ng/g for rice, bread and wheat flour and 2.7 ng/g for puffed corn snack. The retention time of zearalenone was 2.6 min. All samples had a contamination level lower than the maximum tolerated level of ZEA in foods in Iran.	Yazdanpanah et al. 2012
The Soil of pistachio orchards	100	Different regions	Approximately 59%, 81%, and 61% of the isolates were capable of producing aflatoxins (AFs), cyclopiazonic acid (CPA), and sclerotia, respectively.	Jamali et al. 2012
Maize (corn)	373	Ardabil, Khuzestan, Fars	The amount of aflatoxin (AFB_1) in maize samples varied across the years, the percentage of contamination in 2006, 2007 and 2008 were 86.7%, 71.4%, and 100% respectively for Ardebil, 63.4%, 41.3% and 24.5% for Khuzestan, 17%, 14,8% and 27.6% for Fars province. The mean of contamination was 15.63, 57.67 and 154.13 µ/kg for Ardabil; 14.41, 35.93 and 1.61 µ/kg for Khuzestan; 0.9, 2.12 and 36.39 µ/kg for Fars province.	Karami–Osboo et al. 2012
Sunflower and safflower seeds	173	Khorasan	AFB_1 was determined by HPLC with immunoaffinity column (IAC) clean–up and fluorometric detection. Aflatoxin B1 contamination was found in 111 samples: in 8 of the sunflower seed samples (16%) at a mean level of 40.68 ng/g and in 103 safflower seed samples (83.7%) at a mean level of 2.81 ± 0.44 ng/g. In 5 sunflower seed samples and 1 safflower seed sample, aflatoxin B_1 levels were higher than the maximum levels of AFB_1 under Iran regulations (5ng/g). Aflatoxin B_1 levels in 5 sunflower and 2 safflower seed samples were higher than the European Union maximum limit (2 ng/g).	Beheshti & Asadi 2013
Milk and Cheese	121	Ahvaz	Analytical results showed that 61 (50.4%) samples were contaminated with AFM_1 ranging from 11 to 209 ng/l and consisted of 24 (40%) pasteurized milk samples (range: 11–94 ng/l and mean ± SD: 34.7 ± 19.9 ng/l) and 37 (60.7%) white cheese samples (range: 32–209 ng/l and mean ± SD: 103.6 ± 54.1 ng/l).	Rahimi et al. 2012
Pistachio	–	Tehran	The results indicated *B. subtilis* UTBSP1 could considerably remediate AFB_1 from nutrient broth culture and pistachio nut by 85.66% and 95%, respectively. Cell-free supernatant fluid caused an apparent 78.39% decrease in AFB_1 content.	Farzaneh et al. 2012
Breast milk	132	Hamadan	AFM_1 was detected in eight samples (6.06%) at mean concentration of 9.45 ng/L. The minimum and maximum of concentration was 7.1 to 10.8 ng/L, respectively. Although the concentration of AFM_1 in none of the samples was higher than the maximum tolerance limit accepted by the USA and European Union (25 ng/kg) however, 25% had a level of AFM_1 above the allowable level of Australia and Switzerland legal limit (10 ng/L).	Ghiasian et al. 2011
Broiler feeds	50	Kermanshah	Out of 50 feed samples, 46 samples (92%) in dilution 1, 38 samples (76%) in dilution 2, 28 samples (56%) in dilution 3 and 20 samples (40%) in dilution 4, were contaminated to Aspergillus species. The most prevalent species was *Aspergillus flavus* followed by *Aspergillus niger* and *Aspergillus fumigatus*.	Azarakhsh et al. 2011
Milk	100	Urmia	The samples were analyzed with a commercial competitive enzymelinked immunosorbent assay (ELISA) kit. All samples (100%) were found to have levels that exceed the legal limits of 50 ng/l established by the EU/Codex.	Panahi et al. 2011
Bean	30	Khorasan	Janati et al. (2011) A study was undertaken to determine levels of aflatoxins and ochratoxin A in bean, using a technique preceded by an immunoaffinity clean–up step. For this purpose, a total of 30 bean samples were analyzed. 16.67% and 10% of examined bean samples contained Aflatoxin B1 and ochratoxin A more than 0.2 ng g–1. Recoveries were found to be 91.1% and 98.5% for Aflatoxin B1 and ochratoxin A, respectively, while the detection limit was 0.2 ng g–1 for both mycotoxins.	Janati et al. 2011
Sesame	182	Khorasan	Detection limits for AFB1, AFB 2, AFG 1 and AFG2, were 0.45, 0.19, 0.61, and 0.22 ng/g, respectively. AFB1 was detected in 33 samples (18.1%), at a mean level of 1.62 ± 1.32 ng/g, and a maximum level of 5.54 ng/g. AFB_1 levels exceeded the European Union (EU) maximum tolerated level (MTL,2 ng/g) in 9 samples, and the Iran MTL (5 ng/g) in 1 sample. Regarding total aflatoxins (AFT), the mean level was 0.92 ± 1.36 ng/g, and the maximum level was 5.54 ng/g. No sesame sample exceeded the Iran MTL (15 ng/g), but two samples exceeded the EU MTL (4 ng/g) for AFT.	Asadi et al. 2011
Maize	35	Mazandaran	Data revealed that incidence of AFB_1, AFB_2 and total aflatoxins (AFT) in maize samples were 66%, 54% and 63% with mean of 9.5 ± 16.3, 1.7 ± 2.6 and 10.4 ± 18.4 ng/g, respectively. Conclusion: The mean level of AFB_1 in samples was higher than the Iranian maximum tolerated level (MTL). But, AFT mean level (10.4 ng/g) was lower than the Iranian and US (20 ng/g) MTLs.	Hadiani et al. 2009
Milk	98	Hamedan, Shiraz, Rasht, Tehran	The overall mean of all samples was 0.041–0.065 µg/L (95% confidence) and the adjusted mean based on statistical modification was 0.039 ppb: 61 samples had 0.000–0.050 µg/L, 29 samples were contaminated with 0.05–0.10 µg/L, and the remaining 8 samples had 0.1 – 0.39 µg/L. All of the samples were lower than Codex Alimentarius and FDA standards (0.5 µg/L). Levels of aflatoxin M_1 were higher in winter and spring than in summer and autumn.	Tajkarimi et al. 2007

Table 1. Continued.

