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Full Length Research Paper

Isolation and identification of the mycotoxigenic and non-mycotoxigenic fungi from foodstuff and feedstuff in Mazandaran Province, Northern Iran

I. Gholampour Azizi*, S. Shateri and M. Mousavizadeh

Faculty of Veterinary Science, Babol Branch, Islamic Azad University, Babol, Iran.

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Mycotoxin contamination in agricultural products is seriously dangerous to humans and animals. In this study samples were randomly selected from wheat flour, rice, spices, grape, raisin, cattle's farm and poultry feed in Mazandaran Province, Iran. They were isolated moulds from these samples in Sabouraud Dextrose Agar and Potato Dextrose Agar. In this study *Aspergillus niger*, *Aspergillus flavus, Aspergillus fumigatus, Mucor* spp., *Rhizopus* sp., *Mortirella* sp., *Penicillium* spp., *Fusarium* spp., *Acremonium* sp., *Alternaria* sp., *Cladosporium* spp., *Geotrichum* sp. and *Absidia* sp. were isolated from food and feed. The highest contaminations were associated to mycotoxigenic fungi. In northern Iran due to favorable aflatoxigenic moulds growth especially in cold seasons, some measures should be taken in production, processing and storage of food and feed.

Key words: Food, feed, fungi, mycotoxigenic.

INTRODUCTION

The more important mycotoxins belong to species of *Aspergillus*, *Fusarium*, *Penicillium* and *Claviceps*. These toxigenic fungi contaminate food products in different phases of production and processing especially in suitable heat and moist conditions (Richard, 2007; Shrif et al., 2008). The most important mycotoxins are Aflatoxins (AF), Ochratoxin (OT), Zearalenone (ZEA), Fumonisin (FB), Deoxynivalenol (DON), Patulin, Stergmatocystin, Trichothecence, T2-toxin (T2) and Ergot (Kumar et al., 2008). Mycotoxins can cause vomiting, abdominal pains, pulmonary edema, convulsions, coma, carcinogenic effects, immunosuppression, gene toxicity, immunological cyto-toxicity, mutagenic effects, low appetite, weight loss, faintness, depression and death (Paterson and Lima, 2010; Kumar et al., 2008). Aflatoxins are structurally related to a group of toxic compounds found in most plant products such as wheat, peanut, copra, soya, maize and rice. Main Aflatoxins are B_1, B_2, G_1 and G_2. They are generally produced by special strains of *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius* (Bankole et al., 2003; Richard, 2007). The most important species producing Fumonisin are *Fusarium moniliforme, Fusarium nygamai* and *Alternaria alternaria* (Richard, 2007). The Ochratoxins are produced by *Aspergillus ochraceus, Aspergillus niger, Penicillum viridicatum* and *Penicillum verrucosum*. The Ochratoxin is the most important mycotoxin which may cause disorder endocrine, chronic and acute toxicity, immune toxicity and carcinogenic in human (Duarte et al., 2010; Richard, 2007; Abrunhosa et al., 2010). *Fusarium* genus is the most prevalent one producing toxin. The most important toxin of this genus is Zearalenone which is produced by *Fusarium graminearum* (Zinedine et al., 2007). Feeding milk cow with Zearalenone-contaminated feedstuff helps this toxin to enter the milk which is dangerous to the public health. In human beings, the toxin has symptoms such as enlarging breasts in young girls, early maturity, hormones imbalance leading to the breast cancer and cervices cancer. Its acute and chronic toxicity, gene toxicity, immunological cyto-toxicity, mutagenic effects, and its carcinogenicity have also been

*Corresponding author: E-mail: fungi_vet2002@yahoo.com. Tel: +9809113141548.

reported (Glenn, 2007; Zinedine et al., 2007).

