Iranian J Publ Health, Vol. 40, No.4, 2011, pp.1-30

Review Article

Aflatoxins in Iran: Nature, Hazards and Carcinogenicity

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(Received 22 Jun 2011; accepted 17 Oct 2011

Abstract

Many studies have shown that mycotoxin contamination of agricultural products is a challenge for individual's health especially in developing countries. Improper production and storage of foods, prepare conditions for aflatoxin production in crops, especially rice, wheat, pistachio, walnut, almond, etc which are the main sources of foods for people. Feeding livestock by contaminated bread is another way of human exposure to mycotoxins, especially aflatoxin and because of expensive methods for detecting and analyzing aflatoxin in laboratory; it is not measured in foods. This manuscript is a review of some Iranian and nonIranian reports about aflatoxin, its exposure ways, its adverse effect on human health and nutrition, as well as methods for reducing its exposure. Based on studies on foods, aflatoxin exposure is high in Iran. Since livestock feeding by contaminated bread is one of the potential ways for milk contamination, we should control and reduce aflatoxin contamination by improving production process, storage condition and livestock feeding as soon as possible. Pistachio is one of the most important exporting products of Iran and to maintain Iran's position in exporting of this product, specific regulations on lowering its contamination with aflatoxin should be considered seriously. Finally, effective controlling of all food and feedstuffs which are vulnerable to aflatoxin contamination is necessary to prevent its effects.

Keywords: Aflatoxicosis, Mycotoxins, Teratogenicity, Oxidative stress, Cytochrome P450, Congenital malformations, Iran

Introduction

The aflatoxins are a group of mycotoxins produced by certain *Aspergillus* species and can contaminate foodstuff especially in developing countries. These toxins are produced by fungi during production, storage and food processing. According to FDA although it is an unavoidable foodstuff contamination but it could be minimized by supervising systems (1).

Small factories with limited production and lack of food safety measures make contamination control, impossible economically and experimentally. In developed countries food production is in control and thanks to good economic situation food quality processing is effective and law is obeyed.

Knowing which and how many foods are contaminated is not enough because in many areas the people do not have other chances for food choice. In developing countries the maximum level of cutoff points for toxins are not useful because consumption traded food is very low and food laboratory control is expensive. In these countries the consumptive food is usually food that is produced, prepared and stored by family without considering aflatoxin danger. In some food production areas the low-contaminated foods are usually exported and high-contaminated foods are remained to consume by people who have been in exposure to toxin. Comprehensive biological information about amount and incidence of human exposure to aflatoxin are not available for evaluation, and direct measurement of human exposure exist just in a few countries (1).

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Toxicology and pathogenesis

Aflatoxins belong to a group of mycotoxins and they are secondary metabolites which are produced commonly by certain *Aspergillus* species such as *Aspergillus flavus, A. parasiticus,* and rarely *A. nomius* and contaminate plants and their products (2).

Aflatoxins have high toxic, mutagenic, teratogenic and carcinogenic effects and cause liver and other organs cancer (3).

It is necessary to say that several materials have registered up to now that have one of mutagenic, carcinogenic or teratogenic effects but just a few of them have all of these effects and aflatoxin is one of them. Aflatoxins not only contaminate our foods but also enter milk, eggs and livestock products through their feed (4).

Four types of aflatoxins are B1, B2, G1, G2. B and G refer to fluorescence characteristics of aflatoxin under UV light (Blue and Green) and 1 and 2 refer to their position on layer chromatography.

Aflatoxin B1 and G1 are more toxic than B2 and G2 but aflatoxin G1 is more toxic than B2. Liver and kidney are the main toxic targets of aflatoxins and their carcinogenic effects (5).

Mutagenic and DNA damage

Aflatoxin B1 in first phase of metabolism is converted to different metabolites like aflatoxin B1-epoxide and hydroxylated metabolites like as aflatoxin M1 by cytochrome P450 enzymatic system. Epoxide form of aflatoxin B1 is very active and can attach to DNA, RNA and proteins. This attachment is related strongly to carcinogenic effects of aflatoxin in animal and human (5).

In most cases aflatoxin connect to DNA in *N7*guanine position in hepatic cells. Aflatoxin M1, hydroxylated form of aflatoxin and some other metabolites make water soluble esters and excreted in urine (5).

The degree of species vulnerability mostly depends on amount of toxin enters to any chemical pathway. The harmful effect is seen when epoxide forms is produced and react with DNA. On the other hand there is some evidence that show the amount aflatoxin which enters to each pathway depends on ingested aflatoxin amounts, which may be determined by competitive chemical processes (6).

First in the body aflatoxin is changed through enzymatic reactions in the liver by microsomal oxidase. This enzyme mostly exists in the liver but can be found in other organs like lung, kidney, etc. About 80% of ingested aflatoxin dose is excreted from the body in a week. Among detoxified products just 8-9-exoepoxide has mutagenic effects. This form of aflatoxin epoxide mostly is considered to active electrophilic form of aflatoxin which maybe attack to nitrogen, oxygen and nutrophilic sulphur that exist in different cellular components. These very active substances may be connected to DNA bases like guanine and cause them changing. Aflatoxin epoxide may be the most important carcinogenic substance. Formation of these materials and their attachment to DNA leads to cell function impairment and finally loss of cell control and division. However both human and animals have enzymatic systems which reduce effect of aflatoxin epoxide on DNA. For example glutathione-S-transferase is an enzyme that neutralize aflatoxin epoxide toxicity and its activity is lower in human than rats and mice so it is suggested that human has less ability to neutralize this toxin (7).

The aflatoxin levels in different people depend on ingested amount, exposure time and the body's physiologic situation. The unmetabolized forms of aflatoxin like B1, B2, G1, G2 and metabolized forms like M1, M2 and aflatoxicol are excreted in urine, stool and milk (8).