Food products	Samples	Province	Important results	Reference
Milk	328	Tehran	The samples of pasteurized liquid milk ($n = 128$), infant formula ($n = 120$) and milk–based cereal weaning food ($n = 80$) showed that the incidence of contamination with AFM_1 is 96.3%, the presence of AFM_1 in each group was 72.2 ± 23.5, 7.3 ± 3.9 and 16.8 ± 12.5 ng/kg, ranging between 31–113, 1–14 and $3 - 35$ ng/kg, respectively. In general, the amount of AFM_1 in 100 (78%) of liquid milk samples and 24 (33%) of milk–based weaning food was higher than the maximum tolerance limit accepted by European Union	Oveisi et al. 2007
Pistachio	63	Kerman, Semnan	Aflatoxin (AFB_1) concentration in the inoculated nuts estimated by enzyme–linked immunosorbent assay (ELISA) ranged between $0.0 - 5034.3$ ppb. High correlation ($r = 95\%$) was observed between sclerotia production and aflatoxin concentration. Isolates that produced less sclerotia had low aflatoxin levels and isolates that produced more sclerotia could produce high amount of aflatoxin. This study showed that not all isolates of *A. flavus* infecting pistachio produce sclerotia and there is variability in rate of sclerotia production, which in turn was directly proportional to the toxigenic potential of an isolate.	Mirabolfathy et al. 2005
Milk	90	Gonabad	34 samples (75, 55%) out of 45 obtained in autumn and winter and 18 samples (40%) of 45 obtained in spring and summer were higher than the maximum tolerance limit (50 ng/L) accepted by some European countries.	Mokhtarian et al. 2005
Pistachio	–	Jiroft	Akbari pistachio treated with propolis extracts had lower aflatoxin concentration than the critical level. Total aflatoxin levels were 1.28 ppb and 7.3 ppb for treatment and control, respectively.	Khezri, et al. 2006
Cheese	80	Tehran	AFM1 was found in 82.5% of 80 of the cheese samples examined. The range of contamination levels varied among different months. AFM1 in May, August, November, February samples ranged from 0.17 to 1.30, 0.15 to 2.41, 0.16 to 1.11, and 0.19 to 2.05 µg/kg, respectively, while the mean values were 0.41, 0.35, 0.36, and 0.52 µg/kg, respectively. The highest mean concentration of aflatoxin M1 (AFM_1) was registered in February samples (0.52 µg/kg). The lowest mean concentration of aflatoxin M1 was registered in August samples (0.35 µg/kg).	Kamkar et al. 2006
Cornfield soils	100	Semnan and Mazandaran	*Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* in both locations Mazandaran and Semnan. *A. flavus* (87.9%) was found to be the prominent species followed by *A. nomius* (9.1%) and *A. parasiticus* (3.0%). Only 27.5% of *A. flavus* isolates were aflatoxigenic (B_1 or B_1 and B_2), out of which approximately 75% were capable to producing CPA. All the *A. parasiticus* and *A. nomius* isolates produced AFs of both B (B_1 and B_2) and G (G_1 and G_2) types, but did not produce CPA. Sclerotia production was observed in only 4 isolates of *A. flavus* among all 66 isolates from three identified species. *A. flavus* isolates were classified into various chemotypes based on the ability to produce aflatoxins and CPA.	Razzaghi–Abyaneh et al. 2006
Milk	624 pasteurized milk	Shiraz	Aflatoxin M_1 was found in 100% of the examined milk sample. 390 sample (62.5%) had contamination less than 45 ng/l of AFM_1, 123 samples (19.7%) contained 45–50 ng/l, 94 samples (15.1) contained 50–80 ng/l and the remaining 2.7% of samples contained more than 80 ng/l of AFM_1.	Alborzi et al. 2006

orchards in Iran indicated that contamination with toxigenic fungi occurred mostly during the late stages of nut development.

Members of the genera *Aspergillus* and *Penicillium* occurred most frequently. Of the Aspergilli, the species *A. niger*, *A. flavus* and *A. fischeri* var. *spinosus* occurred most frequently, followed by *A. terreus*, *A. tamarii* and *A. nidulans*. Twenty–two isolates comprising 13 species were tested for toxicity to ducklings. Isolates of known toxic fungi belonged to *A. flavus*, *A. niger*, *A. parasiticus*, *A. ochraceus*, *A. versicolor*, *A. nidulans* and *A. terreus*. The toxicity of *A. fischeri* var. *spinosus* was reported. Chemical analysis showed that all isolates of *A. flavus* and *A. parasiticus* produced AFB_1, the isolates of *A. versicolor* and *A. nidulans* produced sterigmatocystin while the toxic isolate of *A. ochraceus* did not produce ochratoxins. Toxic fungi were shown to occur in a variety of nuts and aflatoxin contamination of pistachio nuts was proven to lead to the rejection of consignments of Iranian pistachio nuts (Mojtahedi et al. 1987).

According to the report of Mirabolfathy et al. (2005), aflatoxin contamination of pistachio nuts is undoubtedly an important economic problem for Iran. The European Union's ban on pistachio imports from Iran because of high levels of aflatoxin. The existence of aflatoxins and aflatoxigenic fungi in pistachio were reported in Iran in 1970 (Houshyarfard et al. 2012). In several research conducted to find the fungal species produced aflatoxins, it was reported that up to 13 species were isolated from pistachio kernels and shells collected from the orchard (Mirabolfathy et al. 2005). The probability of contamination incidence in orchard, processing stages, at storage and shipment, were studied. Throughout this project, pistachio nuts were collected from the different storages and terminals of pistachio producing areas of Kerman province including Rafsanjan, Zarand, Kerman, Noogh, and Sirjan. One–hundred and twenty–two isolates of *A. flavus* were isolated using suspension of surfactant contamination of pistachio nuts. To detect fungal contamination at different parts of pistachio

nuts, 51 samples of shells and kernels collected from 51 different storages were disinfected using hypochlorite 0.5 % and cultured on artificial media separately. A total of 86 % of the samples were contaminated to *A. flavus* in a range of 0– 100 %. Due to aflatoxin contamination, 32 % of the samples had aflatoxin more than the accepted level of 2 ppb.