Food contaminations with moulds and mycotoxins have been considered by numerous researchers: Gnonlonfin et al. (2012) isolated *from* cassava chips of the *Rhizopus oryzae*, *Nigrospora oryzae*, *Chrysonilia sitophila*, *Cladosporium resinae*, *Cladosporium herbarum*, *A. niger* and *A. flavus*. Also, Romero et al. (2005) isolated toxigenic fungi from dried vine fruits which the most predominant fungi were *Aspergillus* (50.2%), *Eurotium* (21.4%) and *Penicillium* (13.5%). *Aspergillus* section *Nigri* samples such as *Aspergillus, Fusarium* and *Penicillium Alternaria, Absidia spp., Mucor spp.* ("black aspergilli"). Venturini et al. (2011) studied microbiological quality of mushrooms in Spain. Also, Copetti et al. (2011) detected fungi in cocoa *Absidia corymbifera*, *Aspergillus* sp. *nov*., *A. flavus*, *Penicillium paneum* and yeasts. Toxigenic fungi were *A. flavus*, *A. parasiticus*, *A. nomius*, *A. niger* group, *Aspergillus carbonarius* and *A. ochraceus* group. Abou (2008) was found fungi in all of the collected crude herbal materials*, Rhizoctonia* and *Cladosporium spp.* But, Sreenivasa et al. (2011) isolated *Fusarium* and *Aspergillus* at high levels in maize grains produced in Karnataka (India) for the post harvest. The other fungi included *Penicillium, Drechslera, Nigrospora, Curvularia, Alternaria, Chaetomium and phoma*. Somashekar et al. (2004) studied 9 kind of food, of which 32 samples were contaminated by aflatoxigenic fungi especially *A. flavus*. Moreover, Bankole et al. (2004) of melon seeds isolated *Penicillium, Botryodiplodia, Cladosporium and Rhizopus, Fusarium, Macrophomina, Mucor* and *Paecilomyces* of which the most prevalent was associated to *A. flavus*. Lutfullah and Hussain (2012) analyzed total aflatoxins in rice (25%), broken rice (15%), wheat (20%), maize (40%) barley (20%) and sorghum (30%), while in red kidney beans they were (20%), split peas (27%), chick pea (10%), cow pea (20%), and soybean (15%). Al-Hazmi (2011) from wheat samples from Jeddah market isolated *Aspergilli* with the high incidence. Moreover, Soleimany (2012) reported the occurrence of mycotoxins in cereal samples in morocco; 70, 40, 25, 36, 19, 13, 16 and 16% for Aflatoxins, OTA, ZEA, DON, FB_1, FB_2, T2 and HT2-toxin, respectively. Toteja et al. (2006) determined AFB_1 in wheat ≤5 ppb in 40.3% of samples in India. Also, Tam et al. (2006) observed more than 0.1 ppb AFB_1 in 4% and 1% in breakfast corn and children corn respectively in Canada (European standard limit for AFB_1 in children corn is 0.1 ppb). But, in Ankara, Turkey, Baydar et al. (2005) isolated 0.03-3.16 ppb AFB_1 from wheat flour. Heperkan et al. (2012) reported Cyclopiazonic acid in all samples of dried figs that contained Aflatoxin. Cyclopiazonic acid and Aflatoxin co-occurred in 23% of the samples. In study Belakova et al. (2011) Ochratoxin A was detected in one barley sample (0.3 µg/kg), one malt (0.7 µg/kg) and one hop sample (0.6 µg/kg) out of 237 samples of malt barley, malt, hop, wort, and beer.