Some studies have reported the presence of these 7 kinds of aflatoxin in saliva. This secreted aflatoxin in saliva is absorbed in gastrointestinal tract again and it is a sort of aflatoxin recycling in the body (7). The first effect of aflatoxin in the cell is reduction of protein biosynthesis through forming adduct with DNA, protein and RNA to inhibit RNA synthesis and DNA dependent RNA polymerase activation and reticulum endoplasmic degranulation (7). In carcinogenicity process in the liver, aflatoxin B1 attack preferentially to mitochondrial DNA than nuclear DNA. It is possible that an increase in 8-9-aflatoxin epoxide cause a dramatic increase in liver lipids peroxidation levels. Cell membrane peroxidation initiates destroying cell membrane integrity, loss cell membrane bound enzymes activity and finally cell lysis. When produced active species of oxygen (O2•, H2O2, OH•) levels exceed cell neutralizing ability the oxidative damage occurs. So non-enzymatic antioxidant levels (for example vit C, vit E and glutathione) and enzymatic oxidants (superoxide dismutase, glutathione peroxidase and catalase) reduction are main determinant of cell defense. Antioxidant enzymes activity reduction may be due to protein biosenthesis reduction. In a study after 45 days aflatoxin administration, gluthatione levels reduced considerably in the liver, kidney and testis that may be due to rapid gluthatione oxidation. Gluthatione can inhibit peroxidation, scavenge free radicals and protect cell membrane so considerable reduction in gluthatione levels can aggravate aflatoxin toxic effects. Studies show that free radicals produced in biologic membranes react to alpha- tocopheryl radicals rapidly (7). Cytosolic gluthatione and ascorbic acid help alpha tocopheryl regeneration. Some studies show that oncogenes are critical targets of aflatoxin. Several mutations in p53 tumours suppressor genes in hepatocellular carcinoma in patients from high contaminated areas with aflatoxin and areas with high prevalence liver cancer have been reported (7).

The fungi that produce aflatoxin can grow especially in proper situation like 15% humidity, minimum temperature 25°C, enough air wheat, barley, rice, soya, corn, peanut, fish powder, cotton-meal, chestnut, apricot, peach, almond, spices, livestock feed, walnut, millet, sesame, pistachio, pumpkin seed, juices, mouldy jam, wheat flour, noodle, mouldy bread, potato, pea, all kinds of bean, sorghum, peanut meal, coconut, cotton seed, all kinds of hazelnut, liver, meat, milk and milk products, egg, raisin, fermented sausages, processed meats, etc and contaminate these foods by aflatoxin and finally can damage liver, kidney, pharynx, sub skin tissues, glands and stomach after feeding (9).

Although the main mechanism of aflatoxin effect is addition of its oxidate products to DNA but some evidence show that oxidative damage has a role in its toxicity and carcinogenicity too (10).

Teratogenicity

Devries observed that in 54% of 125 Kenyan pregnant women with aflatoxin in their blood, the birth weights of their children decreased up to 255g. He also showed the presence of aflatoxin in 37% of umbilical cord blood species. Although he did not observe any increase in abnormalities but two intra uterus deaths occurred in persons whose blood had aflatoxin. New formed fetuses had 30% abnormalities but the fetuses that remained live showed 6% abnormality. Neural tube defect, microcephaly, umbilical hernia, cleft palate have been reported (11).

Neural tube is normally closed in fourth week of gestation. Defect in closing and reopening of neural tube leads to NTD. It seems that these anomalies are due to environmental and genetic factors combination (12).

Administration of 20ppm aflatoxin in second half of gestation in rats caused hepatic tumoral hyperplasia in 4 cases and collangiocarcinoma in 1 case of offsprings (13).

In a study in Nigeria, 327 neonatal serums with jaundice and 80 serums of their mothers, 60 normal neonatal serum and 7 of their mothers' serum were tested to investigate unknown causes of neonatal jaundice. Blood group, se-

rum bilirubin levels, red blood cell G6PD levels, aflatoxin and naphtol levels were evaluated. 30. 9% of children with jaundice had G6PD deficiency but just only 13. 3% of control group had this deficiency. Aflatoxin was observed in 27. 4% of children with jaundice, 17% of their mothers and 16. 6% of children in control group and 14. 4% of their mothers. Data analysis showed that G6PD deficiency or presence of aflatoxin in serum is risk factors for neonatal jaundice (14).
According to WHO reports the prevalence of G6PD deficiency in Iran is 10%-14. 9%. The most common form of G6PD deficiency in Iran is Mediterranean form (15).

Aflatoxicosis

According to amount and exposure duration by aflatoxin a range of complication can be observed (1):

Acute aflatoxicosis

Acute aflatoxicosis leads to death in 25% of cases when large doses exposure occurs. Death and serious illness due to aflatoxin usually are reported in developing countries. The number of acute poisoning is not high because people usually do not use moldy foods and human beings usually are resistant to toxin. However in food scarcity or because of poverty people are forced to consume contaminated foods.
Severe aflatoxicosis symptoms are hemorrhagic necrosis of liver, bile duct proliferation, edema and lethargy. In human, adults are more tolerant to aflatoxin than children and in acute exposure the children usually die (1).

Chronic aflatoxicosis

Chronic exposure to aflatoxin has considerable effects on animals’ nutrition status.
Soon after aflatoxin exposure covalent binding occurs between aflatoxin and DNA and protein synthesis decreases and this status remains for almost 5 days. The efficiency of food use is less considerably in aflatoxin exposed animals than those are not exposed. It is clearly shown that presence of aflatoxin in animals diet causes reduction in growing rate and other productivity factors. Recent studies on human have confirmed that these effects also occurred in human. As a logical consequence of aflatoxin effects on protein synthesis, aflatoxin exposure delays people recovery from protein malnutrition. Aflatoxin affects on vit A status in poultry and camels. If aflatoxin exposure affects on vit A status, prevention of aflatoxin exposure could be a way for reduction in vit A deficiency occurence. In chicken vitD status is affected by aflatoxin exposure too. Like vitA, vitD is involved in immune system efficiency. Selenium and zinc statues are affected by dietary aflatoxin and these minerals are essential for normal immune system function (1).

Cumulative aflatoxicosis

Hepatocellular carcinoma is the fifth common cancer around the world and one of the most important worldwide health problems. In 1990 new cases of hepatocellular carcinoma in the world was estimated more than 300000 and 120000 for men and women respectively (16).
Hepatocellular carcinoma incidence varies based on different geographical areas. According to data, the hepatocellular carcinoma incidence is increasing around the world. The difference in incidence may be related to different exposure to carcinogenic risk factors of this cancer like hepatitis B virus and aflatoxin in developing countries, smoking and alcohol in developed countries (17).
These factors as well as male androgen hormones trophic effects make men more susceptible than women to this cancer. The most cases of hepatocellurar carcinoma occur in old people with liver chronic infections. However, in regions with high hepatitis B virus carriers and high aflatoxin exposure like south African sahara, the initiation age of this disease goes down to 33 years. When there are several risk

factors in the same period, like hepatitis C plus alcohol or hepatitis B plus aflatoxin exposure the incidence rate will increase. The hepatocellular carcinoma incidence in developing countries is 16-32 times more than developed countries.