It has been shown that the distribution of aflatoxins in pistachio bulks and their sources are the main factors in pistachio nuts contamination by aflatoxins (Ghadarijani & Javanshah 2005). Sampling was done from final, small, yellow shell discoloration and floating pistachios at pistachio processing terminals in Kerman (6 terminals) and Yazd (1 terminal) provinces in 2002 and 2003. To determine the role of re–hulled pistachios in contamination, sampling was done from several terminals and samples were analyzed in stained and non–stained pistachios. The results indicated that the concentration of AFB_1 increased in final, small, and yellow shell discoloration pistachios and decreased in yellow shell discoloration, stained and deformed pistachios (Hadavi et al. 2017). The existence and percentage of yellow shell discoloration, stained and deformed pistachios can be used to determine contamination of pistachio bulks. Re–hulled, stained and non–stained pistachios were contaminated with AFB_1 and 66 % of non–stained pistachios contained AFB_1 higher than the permitted limit. Aflatoxin concentration varied according to the type of processing terminal and location. The least amount was observed in a semi–mechanized terminal and pistachio sample in Yazd province (Ghadarijani and Javanshah 2005).

Arjmand et al. (2005) compared aflatoxin contamination in different pistachio processing stages, in a completely randomized design with 6 replicates during 2003–2004 in the Pistachio Research Institute. Aflatoxin contents in samples of pistachio were determinate with HPLC. Analysis of variance revealed that there was a significant difference between different parts of the processing procedures. The mean contamination in re–hulled pistachio nuts, stained nuts, very small screened nuts and final pistachio were 286, 210, 140, and 1.04 ppb, respectively. Results indicated that it is necessary not to mix re–hulled pistachio nuts with pistachios, which hulled easily on the first stage, and separating stained and very small nuts to decrease aflatoxin in final pistachio.

Aflatoxins in cheese and milk

The carryover rate of aflatoxins from contaminated feed into milk in dairy cows is considered to average 1–2% (Suzangar et al. 1976, Hadavi 2005). However, in high yielding cows which consume significant amounts of concentrated feeds, the carryover rate of AFM_1 into the milk can reach 6.2%. In Iran, the first indicator of a problem associated with aflatoxins observed around the 1970s in Isfahan province (Suzangar et al. 1976).

Parvaneh et al. (1981) investigated the aflatoxin contamination of Iranian white cheese. In their study, aflatoxin contamination of eighty different Iranian white cheese was determined. The result showed that 97.5% of the samples contained aflatoxins M_1 and M_2. As far as direct contamination, 30% of the samples contained aflatoxins B_1, G_1, and G_2, from which 2.5% contained AFB_1, 5% contained AFB2, 20 % contained AFG_1 and 7.5 % contained AFG_2. In determining the amount of AFM_1 in twenty–one different samples, the maximum amount of 250 $\mu g.kg^{-1}$ was found (Parvaneh et al. 1981).

Karim et al. (1982) studied aflatoxin contamination of milk in Tehran. Aflatoxin contamination of 52 samples of raw milk and nine samples of commercial milk was evaluated. The results showed that 92.31% of the samples of raw milk and all the samples of commercial milk were contaminated by aflatoxins M_1 and M_2. None of the samples were reported to contain aflatoxins B_1, B_2, G_1, and G_2. The maximum amounts of 23 $g.l^{-1}$ and 20.1 $g.l^{-1}$L of AFM_1 were found in raw and commercial milk, respectively.

In another study on the occurrence of AFM_1 in raw milk produced in Sarab city of Iran, the presence of the toxin in 111 samples of raw milk obtained from dairy plants was analyzed (Kamkar 2005, 2006). In 85 of the 111 samples (76.6 %), the presence of AFM_1 was detected in the range of 0.015 to 0.28 $\mu g.l^{-1}$. AFM_1 level in 40 % of positive samples was higher than the maximum tolerance limit (0.05 $\mu g.l^{-1}$) accepted by the European Union. The lowest mean of AFM_1 concentration was found as 0.024 $\mu g.l^{-1}$ on August and the highest level of AFM_1 as 0.118 $\mu g.l^{-1}$ on December. Incidence level of AFM_1 on January, February, April, and December, was higher than the other months. Statistical evaluations showed that mean contamin–ation level of AFM_1 in autumn and winter samples were significantly higher than those of spring and summer ($P<0.01$). In addition, the results showed that there were no statistical differences between AFM_1 contents of spring and summer samples.

Alborzi et al. (2006) studied AFM_1 contamination in pasteurized milk samples in Shiraz city. Six hundred and twenty–four pasteurized milk samples from different supermarkets were collected during 6 months (April to September 2003). AFM_1 was found in 100 % of the examined milk samples. Around 17.8 % of the samples had AFM_1 greater than the maximum tolerance limit (50 $ng.l^{-1}$) accepted by the European Union.

Rahimi et al. (2012) studied 121 dairy product samples consisting of pasteurized milk (60 samples) and white cheese (61 samples), collected from retail markets during August 2010 to September 2011 in Ahvaz, for AFM_1 by enzyme–linked immunosorbent assay (ELISA). Analytical results showed that 61 (50.4%) samples were contaminated with AFM_1 ranging from 11 to 209 $ng.l^{-1}$ consisted of 24 (40%) pasteurized milk samples (range: 11–94 $ng.l^{-1}$ and

mean ± SD: 34.7 ± 19.9 ng.l^{-1}) and 37 (60.7%) white cheese samples (range: 32–209 ng.l^{-1} and mean ± SD: 103.6 ± 54.1 ng.l^{-1}). Considering the US FDA and Iranian national standard limits for AFM$_1$ in milk (500 ng.l^{-1}), none of the samples had levels above the maximum tolerance limit. However, the concentration level of AFM$_1$ in 3 (5%) samples of pasteurized milk was higher than the European Commission limit (50 ng.l^{-1}). No significant differences in the concentration of AFM$_1$ were observed for pasteurized milk samples taken in different seasons in Ahvaz; however, significantly higher concentration of AFM$_1$ (P < 0.05) were found in white cheese samples taken in cold seasons. Although the concentration of AFM$_1$ in none of the examined samples exceeded the Iran regulation, by attention to high consumption and significance of milk and its products in Iranian dietary, survey and control of milk and its products continuously for AFM$_1$ is seriously important.