Moreover, Abdulkadar et al. (2004) tested 106 samples of corn, nuts, spices, dried fruit and drinks for Ochratoxin and Zearalenone at Qatar supermarkets. They reported that 11 samples contained 0.20-4.91 ppb Ochratoxin and 13 samples contained Zearalenone with concentration of 0.18-6.81 ppb. Many countries have conducted inspection program and controlled mycotoxins for several years to promote public health. Also, In Bulgaria, Zearalenone was measured in 91 crop samples (19 barley, 54 maize and 18 wheat) that were 11.1, 21.1 and 1.9 ppb and their mean concentration were 29, 80.6 and 10 ppb and their maximum contamination were 36.6, 148 and 10 ppb, respectively (Manova and Mladnova, 2009). But, in 2004 to 2005, Zearalenone and Ochratoxin were tested in 209 samples (spices, dried fruit, corn, wheat and barely) at Tunisian supermarkets. Ochratoxin contamination was 59.8% with the mean concentration of 3.5 to 5.3 ng/g, but Zearalenone was detected in 15% of the samples with the mean concentration of 10.4-11.8 ng/g (Ghali et al., 2007) Also, Schollenberger et al. (2005) studied 214 plant origins on *Fusarium* toxin at German supermarkets and separated 38% Zearalenone, whereas Copetti et al. (2011) detected the more numbers and the highest percentage of toxigenic fungi in cocoa samples, especially during drying and storage. Also, Attitalla et al. (2010) during 2003 to 2004 isolated fungi in including cereal types, legumes, fatty seeds and animal feeds in Libya, and 20 fungi species belonging to 10 genera were identified. These included *A. flavus, A. ochraceus, Aspergillus terreus, A. niger, Aspergillus candidus, Aspergillus fumigates, Penicillium chrysogenum, Penicillium canescens, Penicillium waksmanii, F. oxysporum, F. graminearum, Fusarium sporotrichioides, Rhizopus stolonifer, Mucor piriformis, Alternaria tunuisima, Rhizoctonia solani, Pythium ultimum, Phyllactinia rigida and Sccharomyces cerevisiae.* Sorghum grains had highest fungal contamination (45%), while barley had the least contamination (11.17%). Contaminated animal feeds (35%) were over than the diet (30.63%). Kollu et al. (2009, 2011) reported several of the mycotoxigenic of *Penicillium* and Fusarium species in poultry and cattle feeds. Karami-Osboo et al. (2012) in Iran detected AFB_1 in 43.6% maize samples. Whereas, Tavakoli et al. (2012) found AFM_1 in 60% of white cheese samples, ranging from 40.9 to 374 ng/kg, in Tehran, Iran. Hadizadeh et al. (2010) in northern Iran found aflatoxin B_1 in animal feed between 10.4 to 68.8%. Zaboli et al. (2011) detected AFB_1 and the most frequent *Aspergillus* species sach as *A. terreus*, *A. flavus*, *A. niger*, *A. parasiticus* and *A. fumigatus* in rice from northern Iran. Moreover Gholampour and Azarmi, (2009) in the same area detected mycotoxin of Zeralenone and Ochratoxin in human food 5 to 8.3 and 5 to 7.5%, respectively. Also, Aflatoxin M_1 (AFM_1) was found in 100% of the pasteurized yogurt and local yogurt samples collected in Mazandaran province (northern Iran) in autumn 2009 with concentrations of AFM_1 2.1 to 61.7 and 7 to 53 ng/L, respectively (Barjesteh et al., 2010).

Table 1. Incidence of fungi in human food samples in summer 2008, in Mazanderan province, Iran.

Samples	Wheat flour (N=60)		Rice (N=60)		Spices (N=45)		Sum (N=165)		SD	Se
Fungi	F	%	F	%	F	%	F	%		
A .niger	28	43.1	19	29.2	18	27.7	65	100	0.83	0.10
A. flavus	32	46.4	22	31.9	15	21.7	69	100	0.75	0.1
A. fumigatus	21	48.8	15	34.9	7	16.3	43	100	0.75	0.11
Mucor spp.	7	46.7	5	33.3	3	20	15	100	0.8	0.21
Rhizopus sp.	3	30	6	60	1	10	10	100	0.63	0.2
Penicillium spp.	17	47.2	11	30.6	8	22.2	36	100	0.81	0.13
Fusarium spp.	14	45.2	12	38.7	5	16.1	31	100	0.74	0.13
Acremonium sp.	5	41.7	5	41.7	2	16.7	12	100	0.75	0.22
Alternaria sp.	4	36.3	4	36.4	3	27.3	11	100	0.83	0.25
Cladosporium spp.	6	42.9	3	21.4	5	35.7	14	100	0.92	0.25
Geotrichum sp.	0	0	2	100	0	0	2	100	-	-
Unknown	2	40	1	20	2	40	5	100	-	-

F = frequency, SD=standard deviation, Se= Standard Error of Mean.