From 500000-600000 worldwide hepatocellular carcinoma new cases in a year, about 25200 to 155000 cases may be related to aflatoxin (18).

Aflatoxin is one of the most important environment toxins that have a role in hepatocellular carcinoma especially in regions with high food contamination like contaminated pistachio, peanut, Brazil nut, spices, corn and fig. Aflatoxins have several chemical forms like B1, B2, G1, G2 and the most toxic of them is B1 and WHO has classified aflatoxin B1 in carcinogenic first class substances. As mentioned earlier aflatoxin B1 is metabolized in liver by cytochrome p450 system and is converted to very carcinogenic substance AFB1-8, 9 epoxide and finally this substance binds to DNA. The binding to DNA in 7 position of guanine leads to preferal mutation C:G>A:T in 249 codon of p53 tumors suppressor. The mutation in p53 tumor suppressor gene in patients with hepatocellular carcinoma who have been in aflatoxin exposure has been proved. Aflatoxin exposure through foodstuffs have important effect on hepatocellular carcinoma especially in hepatitis B infected people which leads to 50 times increase in hepatocellular carcinoma incidence risk in these people. There are several mechanisms for it. First, hepatitis B virus infection make liver cells susceptible to carcinogenic effects of aflatoxin, second presence of both risk factors together leads to activation of phase II detoxification of enzyme and finally cause cancer (19).

Upper limit allowance of aflatoxin in human and animals foodstuffs according to European food standard of aflatoxin (CODEX), world maximum tolerated levels of mycotoxins, according to different countries regulations based on FAO data, national standard of Iran (Industrial Research and Standard Organization) are gathered in Tables 1-3.

Table 1: Upper limit allowance for aflatoxin in human food and animal feed according to European standard 2010 (20)

Foods item	AflatoxinB1 ng/g new standard	Total aflatoxins ng/g new standard	AflatoxinB1 ng/g up to March2010	Total Aflatoxin ng/g up to March2010
Almond, pistachio, apricot seed ready to eat	8	10	2	4
For more process	12	15	5	10
Hazelnut, brazil nut ready to eat	5	10	2	4
For more process	8	15	5	10
Other tree nuts ready to eat	2	4	2	4
For more process	5	10	5	10

Table 1: Continued…

Peanut ready to eat	2	4	2	4
For more process	8	15	8	15
Other oily seeds ready to eat	2	4	-	-
For more process	8	15	-	-
Corn ready to eat	2	4	2	4
For more process	5	10	5	10
Rice ready to eat	2	4	2	4
For more process	5	10	-	-
Other cereals ready to eat	2	4	2	4

Table 2: World maximum tolerated levels of mycotoxins in human foodstuffs, dairy products and animal feedstuffs in 2002/2003 survey, according to different countries regulations (21)

Country	Food Item	Mycotoxin type	Limit (µg/kg)
Algeria	Peanut, nut, cereals	afla B1	10
		afla B1B2G1G2	20
	Cattle feed	afla B1	20
Antigua and Barbuda	-	-	-
Argentina	see MERCOSUR member state (harmonized regulations)		
Armenia	all foods	afla B1	5
	milk	afla M1	0. 5
Australia (all regulations harmonized with New Zealand)	Peanuts, tree nuts	afla B1B2G1G2	15
Australia[Eu member state])	Other products[outside EU regulations]	afla B1	1
		afla B1B2G1G2	5
Bahamas	-	-	-
Bahrain	-	-	-
Bangladesh	feed (maize, rice polish	afla B1B2G1G2	100µg/kg

Table 2: Continued…

	and mixed feed for poultry)		is used in practice
Barbados	all foods milk all feedstuffs	afla B1B2G1G2 afla M1 afla B1B2G1G2	20 0. 5 50
Belarus	grain, leguminous plants infant food butter, milk protein concentrate infant food	afla B1 afla B1 afla M1 afla M1	5 not allowed 0. 5 not allowed
Belgium (Eu member State)	see EU		
Belize	maize, groundnut	afla B1B2G1G2	20
Benin Bolivia	- -	- -	- -
Bosnia & Herzegovina (FAO 1997)	wheat, maize, rice, cereals beans	afla B1G1 afla B1G1	1 5
Brazil additional regulations of Brazil:	MERCOSUR member state all foodstuffs feed (animal feed and ingredients:hay cotton, peanut, rice, oats, residues of bird bowels, babassu, cocoa, sugar cane (residue-pulp), linhaça, dendě, manioc, sunflower, crisălidas, malt, wheat, soya, yeast (sugar cane subproducts)	 afla B1G1 afla B1B2G1G2	 30 50
Bulgaria	see EU		
Burkina Faso	-	-	-
Cameroon	-	-	-
Canada	nuts and nut products all feeds	afla B1B2G1G2	15

Table 2: Continued…

		aflaB1B2G1G2	20
Chile	all foods	afla B1B2G1G2	5
	milk	afla M1	0. 05
	complete feedingstuffs for poultry,goats and cattle	afla B1B2G1G2	30
	complete feedingstuffs for other animals		10
	all ingredientsfor use in animal feed except peanuts and derivatives, cottonseed and derivatives, maize and deriva-tives		50
	peanuts and derivatives , cottonseed and derivatives, maize and derivatives	afla B1B2G1G2	200
China	maize and maize products, pea-nut and peanut products, peanut oil, irradiated pea nut	afla B1	20
	rice, irradiated rice, edible vegetable oil		10
	soya bean sauce, grain paste, vinegar, other grains, beans, fermented foods, fermented bean products, starch products, fermented wine, red rice, butter cake, pastry biscuit and bread, food additive gluco-amylase preparation, salad oil		5
	infant formula-soybean based, infant formula ´5410´, formu-lated weaning supplementary foods (rice, soybean, wheat flour, milk powder)	afla M1	non-de-tectable
	milk and milk products		0. 5
	food for infants and young children, infant formula milk powder		non-de-tectable