Panahi et al. (2011) studied AFM$_1$ in raw milk. During the spring, 100 samples of raw cow's milk were selected randomly from Urmia city. The samples were analyzed with a commercial competitive enzyme–linked immunosorbent assay (ELISA) kit. All samples (100%) were found to have levels that exceed the legal limits of 50 ng/.l^{-1} established by the EU/Codex.

Ghiasian et al. (2011) used an ELISA kit for the analysis of AFM$_1$ in breast milk samples from 132 lactating mothers referred to four urban Mothers and Babies Care Unit of Hamadan city. AFM$_1$ was detected in eight samples (6.06%) at mean concentration of 9.45 ng.l^{-1}. The minimum and maximum concentrations were 7.1 and 10.8 ng.l^{-1}, respectively. Although the concentration of AFM$_1$ in none of the samples was higher than the maximum tolerance limit accepted by the US and the European Union (25 ng.l^{-1}), 25 % had higher allowable level of AFM$_1$ Australia and Switzerland (10 ng.l^{-1}).

Riahi–Zanjani & Balali–Mood (2013) studied the presence of AFM$_1$ in pasteurized milk samples in Fariman city in Khorasan Razavi province by ELISA. Forty–five samples of pasteurized milk from different supermarkets were collected during 3 months in summer (July to September 2012). AFM$_1$ contamination was detected in all of the milk samples. The mean concentration of aflatoxin M$_1$ was 27.2 ng.l^{-1} ranged from 8.8 to 64 ng.l^{-1}. Thirteen samples (28.8%) had AFM$_1$ levels exceeding the maximum levels (50 ng.l^{-1}) accepted by the European Union.

Mohajeri et al. (2013) studied the occurrence of AFM$_1$ in 82 cheese samples composed of white cheese (45 samples) and Lighvan cheese (37 samples) obtain–ed from supermarkets and retail outlets in Rafsanjan city by a competitive enzyme immunoassay procedure. AFM$_1$ was detected in 39 (47.6%) samples, consisting of 29 (64.4%) white cheese (mean: 135 ng.kg^{-1}; range: 93.3–309 ng.kg^{-1}) and 10 (27%) Lighvan cheese (mean: 90.8 ng.kg^{-1}; range: 70.5–203 ng.kg^{-1}). According to Iranian national standard limit for AFM$_1$ in cheese (200 ng.kg^{-1}), 9 samples (20%) of white cheese and 1 sample (2.70%) of Lighvan cheese had levels above the limit.

Khodadadi et al. (2014) studied the occurrence of AFM$_1$ in pasteurized and traditional cheese marketed in Southern Khorasan, from December 2011 to January 2012. A total of 102 cheese samples (including 43 non–pasteurized traditional and 59 pasteurized cheese) were analyzed. A rapid and sensitive indirect competitive ELISA was used to measure AFM$_1$ concentration in the samples. AFM$_1$ was detected in 14 (32.55%) traditional and 21 (35.59%) pasteurized cheese samples. In total, 25.42% of pasteurized and 27.90% of traditional cheese samples were contami–nated with AFM$_1$ at a level above of the Iranian standard limits (50 ng.kg^{-1}).

Aflatoxins in grains

Rasti et al. (2000) studied the maize contamination in imported and native maize with AFB$_1$ in central feed silos of Isfahan. A total of 70 maize samples were taken according to the FAO procedure and AFB$_1$ was determined by high – performance liquid chromatography (HPLC). The analytical column was a reverse phase column (C8) with 250 × 4 mm dimension. A mixture of water, acetonitrile, methanol (60:20:20) was used as a mobile phase, at a flow rate of 1 mL.min^{-1}, the UV detector was set at 244 mm and 20 µL aliquots were injected into the HPLC. The results showed that most of the samples were contaminated with AFB$_1$ in the range of 0.0 to 9.9 µg.kg^{-1}; the average was 5.1 µg.kg^{-1}, which was much lower than the tolerance level. The lowest and the highest contamination was for the imported and native maize samples respectively, which was significantly lower than the tolerance level recommended in most of the countries. Statistical analysis showed the significant effect of locations (P<0.05) and moisture (P<0.01), however, no significant effect of temperature was observed.

Yazdanpanah et al. (2001) studied the natural occurrence of aflatoxins in corn and barley from Mazandaran and Golestan in the North of Iran. Fourteen barley and nine corn samples, destined for animal feed were analyzed for aflatoxins using HPLC. In corn samples, AFB$_1$ and AFB$_2$ were detected in 8 (88.8%) and 6 (66.6%) samples at a mean level of 15.83 and 2.99 ppb (median 1.72 and 1 ppb), respectively. None of the corn samples contained detectable amounts of aflatoxins G$_1$ and G$_2$. Only one of the AFs–contaminated samples was co–contaminated with ochratoxin at a concentration of 0.35 ppb.

Saberi–Riseh et al. (2003) studied the importance of *A. flavus* as a contaminant of wheat grains in store–pits of Markazi province, Iran. Several samples were randomly collected from each of five store–pits located in different areas of Markazi Province including Arak, Mahallat, Khomein, Saveh, and Sarband. At least 100 grains per each sample were randomly used for each test and treatment. Different

fungi were isolated from examined samples of which *A. flavus* was noticed due to the ability for producing aflatoxins.

Chehri et al. (2015) studied the natural occurrence of *Aspergillus* species in corn grains in Kermanshah province during 2006–2013 growing seasons. One–hundred samples, mostly from diseased corn grains for human and animal consumptions were collected from different geographic regions of the province. One–hundred *Aspergillus* isolates were collected and identified and classified into six species in which *A. flavus* was the second most prevalent species with 27 % distribution among the isolates. Authors studied AFB_1 contamination by ELISA. AFB_1 was detected in 66 samples ranging from 0.046–10.776 µg.kg^{-1}. The highest AFB_1 levels were detected in samples from Bisetoon and Sarpol Zehab (up to 10.000 µg.kg^{-1}).

Karami–Osboo et al. (2012) studied AFB_1 contamination in 373 samples collected during 2006–2008 at harvest stage, from different agro–climatic regions of the major maize production area of Iran, including Ardabil (Northwest), Khuzestan (Southwest) and Fars (South). AFB_1 determined by CD–ELISA was detected in 146 samples (43.6%) in which only 22.5% were contaminated to higher than MRL level. The amount of aflatoxin in maize samples varied across the years, the percentage of contamination in 2006, 2007 and 2008 were 86.7%, 71.4%, and 100% respectively for Ardebil, 63.4%, 41.3% and 24.5% for Khuzestan, and 17%, 14.8% and 27.6% for Fars province. The mean contamination was 15.63, 57.67 and 154.13 µ.kg^{-1} for Ardabil; 14.41, 35.93 and 1.61 µ.kg^{-1} for Khuzestan; and 0.9, 2.12 and 36.39 µ.kg^{-1} for Fars province.

Tavakoli et al. (2014) studied mycotoxin contamination of imported and local rice samples which were being consumed in military centers of Tehran city. In this study, 80 rice samples (62 imported and 18 local) were randomly collected and their contamination with aflatoxin B_1, B_2 and G_1, ochratoxin A and zearalenone was determined using HPLC. Ochratoxin A and zearalenone were not found in any of the samples. However, 54.8 % samples of imported rice and 22.2 % samples of local rice were contaminated with aflatoxins. The highest and the lowest amounts of aflatoxins were 2.46 and 0.34 ng.g^{-1} in imported rice and 1.09 and 0.79 ng.g^{-1} in the local rice respectively. The most abundant aflatoxin in both imported and local samples was AFB_1. The level of AF contamination of the evaluated samples was lower than maximum tolerated levels established by Iranian National Standards Organization (Table 3).

Nazari et al. (2014) used a liquid chromatogramphy/tandem mass spectrometry (LC–MS/MS) method for the simultaneous detection and quantification of a broad spectrum of aflatoxins and fungal metabolites in domestic rice in Iran. A total of 20 fungal metabolites were detected in 65 rice samples. Aflatoxins were observed in 9.2 % of samples.

Hadiani et al. (2009) studied pre–harvest maize samples from Mazandaran province, north of Iran for AFs contamination. Thirty–five pre–harvest maize samples were analyzed using immunoaffinity column and reversed–phase liquid chromatography with post–column derivatization. Data revealed that incidence of AFB_1, AFB_2 and total aflatoxins (AFT) in maize samples were 66 %, 54 % and 63 % with mean of 9.5, 1.7 and 10.4 ng.g^{-1} respectively. The mean level of AFB_1 in samples was higher than the Iranian maximum tolerated level (MTL). However, AFT mean level (10.4 ng.g^{-1}) was lower than the Iranian and US (20 ng.g^{-1}) MTLs.

Nazari et al. (2015) studied the occurrence of AFs in the rice currently sold at the supermarkets in the city of Ahvaz, Khuzestan. The levels of AFs in 90 collected imported rice samples were measured by HPLC. The results showed that the highest concentration of AFB_1 and total AFs in the rice samples were 2.35 and 2.70 ng.g^{-1} respectively. The different mean concentration of AFB_1 and total AFs in three brands of the rice samples was significantly lower than the maximum tolerable level (MTL) of AFB_1 (5 ng.g^{-1}) and total AFs (30 ng.g^{-1}) considered by the Institute of Standards and Industrial Research of Iran.

Aflatoxins in oilseeds

Asadi et al. (2011) studied the occurrence of aflatoxins in sesame seeds in Khorasan province between September 2009 and August 2010. Samples (n=182) were analyzed by liquid chromatography (LC), and detection limits for AFB_1, AFB_2, AFG_1 and AFG_2 were 0.45, 0.19, 0.61, and 0.22 ng.g^{-1} respectively. AFB_1 was detected in 33 samples (18.1%), at a mean level of 1.62± 1.32 ng.g^{-1}, with a maximum level of 5.54 ng.g^{-1}. AFB_1 levels exceeded the European Union (EU) maximum tolerated level (MTL, 2 ng.g^{-1}) in nine samples, and the Iran MTL (5 ng.g^{-1}) in 1 sample. Regarding total aflatoxins (AFT), the mean level was 0.92 ng.g^{-1}, with a maximum level of 5.54 ng.g^{-1}. No sesame sample had exceeded level by the Iran MTL (15 ng.g^{-1}), however, two samples exceeded the EU MTL (4 ng.g^{-1}) for AFT.

Hosseininia et al. (2014) studied aflatoxins on sesame seed in Khorasan Razavi. They evaluated the occurrence of aflatoxins (AFs) in five lots of imported sesame seeds before their distribution to the market during a one year period. A total of 269 sub–samples were obtained from a total of 9,321 tons of sesame seeds from five importing companies. Aflatoxins at >1 µg.kg^{-1} were found in 50% of all samples, however at low levels in most cases, it was illustrated by mean AFB_1 and total AF levels of 1.25±3.70 and 1.43±4.38 µg.kg^{-1} respectively. A few samples (1.9%) exceeded the National Iranian Standard maximum accepted level for AFB_1 (5 µg.kg^{-1}) or total AFs (15 µg.kg^{-1}); the maximum total AFs level was found in one sample as 48 µg.kg^{-1}.

Beheshti & Asadi (2013) reported aflatoxin content of 173 sunflower and safflower seeds by HPLC. AFB_1 contamination was found in 111 samp–les: eight of the sunflower seed samples (16%) were

contaminated at a mean level of 40.68 ng.g^{-1} and 103 safflower seed samples (83.7 %) at a mean level of 2.81±0.44 ng.kg^{-1}. In five sunflower seed samples and one safflower seed samples, AFB$_1$ levels were higher than the maximum levels under Iran regulations (5 ng.kg^{-1}). AFB$_1$ levels in 5 sunflowers and 2 safflower seed samples were higher than the European Union maximum limit (2 ng.kg^{-1}).