Table 2: Continued…

CODEX ALIMEN-TARIUS created in 1963 by FAO and WHO in 2003 the codex alimen-tarius commission had 168 member countries	peanut, raw	afla B1B2G1G2	15
	milk	afla M1	0. 5
Colombia	all foods	afla B1B2G1G2	10
		aflaM1	10
		afla M2	10
	maize	afla B1B2G1G2	20
	feed:		
	maize and maize products	afla B1B2G1G2	20
	sorghum		40
	rabbit/trout feeds		10
	poultry/dog/cat/fish feeds		20
	bovine/pig feeds		50
Costa Rica (FAO1997)	maize	afla B1B2G1G2	35
	feed (maize)	afla B1B2G1G2	50
Cote d´Ivory (FAO1997)	straight feedstuffs	afla B1B2G1G2	100
	complete feedstuffs		10
	complete feedstuffs for pigs/poultry except young animals/ducks		38
	complete feedstuffs for cat-tle/sheep/goats		75
	complete foodstuffs for dairy cattle		50
Croatia	cereals, beans, peanuts, coffee tea	afla B1	5
	spices		30
	cocoa, beans, almonds, flours, hazelnuts, walnuts	afla B1B2G1G2	3
	milk, milk products	afla M1	0. 5
Cuba	Cereals, peanuts, cocoa mass	afla B1	5
	all foods	afla B1B2G1G2	5
	all feeds and feed ingredients	afla B1B2G1G2	5
Cyprus	see EU		
Czech Republic	see EU		

Table 2: Continued…

Denmark	see EU		
Dominican Republic	maize (products), groundnut, soya, tomato (products)	afla B1G1	0
	imported maize	afla B1B2G1G2	20
Ecuador	-	-	-
Egypt	peanut and cereals	afla B1 afla B1B2G1G2	5 10
	corn	afla B1 afla B1B2G1G2	10 20
	animal and chicken feed	afla B1 afla B1B2G1G2	10 20
Estonia	see EU		
Ethiopia	-	-	
European Union: (EU)	EU member states:Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Luxemburg, The Netherlands, Portugal, Spain, Sweden, United Kingdom		
	groundnuts, nuts and dried fruit and processed products thereof, intended for direct human consumption or as an ingredient in foodstuffs	afla B1 afla B1B2G1G2	2 4
	groundnuts to be subjected to sorting or other physical treatment, before human consumption or use as an ingredient in foodstuffs	afla B1 afla B1B2G1G2	8 15
	nuts and dried fruit to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuff	afla B1 afla B1B2G1G2	5 10
	cereal (including buckwheat t, *Fagupyrum sp.)*an	afla B1 afla B1B2G1G2	2 4

Table 2: Continued…

	processed products thereof in tended for direct human consumptionor use as an ingredient in foodstuffs		
	cereal (including buckwheat, *Fagupyrum sp.)*, with the exception of maize, to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs	afla B1 afla B1B2G1G2	2 4
	maize to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs	afla B1 afla B1B2G1G2	5 10
	spices:*Capsicum spp. (*dried fruits thereof, whole or ground, including chillies, chilli powder, cayenne and paprika);*Piper spp*. (fruits thereof, including white and black pepper);*Myristica fragrans* (nutmeg); *Zingiber officinale* (ginger);*Curcuma longa*	afla B1 afla B1B2G1G2	5 10
	milk (raw milk, milk for the manufacture of milk-based products and heat –treated milk as defined by Council Directive 92/46/EEC, as last amended by Council Directive 94/71/EC)	aflaM1	0. 05
	all feed materials	aflaB1	20
	complete feedingstuffs for cattle, sheep and goats with the exception of:		
	-complete feedingstuffs for dairy animals		20

Table 2: Continued…

	-complete feedingstuffs for calves and lambs		
	complete feedingstuffs for dairy animals		5
	complete feedingstuffs for calves and lambs		10
	complete feedingstuffs for pigs and poultry (except young animals)		20
	other complete feedingstuffs		10
	complementary feedingstuffs for cattle, sheep and goats (except complementary feed-ingstuffs for dairy animals, calves and lambs		20
	complementary feedingstuffs for pigs and poultry (except young animals)		20
	other complementary feed-ingstuffs		5
Finland	see EU		
France	see EU		
Germany	see EU		
Ghana	-	-	-
Greece	see EU		
Guatemala [FAO1997]	maize, kidney beans, rice, sor-ghum, groundnuts, ground butter	afla B1B2G1G2	20
	feed (concentrate)	afla B1B2G1G2	20
Honduras [FAO1997]	all foods	afla B2G1G2	1
	maize (ground or whole grain)	afla B1	1
	baby food	afla B1B2G1G2	0. 01
	milk (products)	afla M1	0. 02
	cheese	aflaM1	0. 05
			0. 25
Hong Kong	foods	afla B1	15
		afla B1B2G1G2	15
		afla M1	15
	peanuts, peanut products	afla B1	20
		afla B1B2G1G2	20

Table 2: Continued…

		afla M1	20
Hungary	see EU		
Iceland	see EU		
India	all food products	afla B1B2G1G2	30
		aflaM1	30
	feed:peanut meal (export)	aflaB1	120
Indonesia	peanuts, coconuts, spices, traditional drugs/medicine/ herbs	aflaB1B2G1G2	20
	milk, cheese	aflaM1	5
Iran, Islamic republic of	pistachio nuts, peanuts, walnuts, other nuts and edible seeds	afla B1	5
		afla B1B2G1G2	15
	dates, dried grapes (raisins and sultanas), figs and all dried fruits	afla B1	5
		afla B1B2G1G2	15
	babhy food based on cereals	afla B1	1
	with milk	afla M1	1
	barley	afla B1	10
		afla B1B2G1G2	50
	maize, rice	afla B1	5
		afla B1B2G1G2	30
	wheat	afla B1	5
		afla B1B2G1G2	15
	legumes	afla B1	5
		afla B1B2G1G2	10
	milk[raw, pasteurized, sterilized]	aflaM1	0. 05
	milk powder		0. 5
	milk powder for babies (after reconstitution)		0. 01
	cheese		0. 2
	butter, gee		0. 02
	other dairy products		0. 05
	feed:cotton seed meal	afla B1	15
	fishmeal, meat meal, bone meal, blood meal, single cell protein, rice and wheat bran:	afla B1B2G1G2	50
	intended for sheep, goats and beef cattle	aflaB1	10

Table 2: Continued…

	intended for poultry, calf, lamb, kid, dairy sheep, goats and cattle	afla B1 afla B1B2G1G2	5 20
	soya bean meal, sunflower meal, sesame seed meal, olive meal and other meals from oil producing seeds: intended for sheep, goats and beef cattle	afla B1 afla B1B2G1G2	10 20
	intended for poultry, calf, lamb, kid, dairy, sheep, dairy sheep, dairy goats and dairy cattle	afla B1 afla B1B2G1G2	5 20
	maize: intended for sheep, goats and beef cattle, poultry, calf, lamb, kid, dairy sheep, dairy goats and dairy cattle	afla B1 afla B1B2G1G2	5 20
	premixes including vitamins and mineral premixes: intended for sheep goats and beef cattle	aflaB1B2G1G2	10
	intended for calf, lamb, kid, dairy sheep, goats and cattle intended for poultry		5 10
	complete feed: intended for sheep, goats and beef cattle	aflaB1	50
	intended for calf, lamb, kid, dairy sheep, goats and cattle	aflaB1	5
	intended for layers and breeders (broilers and layers)	afla B1 afla B1B2G1G2	10 20
		aflaB1	10
	intended for broilers and pullet intended for parent and grand-parent stocks	afla B1 afla B1B2G1G2	5 20
Iraq	-	-	-
Ireland	see Eu		
Israel	nuts, peanuts, maize flour, figs and their products and other foods	afla B1 afla B1B2G1G2	5 15