Aflatoxins in animal feed

As a general rule, unhygienic animal feedstuffs can lead to nutrient losses and detrimental effect on animal production and public health. There are several reports on contamination of animal feed in poultry, fisheries and livestock industries in Iran.

Ghaemmaghami et al. (2016) studied eighty–five samples of corn, soybean meal, and poultry finished feed collected from nine poultry feed factories in three provinces i.e. Tehran, Alborz and Qom from October 2014 to January 2015. A total of 384 fungal isolates belonging to 7 genera of filamentous fungi and yeasts were obtained from corn (124 isolates), soybean meal (92 isolates), and feed before (72 isolates), and after pelleting (96 isolates). The most prominent fungal isolate in corn, soybean meal, and feed before pelleting (feed as mash form) was *Fusarium*, however in feed after pelleting only *Aspergillus* was observed. Among five *Aspergillus* species isolated, potentially aflatoxi– genic *A. flavus* isolates were predominant in corn (46.6%), soybean meal (72.7%) and poultry finished feed (75%). CFUs results indicated that 9 out of 22 corn samples (40.9%), none of 22 soybean meal samples and nineteen out of forty–one finished feed (46.3 %) were contaminated higher than the standard limit.

Taifebagerlu et al. (2015) studied a total of 89 samples (poultry feed ingredients and poultry feed) received from various parts of the West Azerbijan province by competitive direct ELISA for detection of total aflatoxins (B$_1$, B$_2$, G$_1$, and G$_2$). The Overal incidence of AF was 42%. The average contamination levels of AFs were 2.83 ppb (µg.kg^{-1}), with minimum and maximum levels of 0.1 and 43.8 ppb, respectively. The FDA's Aflatoxin Regulations Policy can support enforcement action if aflatoxin levels exceed 20 ppb for Feed of chicks (such as broiler chickens) and 100 ppb for mature poultry (such as laying hens).

Alinezhad et al. (2011) studied mycobiota and natural occurrence of aflatoxin B$_1$ (AFB$_1$) in pellet feed and feed ingredients used in a feed manufacturing plant for rainbow trout nutrition. A total of 109 fungal isolates were identified and *Aspergillus* was the prominent genus (57.0%), followed by *Penicillium* (12.84%), *Absidia* (11.01%) and *Pseudallscheria* (10.10%). The most frequent *Aspergillus* species was *A. flavus* (60.66%) isolated from all feed ingredients as well as pellet feed. Among 37 *A. flavus* isolates, nineteen (51.35%) were able to produce AFB$_1$ in the range of 10.2 to 612.8 µg.g^{-1} fungal dry weight. HPLC analysis of trout feed showed that pellet feed and all feed ingredients tested except gluten were contaminated with different levels of AFB$_1$ in the range of 1.83 to 67.35 µg.kg^{-1}. Unacceptable levels of AFB$_1$ were reported for feed including soybean, fish meal and wheat. These results indicated the importance of AF contamination of trout feed in amounts higher than the acceptable level as a risk factor for fish farming production.

DETECTION, LIMITS AND CONTROL MEASURES OF AFLATOXINS

Various techniques for the detection of aflatoxigenic fungi and aflatoxins are summarized in Fig. 5. A dual checking could be considered in order to meet food/feed safety concerns and official legislated protocols under the bio-warfare convention. First, the occurrence of fungi having the potential to produce the bio–threat toxin could be tested at critical points during the production of agricultural commodities as well as during the process of food/feed preparation. Early detection of these fungi could prevent contamination in products and protect consumers from aflatoxins. The current trend is toward culture independent PCR–based techniques because they overcome problems related with selective cultivation and isolation of toxigenic fungi and are generally characterized by their simplicity, speed, cost– effectiveness, and reliability (Venkataramana et al.2015). Subsequently, aflatoxins could be detected directly in the food/feed sample using analytical techniques able to perform highly selective measurements. For this reason, chromatographic techniques have been usually taken as reference techniques because of their accuracy and reproducibility (Venkataramana et al. 2015).

To deal with the increasing number of sample matrices and aflatoxins of interest, fast and accurate analytical methods are needed. This demand has led to the development of rapid screening methods for aflatoxins or whole mycotoxin classes based on immunochemical techniques (ELISA), biosensors (protein chips, antibody–/protein–coated electrodes) and noninvasive optical techniques. On the other hand, highly sophisticated multi–mycotoxin methods based on LC coupled to multiple–stage MS are being developed to allow accurate and precise determination and unambiguous identification of toxins without the need for tedious sample preparation and cleanup procedures. Recently, aptamers have been shown to successfully compete with antibodies as biological receptors for analytical tool development. These single–stranded oligonu- cleotides are selected in vitro in a short time compared to the antibody production and are more stable under a wide range of conditions. Furthermore, they can be easily modified or labeled providing flexibility to progress a wide range of assessment assays (Venkataramana et al. 2015). Table 2 shows

the permitted levels of aflatoxins in food and animal feeds with the US Food and Drug Administration. In most countries, permitted level of aflatoxins in human food is 5 to 20 ppb. According to the US Food and Drug Administration (FDA), permitted level of AFB_1 in many foods is 20 ppb and permitted level of AFM_1 in milk is 0.5 ppb.

In most European countries, permitted level of AFB_1 in human food is 3 to 5 ppb. Europe Union follows a limit of AFM_1 as 0.05 ppb in milk and 0.025 ppb in infant formula. In animals, LD_{50} (lethal dose 50%) of AFB_1 depending on the type of animal has been reported from 0.3 to 10 mg.kg^{-1} body weight. LD_{50} of AFB_1 in rats and trout is considered as 1.2 mg.kg^{-1} and 0.5 mg.kg^{-1}, while in mice and guinea pigs it is around 9 mg.kg^{-1} and 10.2 mg/.kg^{-1}, respectively (Chehri et al. 2015). Table 3 summarizes the maximum tolerated levels of aflatoxin in human foodstuffs, dairy products and animal feedstuffs in Iran. Post and pre–harvest methods have been used to control aflatoxin contamination of agricultural commodities. Sodium bisulfite, alkaline substances (ammonia, soda) and oxidants are effective in inactivating the aflatoxins. Oxidizing agents such as ozone and hydrogen peroxide can reduce the aflatoxin contamination in some foods. In some countries such as the US and France, ammonia gas under high pressure and temperature is used for the degradation of aflatoxins in livestock feed. Food especially cereals and dried fruits should be stored in the cool and dry environment. Grain storage in less than 5 °C and 20% atmospheric oxygen, 60% carbon dioxide and 20% nitrogen reduce the risk of aflatoxin production. The use of antifungal agents such as sorbic acid, potassium sorbate, and propionic acid also prevents fungal growth. Studies have shown that a diet rich in protein increases resistance against aflatoxin poisoning in some animals (Basappa & Murthy 1974, Park & Liang 1993). Adding antioxidants such as Butylated Hydroxy Toluene (BHT) to feed poultry, protects them against the aflatoxin effects with an unknown mechanism. It is assumed that BHT inhibits CYP activity or prevents the conversion of aflatoxin B_1 into its active form (Mahmoudi et al. 2013).
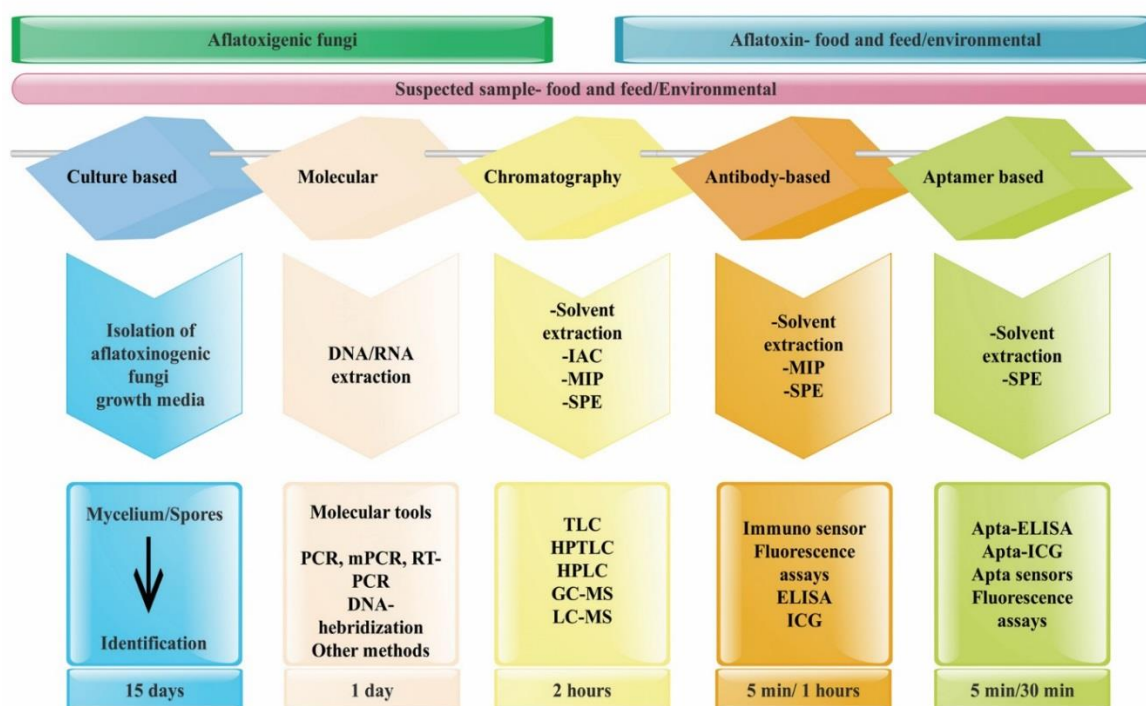

Fig. 5. Traditional and modern techniques for detection of aflatoxins and aflatoxigenic fungi in biologic and environmental samples.

Table 2. US Food and Drug Administration limits of total aflatoxins in food and feed.

Food or animal feed	Total aflatoxins (ng/g)
All products except milk	20
Milk	0.5
Immature corn for animal feed and dairy cattle	20
Corn for cattle, pigs, and poultry in the early stages of growth	100
Corn for feeding pigs in the final phase of growth	200
Corn for cattle and calves in the final phase of growth	300
Cottonseed Meal	300
Other foods except corn	20

Table 3. Maximum tolerated levels of aflatoxin in human foodstuffs, dairy products and animal feedstuffs in Iran.

Substrate	Aflatoxin type	Limit (μg/Kg)
Pistachio nuts, peanuts, walnuts, other nuts and edible seeds	AFB_1	5
	AFB_1, B_2, G_1, G_2	15
Dates, dried grapes (raisins and sultanas), figs and all dried fruit	AFB_1	5
	AFB_1, B_2, G_1, G_2	15
Baby food based on cereals with milk	AFB_1	1
	AFM_1	1
Barley	AFB_1	10
	AFB_1, B_2, G_1, G_2	50
Maize, rice	AFB_1	5
	AFB_1, B_2, G_1, G_2	30
Wheat	AFB_1	5
	AFB_1, B_2, G_1, G_2	15
Legumes	AFB_1	5
	AFB_1, B_2, G_1, G_2	10
Milk[raw, pasteurized, sterilized]	AFM_1	0.05
Milk powder	AFM_1	0.5
Cheese	AFM_1	0.01
Butter	AFM_1	0.2
Other dairy products	AFM_1	0.02
Feed: cottonseed meal fishmeal, meat meal, bone meal, blood meal, single cell	AFB_1	10
protein rice and wheat bran: intended for sheep, goats and beef cattle	AFB_1, B_2, G_1, G_2	50
Intended for poultry, calf, lamb, kid, dairy sheep, goats and cattle	AFB_1	5
	AFB_1, B_2, G_1, G_2	20
Soya bean meal, sunflower meal, sesame seed meal, olive meal and other meals from	AFB_1	10
oil-producing seeds: intended for sheep, goats and beef cattle	AFB_1, B_2, G_1, G_2	20
intended for poultry, calf, lamb, kid, dairy, sheep, dairy sheep, dairy goats and dairy	AFB_1	5
cattle	AFB_1, B_2, G_1, G_2	20
Maize: intended for sheep, goats and beef cattle, poultry, calf, lamb, kid, dairy sheep,	AFB_1	5
dairy goats and dairy cattle premixes including vitamins and mineral premixes: intended for sheep goats and beef cattle intended for calf, lamb, kid, dairy sheep, goats and cattle intended for poultry complete feed: intended for sheep, goats and beef cattle	AFB_1, B_2, G_1, G_2	10
Intended for broilers and pullet intended for parent and grandparent stocks	AFB_1	5
	AFB_1, B_2, G_1, G_2	20