Table 2: Continued…

	milk and milk products feed (all grains)	aflaM1 afla B1B2G1G2	0. 05 20
Italy	see Eu		
Jamaica[FAO1997]	food, grains	afla B1B2G1G2	20
Japan	all foods feed: compound feeds for cattle (except calves, dairy cows), pigs (except piglets), chicken (except young chicken, broilers)and quails	afla B1 afla B1	10 20
Jordan	almonds, cereals, maize, peanuts, pistachio nuts, pine nuts, rice all feedstuffs	afla B1 afla B1B2G1G2 afla B1 afla B1B2G1G2	15 30 15 30
Kenya[FAO1997]	peanut (product)s, vegetable oils	afla B1B2G1G2	20
orea, Republic of	grains, soy-bean, peanuts, nuts, wheat and the products made from these by simple processing such as grinding and cutting milk and milk products feed: compound feeds for calfs, chicken, piglets, broilers (early stage)and dairy cattle other compound feeds (except premixes) feed ingredients:vegetable proteins, grains, by-products of grains and food	afla B1 aflaM1 aflaB1	10 0. 5 10 20 50
Kuwait	infant and children food liquid milk and milk products[except dried milk]	afla B1B2G1G2	0. 05 0. 2
Latvia	see EU		
Liechtenstein	see EU		
Lithuania	see EU		
Luxembourg	see EU		
Macedonia	wheat, maize, rice, bean	aflaB1G1 afla B1G1	1 5

Table 2: Continued…

Malavi[FAo1997]	peanuts (export)	aflaB1	5
Malaysia[FAO1997]	all foods	aflaB1B2G1G2	35
Malta	nuts, dried fruit, cereals milk	afla B1 afla B1B2G1G2 aflaM1	2 4 0. 05
Mauritius [FAO 1997]	all foods groundnuts	aflaB1 afla B1B2G1G2 aflaM1M2 aflaB1 afla B1B2G1G2 aflaM1M2	5 10 10 5 15 15
MERCOSUR:	member states:Argentina, Brazil, Paraguay, Uruguay peanuts, maize and products thereof fluid milk powdered milk	 afla B1B2G1G2 aflaM1	 20 0. 5 5
Mexico	cereals and products corn flour for tortillas feed:cereals for bovine and porcine fattening feedstuffs feedstuffs for dairy cattle/poultry	afla B1B2G1G2	20 12 200 0
Moldova, republic of	cereals, legumes, flour, cocoa, nuts, coffee, sunflower, tea milk, cottage cheese, butter	alfaB1 aflaM1	5 0. 5
Morocco	all foods peanuts, pistache nuts, almonds, vegetable oils in pasta,children foods wheat meal wheat bran vegetable oils, cereals, wheat meal (complete) milk (product) milk (product) forinfants under3 years milk powder milk powder for infants under 3 years	aflaB1 aflaM1	10 1 3 10 5 0. 05 0. 03 0. 5 0. 3

Table 2: Continued…

	feed: simple feedstuffs (except peanuts, copra, cottonseed, babassu, maize and their products)	aflaB1	50
	peanuts, copra, cottonseed, babassu, maize and their products		20
	complete feedstuffs for cattle, sheep and goats (except for dairy animals, calves and lambs)		50
	complete feedstuffs for dairy animals		5
	complete feedstuffs for calves and lambs		10
	complete feedstuffs for pigs and poultry (except young animals)		20
	other complete feedstuffs		10
	complementary feedstuffs for cattle, sheep and goats (except for dairy animals, calves and lambs)		50
	complementary feedstuffs for pigs and poultry (except young animals)		30
	other complementary feedstuffs, especially dairy animals		10
Mozambique	peanut, peanut milk	aflaB1B2G1G2	10
	milk	aflaM1	Unknown
	feed:peanut, maize, peanut	aflaB1B2G1G2	10
	butter	aflaB1B2G1G2	10
	cereals and feedstuffs	aflaB1	unknown
	feedstuff		
Myanmar	-	-	-
Nepal	cereals	aflaB1	20
	feedstuffs		50
Netherlands	see EU		
New Zealand	see Australia		
Nicaragua	-	-	-
Nigeria	foodstuffs	aflaB1	20
Norway	see EU		
Oman	complete foodstuffs	aflaB1	10
	complete feedstuffs for poultry		20
Pakistan	-	-	-

Table 2: Continued…

Panama	-	-	-
Paraguay	see MERCOSUR		
Peru	raw and processed peanuts milk	aflaB1B2G1G2 aflaM1	15 0. 5
Philippines	nut (products) feed: mixed feed copra and copra products	aflaB1B2G1G2 aflaB1	20 20 20
Poland	see EU		
Portugal	see EU		
Qatar	-	-	-
Romania	see EU		
Russian Federation	bread grains, grain legumes, cereals, oat flour, flakes, wheat flour, pasta, bread, bakery products, cocoa, nuts, tea, coffee,oil seeds, vegetable oils, wheat germ flake cow butter milk and milk products	aflaB1 aflaM1	5 0. 5 0. 5
Salvador, El	foods all feedstuffs supplementary feeds for porcine/poultry/dairy cattle; single composite feedstuffs;bovine/caprine/ovine feedstuffs	aflaB1B2G1G2 aflaB1	20 10 20
Saudi Arabia	infant and children food liquid milk and milk products[except dried milk]	all afla	0. 05 0. 2
Senegal[FAO1997]	peanut products (straight feedstuffs) peanut products (feedstuff ingredients)	aflaB1	50 300
Serbia and Montenegro	wheat, corn, rice, barley, bean, peas, rosted coffee, roasted peanut, tea meat and meat products spices milk and milk products feed for chicken, pigs (until 50kg), calf, young turkey, duckling, cow feed for ox,	aflaB1 aflaB1 aflaM1 aflaB1B2G1G2	5 0. 5 30 0. 5 0. 5 10