FUTURE PERSPECTIVES IN AFLATOXIN RESEARCH

It is a very essential duty to observer to control the existence of aflatoxin in the human foodstuff resources as it involves various aspects. The regulation of rule for example by situation a convinced limit can be considered as the "first protection" to avoid aflatoxin exposure to animal and human. This law was resulting in the huge epidemic of diseases related with aflatoxin back in the 1960s as talk about later. However, a practical, valuable, safe and generally–accepted method is not available for detoxification.

Institute of Standards and Industrial Research of Iran is responsible for codification of the law to limit the amount of aflatoxins in products. The previous attempt in control of aflatoxins relied on the removing toxin from contaminated food and feed and the prevention of their post–harvest contamination. Studies in recent years have shown that pre–harvest practices such as biocontrol with non–toxigenic strains of *A. flavus* are more effective (Cotty 1994, Mauro et al. 2018). Due to the importance of aflatoxins in agricultural economics and human health, it is quite essential to control their natural occurrence in susceptible substrates such as peanuts. Likewise, to minimize barriers to international trade, following rules of standards at the international level is an urgent need. All operations related to food storage in the warehouse and the optimal condition such as temperature and humidity control as well as the way picking up and putting food in stock to prevent the growth of aflatoxigenic fungi in food must be in control and be respected.

CONCLUDING REMARKS

Despite sporadic reports about natural occurrence of aflatoxin producing fungi and contamination of food and feed with them and various aflatoxins, a comprehensive information regard to the past and current situation of aflatoxin risk for the public health is in demand in Iran. There are very limited reports on the aflatoxin exposure of humans because of the absence of integrated studies on assessment of the toxin in clinical samples (Habibi et al. 2018). To our knowledge, aflatoxin contamination of food and feed in Iran is a problem mostly occur during storage and the major substrates involve are nuts (peanuts, pistachio, etc.) and cereals (maize and rice). Regard to aflatoxin metabolites, the major problem is

contamination of milk and dairy products by AFM_1 which should be considered as a potential public health hazard due to the high resistance of the toxin to the most physic–chemical methods of inactivation. The future of aflatoxin issue in Iran awaits the careful assessment of country's mycotoxin condition through proper scientific research and methods with the subsequent formulation of action plans for prioritization and application of defined organiz-ational and technical strategies.

Taken together, emphasis should be placed on devising accurate, sensitive, specific and rapid procedures for detection and determination of aflatoxin contamination in various foods and feeds and effective strategies for prevention of contamination and possible safe decontamination procedures. National and global networking is important to collect information for organizing suitable strategies to avoid continuing the problem of aflatoxin exposure in the future.

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آفلاتوکسین ها و قارچ های مولد آفلاتوکسین در ایران: مرور سیستماتیک وضعیت گذشته، حال و آینده

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چکیده: آفلاتوکسین ها ترکیبات بسیار سمی با ساختار متابولیت ثانویه هستند که بوسیله برخی از گونه های آسپرژیلوس و امریسلا تولید می شوند. علیرغم پیشرفت های قابل ملاحظه در زمینه تحقیقات آفلاتوکسین در ۵۰ سال گذشته، معضل آلودگی اغذیه و محصولات کشاورزی با آفلاتوکسین به عنوان یک مشکل بهداشتی در سطح جهان همچنان به قوت خود باقی است. آفلاتوکسین ها به عنوان ترکیبات جهش زا و سرطان زا از عوامل مهم ایجاد هپاتوسلولار کارسینوما در انسان و حیوانات به حساب می آیند. گروه متنوعی از بسترهای غذایی شامل مغزها، دانه های روغنی، غلات و میوه های خشک مستعد آلودگی آفلاتوکسینی هستند که یک چالش مهم بهداشت عمومی بویژه در کشورهای در حال توسعه محسوب می شود. ذخیره سازی نامناسب اغذیه انسانی و دامی شرایط را برای تولید آفلاتوکسین در محصولات کشاورزی شامل برنج، گندم، پسته، گردو و بادام به عنوان منابع اصلی تغذیه ای در زنجیره غذایی انسان مهیا می کنند. همچنین، آفلاتوکسین ها میتوانند بدنبال مصرف اغذیه آلوده در بافت های حیوانی و شیر حیوانات تجمع یابند. در میان مغزها، پسته یکی از مهمترین محصولات صادراتی ایران است که موارد آلودگی آن به آفلاتوکسین مکررا گزارش گردیده است. در مجموع، علی رغم گزارشات متعدد از حضور قارچ های مولد آفلاتوکسین و آلودگی اغذیه انسانی و دامی به این سم مهلک قارچی در ایران، اطلاعات محدودی در خصوص مواجهه انسان و حیوانات با آفلاتوکسین ها و تبعات این مواجهه در دسترس می باشد. مطالعه مروری حاضر اطلاعات جامع و کاملی در خصوص شرایط موجود و دورنمای آینده حضور آفلاتوکسین ها در اغذیه انسان و دام و محصولات کشاورزی در ایران، اثرات زیان بار آفلاتوکسین ها بر بهداشت عمومی، و روش های عملی اندازه گیری و کنترل آلودگی با آفلاتوکسین ها را در دسترس قرار می دهد.

کلمات کلیدی: آفلاتوکسین ها، آسپرژیلوس فلاووس، آلودگی مواد غذایی، هپاتوسلولار کارسینوما