Table 2: Continued…

	sheep, goat		50
	feed for swine and poultry		20
Singapore	corn, nuts, and cereal products	aflaB1	not given
		aflaB1B2G1G2	5
	milk and cheese products	aflaM1	0. 5
Slovakia	see EU		
Slovenia	see EU		
South Africa	all foodstuffs	aflaB1	5
		aflaB1B2G1G2	10
	milk	aflaM1	0. 05
Spain	see EU		
Sri Lanka	all foods	all afla	30
	food for children up to 3 years		1
Sudan	Oil seeds	aflaB1B2G1G2	see codex
Suriname[FAO199]]	maize	aflaB1B2G1G2	30
	groundnut (products), legumes	aflaB1	5
	feedstuffs	aflaB1B2G1G2	30
Sweden	see EU		
Switzerland	nutmeg	aflaB1	10
		aflaB1B2G1G2	20
	spices	aflaB1	5
		aflaB1B2G1G2	10
	infant formulae and follow-on formulae	aflaB1B2G1G2	0. 01
		aflaM1	0. 02
	processed cereal-based foods and baby foods for infants and young children	aflaB1B2G1G2	0. 01
		aflaM1	0. 02
	all foodstuffs	aflaB1	2
		aflaB1B2G1G2	4
	milk and milk products	aflaM1	0. 05
	cheese		0. 25
	feed:		
	babassu seed, cotton seed, peanut, coconut, maize kernel	aflaB1	200
	complementary for lactating bovine animals, lactating sheep and lactating goats		5
	other complete and complementary feeds		10
Syrian Arab Republic	peanuts and pistachio	aflaB1	5
	baby food	aflaB1B2G1G2	005
	pulses, mixed nuts, oil seeds		

Table 2: Continued…

	and products thereof		20
	liquid milk	aflaM1	0. 2
	dried milk[not used in baby food]		0. 05
	domestic feed	aflaB1B2G1G2	20
	livestock cattle feed		10
Taiwan Province of China	peanut, corn, maize	aflaB1B2G1G2	15
	rice, sorghum, legumes, nuts, wheat and barley, oats		10
	edible oils and fats		10
	other foods		10
	infant food		not detectable
	milk	aflaM1	0. 5
	milk powder		5
	maize (raw material)	aflaB1B2G1G2	50
	all feedstuffs	aflaB1	25-100
Tanzania, United Republic of	cereals, oil seeds	aflaB1	5
		aflaB1B2G1G2	10
	feeds	aflaB1	5
		aflaB1B2G1G2	10
Thailand	all food products	aflaB1B2G1G2	20
Trinidad and Tobago	-	-	-
Tunisia	all products	aflaB1	2
		aflaB1B2G1G2	unknown
	milk	aflaM1	unknown
Turkey	hazelnut, peanut and other nuts; oily seed;dried fruits(fig, raisin, etc.) and foodstuffs prodused of these, spices,and other foods	aflaB1	5
		aflaB1B2G1G2	10
	cereals and cereal flour	aflaB1	2
		aflaB1B2G1G2	4
	baby food	aflaB1	1
		aflaB1B2G1G2	2
	milk	aflaM1	0. 05
	milk powder		0. 05
	cheese		0. 5
	feed ingredients, mixed feed for		0. 25
	rominants except young animals	aflaB1	50
	mixed feed for poultry except young animals		20

Table 2: Continued…

	other mixed feeds		10
Uganda	-	-	-
Ukrain	milk and dairy products;grain based baby food	aflaB1	1
	meat products, sausages, poultry;egg, grains, beans, flour, bread, all nuts, cocoa, coffee, tea, vegetable oil		5
	grain based babyfood products, babyfood for early born babies, milk and dairy products, dairy products for babyfood	aflaM1	0. 5
	combined feed for non-productive animals	aflaB1	10
	combined feed for poultry		25
	combined feed for cows in the period of lactation		
	combined feed for calves and sheep older than 4 months,		50
	animals for meat, breeding bulls		100
United Arab Emirates	-	-	-
United Kingdom of Great Britain and Northern Ireland	see EU		
United States of America	all foods except milk	aflaB1B2G1G2	20
	milk	aflaM1	05
	feed:		
	corn and peanut products intended for fishing (i. e., feedlot)beef cattle	aflaB1B2G1G2	300
	cottonseed meal intended for beef cattle, swine, or poultry		300
	corn and peanut products intended for finishing swine of 100 pound or greater		200
	corn and peanut products intended for breeding beef cattle, breeding swine, or mature poultry		100
	corn and peanut products and		20

Table 2: Continued…

	other animal feeds and feed ingredients, excluding cottonseed meal, intended for immature animals		
Uruguay	MERCOSUR member state		
Venezuela	corn, corn flour, peanuts, peanut butter	aflaB1B2G1G2	20
	fluid milk	aflaM1	0. 5
	milk powder		5
Viet Nam	Foodstuffs	aflaB1B2G1G2	10
		total of other mycotoxins	35
	milk and milk products	aflaM1	0. 5
Yemen	no official regulations, but some control takes place		
Zambia	-	-	-
Zimbabwe	foods	aflaB1	5
	groundnuts, maize, sorghum	aflaB1	5
		aflaG1	4
	feedstuffs	aflaB1B2G1G2	unknown
	poultry feed	aflaB1G1	10

Table 3: Upper limit allowances for aflatoxin in human and animal foodstuffs according to national standard of Iran (22)

Food item	**Aflatoxin B1 ng/g**	**All kind of aflatoxins ng/g**
Wheat	5	15
Barley	10	50
Corn	5	30
Rice	5	30
Legumes	5	15
Whole pistachio and its nut walnut, peanut, cashew nut other nuts	5	15
Date, raisin, fig, different dried fruits	5	15
Infant formula based cereal without milk	1	
Infant formulas based milk	0. 5	
Fish powder, meat powder, bone powder,blood powder, one cellular protein, rice	10	20
Bran, corn bran, wheat bran,	5	20

Table 3: Continued…

barley bran,		
Wheat and barley used in:		
a)sheep, goat, steer feed		
b)poultry, calf, lamb, yeanling lactesent sheep, goat and cow		
Cotton seed meal	15	50
Supplements including mineral and vitamin		
supplement which are used in :		
a)sheep, goat, steer feed		10
b)poultry, calf, lamb, yeanling lactesent sheep, goat and cow		5
c)poultry		10
Whole feed used in :		
a)sheep, goat, steer feed	50	
b)poultry, calf, lamb, yeanling lactesent sheep, goat and cow	5	
c)poultry (oviparous, fowl and oviparous)	10	20
d)fowl poultry, semi oviparous	10	

Milk and milk products	Aflatoxin M1
Crude, pasteurized and sterilized milk	0. 05
Industrial powdered milk	0. 5
Cheese	0. 2
Butter, butter oil	0. 02
Other milk products	0. 05

Aflatoxin contamination in Iran

According to published data there are limited numbers of studies about aflatoxin contamination of food in cities and different regions during different times in Iran. These researches show that contamination quantity is considerably high. Here are some research results about aflatoxin contamination in Iran.

The mean bread consumption in Iran per person is estimated 180-200 kg yearly that about 20%-30% of it is thrown away from food cycle as waste bread. In Iran almost all of this wasted bread is used as livestock feed (23).

Nutritional and chemical toxicology bulletin published a new report in December 2006 in which Iranian researchers have shown that although a lot of efforts have achieved to aflatoxin contamination control but Iranian pistachio contamination is high up to now. Aflatoxin can be found in pistachio more than other foodstuff in Iran. Because of international competition in pistachio market, every contamination levels higher than standard lead to substitution of other countries for Iran in this market. According to this new research Iran's pistachio contamination is in the American standards range but is higher than European standards (24).

American standard for aflatoxin is 20ng/g pistachio and European standard is 2-4ng/g pistachio but total aflatoxin contamination of Iranian pistachio is almost 16ng/g pistachio. The worry about increasing hepatocellular carcinoma cases in Europe where have lower incidence of diseases like hepatitis B cause more restrictions for Iranian pistachio (24).

A survey on distribution of *Aspergillus* section *flavi* in corn field soils collected from Mazandaran and Semnan with totally different climatic in Iran showed a relatively larger isolated proportion of aflatoxigenic *A. flavus* strains were from corn field soils of Mazandaran province. These results indicate a possible relationship between humidity and higher rate of aflatoxin producing fungi (25).

Examination of 32 milk samples collected from Babol City, north of Iran on Caspian littoral, in 2007 and revealed that all of them had aflatoxin M1 contamination (26).

In a study in Tehran 5 (11. 11%) of 45 clinical isolates (4 from sinusitis, 1 from onychomycosis) showed to be aflatoxigenic by TLC (27).

A study on 52 milk samples collected from Tehran city has shown that all of them had contamination levels higher than European upper limit allowance (28).

In a survey on 180 dried bread samples collected from Lorestan Province in Iran it was found that aflatoxin B1amount in 29 samples and total aflatoxin amount in 18 samples were higher than upper limit allowance (9).

In a study on 43 isolates of *A. flavus* from cultured green tiger shrimps of Persian Gulf, it was found only 8 (18. 7%) isolates to be aflatoxin producers by HPLC method. Aflatoxin B1 in these isolates ranging from 0.32 to 12. 18 ppb. One (2. 3%) of isolates from hepatopancreatic sample produced 18. 88ppb and 0. 36 ppb of aflatoxin B1 and aflatoxin B2 respectively, but no histopathological change was observed in that tissue (29).

Investigators tested 71 rice samples which were imported to Iran during March 2006 – March 2007. Among 71 rice sample, AFB1was detected in 59 samples (83% of total). AFB1 level in two samples (2. 8%) was above the maximum tolerated level of AFB1 in Iran. Aflatoxin total level was lower than maximum tolerated level in Iran and was lower than maximum level of EU for aflatoxin total level. Only 9 samples had levels above the maximum tolerated level of Eu in aflatoxin total level (30).

Results of another study on 428 crude and pasteurized milk and livestock feed samples in different seasons in Shiraz city shows that 43. 35% of livestock feed had aflatoxin B1higher than upper limit allowance. Crude milk samples had 38. 03% and pasteurized samples had 14. 42% aflatoxin contamination levels higher than upper limit allowance. Contamination amount in summer and fall were more than winter and spring which may be due to high humidity in fall and high temperature in summer (31).

A study on 210 sterilized milk sample collected from Tehran city supermarkets in 2010, showed aflatoxin M1 (detoxified product of aflatoxin B1 which is excreted in urine but this product has also considerable effects on newborn immune system and nutritional status) in 52. 2% of samples and in 33. 3% of samples with more than allowance levels. The contamination amount was different according to month which was highest in February and least in August. This difference is likely due to livestock feed (32).

Other researchers tested 90 milk samples obtained from Ardebil city in 2010, showed that 100% of them had aflatoxin M1 contamination which in 33% of these samples contamination was higher than upper limit allowance (33).

In a research by Safara for determination aflatoxin levels in different seeds (rice, barley, bean, sesame, Japanese seed) she found out rice, bean, sesame were healthy according to

Iranian standard but were not according to European standard and Japanese seeds were not healthy according to both Iranian and European standards (34).
Investigators measured the amount of aflatoxin M1 in 50 samples of pasteurized milks collected from two dairy factories that provide one of Tehran university dairy needs. 84% of the milk samples (42 samples) examined by ELISA were contaminated with AFM1 by measurable amounts. AFM1contamination was higher than Iran national standard (50 ng/l) only in two (4%)of the milk samples. The mean concentration of AFM1 was higher in no. 1 company's products in comparison with No. 2 Company. The No. 1 company's winter products were more contaminated than its summer products (35).
In another study, 72 pasteurized milk packages collected from Mazandaran province (Babol City) supermarkets and tested for AFM1 with the competitive ELISA method. The results showed that all the samples (100%) had Aflatoxin M1 contamination. The contamination means in January, February and March were 227. 85, 229. 64, and 233. 1 ng/l, respectively. The highest contamination was observed in March and the lowest in January. AFM1 contamination level ranged from 178. 8 to 253. 5ng/l (mean value 230. 2), which shows that the contamination level in all samples (100%) exceeded the European community regulations (50 ng/l). There was no significant relationship between AFM1 contamination level and different months of winter ($P \leq 0.05$) (36).

Prevention of aflatoxin exposure

Food production

Drought has a very important role in prevention of crops contamination so irrigation is an important mean of food quality ensuring (37).
Technological developments have achieved recently, in one of them some genes which cause prevention of aflatoxin production by fungi or reduce fungal action are used. In developing countries some of these technologies are not used in preharvest period for minimizing contamination. Insect damage is not controlled by insecticide or other cultural strategies and drought is a common phenomenon and correct ways of irrigation are not used as preventive tool. Mechanized harvesting is not also used and drying is achieved inefficiently and depends on weather situation (1).

Food storage

The most aflatoxin contamination is occurred during storage so for prevention of this contamination, biologic activity should be prevented through adequate drying (less than 10% humidity), insect activity elimination (which can increase humidity by respiration), low temperature and inert atmosphere, but these processes are not easy to carry out in developing countries. Most people in rural areas grow and store their foods by themselves and most foods are stored in small and traditional granaries and there is low investment in contamination control and management field (38).

Food processing

Aflatoxin detoxifications include physical and chemical methods. Physical methods include separation, thermal inactivation, irradiation, solvent extraction, adsorbtion from solution, microbial inactivation, and fermentation. Two chemical methods for detoxification of aflatoxin are ammoniation and reaction with sodium bisulfate (39).
Food processing could be achieved to reduce aflatoxin contamination. There are 3 main ways in this field: dilution, decontamination and separation. The easiest way is to mix contaminated crops with efficiently controlled low aflatoxin contaminated crops. Through this way the toxin amount could be diluted, although total toxin amount per person is decreased but these persons are still in exposure. Unfortunately, this way usually fails because

there is not enough clean grain for dilution and there are not proper instrument for mixing (40).
Sodium bisulfate has been shown to react with aflatoxins (B1, G1, and M1) under different conditions like temperature, concentration, and time to form water soluble products (39).
For detoxification, ammonia, alkaline substances and ozone are used to denature the aflatoxin but whether this change is permanent is not clear. For example use of soda for corn processing which is used by Mesoamericans reduces aflatoxin amount but there are several evidences which show that these chemical changes may be reversible and aflatoxin may be formed in acidic condition of stomach again after consumption (40).
A new method for detoxification of aflatoxin is the addition of chemisorbent materials such as hydrated sodium calcium aluminosilicate (HSCAS) to the diet of animals. This substance can bind tightly to aflatoxin and immobilize it in the gastrointestinal tract of animals, resulting in reduction in its bioavailability (39).
An investigation has shown that 1normal citric acid causes sufficient reduction of aflatoxin in rice. In samples with 4-30ppb aflatoxin contamination, using citric acid leads to destroy 97. 22% of contamination (41).
In separation method, considerable success is obtained when contaminated grain are separated from the bulk but unfortunately poor producers consume these high contaminated grain themselves or feed their livestock with it (1).

Inhibitors of aflatoxin biosynthesis and its toxic effects

Understanding of genetic differences, genome function and components in foods leads to precise manipulating of genes function and stability in life span to maintain human health and prevention of diseases (42).
Medical research has shown that usual diets containing *Apiaceous* family vegetables like carrot, parsley and celery can reduce carcinogenic effects of aflatoxin (43).
As mentioned earlier oxidative damage may have role in toxic and carcinogenic effects of aflatoxin. In a study on Hep G2 (Human hepatoma-derived cell line) and CaCo-2 (Human colonic adenocarcinoma cell line) researchers showed that natural antioxidants in oranges, blackberries, strawberries and cranberries named Cyanidin3-o-β-glucopyranoside have protective effects against oxidative damage caused by mycotoxins like aflatoxin B1 (10).
In a research on blood cultures which were under aflatoxin exposure, researchers showed that antioxidant vitamins like vitA, C and E reduce sister chromatid exchange which induced by aflatoxin and is responsible for mutagenic effects of aflatoxin and this effect is dose dependent which means more amounts of vitamins cause more protective effects. Among these vitamins according to efficacy vit C has the most effects and after that vit E and vitA. VitC probably induces its effects through free radicals scavenging and reaction with mutagenic substances like OH radical and neutralize them before reaching and damaging DNA. Animal studies have shown that low calorie diets cause reduction in carcinogenic effects of aflatoxin. Consumption of vegetables such as cauliflower, cabbage, broccoli and brussels cabbage lead to reduction in bladder, colon and rectum cancer through neutralizing aflatoxin effects (5).
Neem plant is a known inhibitor of aflatoxin production. The effects of different concentrations of aqueous neem leaf extract on fungal growth and aflatoxin production by *Aspergillus parasiticus* was studied at different incubation times and revealed that inhibition of aflatoxin production by neem leaf extract is time and dose dependent. The maximum inhibitory effect of this plant extract was 80-90% in the presence of 50% concentration and was significant in comparing with control samples (44).

Dillapiol which is a specific inhibitor of aflatoxin G1 production is isolated from the essential oil of dill (*Anethum graveolens* L., an Iranian medicinal plant). It inhibits aflatoxin production by *Aspergillus parasiticus*. Apiol and myristicin which both are congeners of dillapiol, have the same activity but in more amounts (45).
Other authors reported the effects of phenolic compounds on aflatoxin control and found out that an increase in the phenolic compounds content of pistachio fruits causes a reduction or control of aflatoxin production in them (46).
Some others studied inhibitory effects of essential oil of *Ageratum conyzoides* on the mycelia growth and aflatoxin B1production by *Aspergillus flavus*. Comparison of fungal cells, control and those incubated with different concentrations of essential oils showed structural changes of fungal cells which were concentration dependent of the essential oil of *A. conyzoides* (47).

Aflatoxin analysis methods

First samples are cleaned up before analysis to remove extra materials that often interfere with determination of main analytes. Then samples are analyzed by one of following techniques (39):

Thin-Layer Chromatography (TLC)

This technique is widely used to separate aflatoxin from other metabolites. It has been considered as the official method to identify and quantitative aflatoxins at levels as low as 1 ng/g.

Liquid chromatography (LC)

This technique is similar to TLC. LC and TLC complement each other.

Immunochemical Methods

TLC and LC methods for determining aflatoxins in foods are hard and time consuming and these methods need knowledge and experience so new techniques such as highly specific antibody based tests are available which can identify and measure aflatoxins in foods in less than 10 minutes. These techniques are based on monoclonal or polyclonal antibodies affinity for aflatoxins. Three type of these methods are radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), and immunoaffinity column assay (ICA).

Conclusion

Analysis for mycotoxins is essential to minimize the consumption of contaminated food and feed. It is necessary to prevent the presence of thereof in milk and milk products intended for human consumption and young children in particular. To control aflatoxins in Iran, food and feedstuffs specific regulations control methods should be established. The amount of aflatoxin levels can be minimized by prevention of contaminated food and feedstuffs consumption and by reduction of fungal growth and production through agricultural phases. When aflatoxin concentration levels can not be reduced to safe levels addition of aflatoxin adsorbents can be recommended.

Ethical Considerations

Ethical issue principles including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc. have been completely observed by the authors.

Acknowledgements

The authors thank, Dr. H. Sadigi, Tehran Genetic Clinic, for reading the manuscript. The authors declare that there is no conflict of interests.

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