Wheat and rice are the most important crops in Iran considering their culture, production and consumption. As these foodstuffs are essential food resources for human beings, their fungal contaminations play a crucial rolein jeopardizing human health. Due to the effects of moulds and mycotoxins and their deteriorating effects on human, animal, and poultry health, conducting such a study to determine their levels in food and feed in the region looks necessary.

MATERIALS AND METHODS

In this study following samples were selected at random 60 samples of wheat flour at bakeries, 60 samples of rice from bit seller stores, 54 samples of spices (curry, pepper and cinnamon), in summer 2008, 135 cattle farm feed samples (wheat bran, beetroot refuse and concentrated feed) and 45 poultry feed samples in winter 2009 and 100 grape samples and 100 raisin samples in spring 2010 in Mazandaran province, northern Iran.

Samples were set in sterile plastic bags and kept at 4 centigrade. At first to isolate the moulds samples were milled then add 1 gr of each sample into a large tube that contained of 10 milliliter of distilled water. After shake it placed in room temperature for one hour. Then 100 microliter of the upper mater were plated on of Sabouraud Dextrose agar and Potato Dextrose agar supplemented with 100 µg/ml chloramphenicol and put in the room temperature for 7 days and were isolated the fungi after that counting colony numbers. Each fungus was detected based on macroscopic and microscopic observations. Data were analyzed by ANOVA utilizing the SPSS software package (Attitalla et al., 2010; Samson and Hoekstra, 1984; Gonzalez et al., 2005). The percentage incidence of individual fungi was calculated using the following formula:

Percentage of incidence= No. of colonies of species in all the plates ÷ Total No. of colonies of all the species in all the plates ×100 (Kollu et al., 2009).

RESULTS

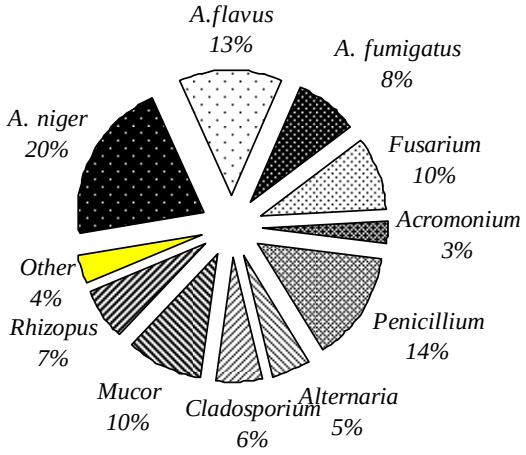
In this present *A. niger* (20%), *A. flavus* (13%), *A. fumigates* (8%), *Mucor* spp. (10%), *Rhizopus* sp. (7%), *Penicillium* spp. (14%), *Fusarium* spp. (10%), *Acremonium* sp. (3%), *Alternaria* sp. (5%), *Cladosporium* spp. (6%) and *Mortierella* sp., *Absidia* sp., *Geotrichum* sp.(other, 4%) were isolated from wheat flour, rice, spices, grape and raisin samples (human food) (Tables 1 and 2, Graph 1). *A. niger* (23%), *A. flavus* (21%), *A. fumigates* (8%), *Mucor* spp.(5%), *Rhizopus* sp.(3%), *Penicillium* spp. (12%), *Fusarium* spp. (11%), *Acremonium* sp. (3%), *Alternaria* sp. (3%), *Cladosporium* spp. (8%) and *Mortierella* sp., *Absidia* sp., *Geotrichum* sp. (other, 3%) were found cattle farm and poultry feed samples. In this present the most importance mycotoxigenic fungi were isolated such as, *A. flavus, Penicillium spp., Fusarium* spp. and *Acremonium* sp. (Table 3, Graph 2). There was a significant relationship between these fungi contamination levels and types of the analyzed food and feedstuffs.

DISCUSSION

Although it is difficult to prevent Aflatoxin formation in food prior to harvesting due to high heat and moisture, it is possible to attain favorable results by correct storage (Zinedine and Maes, 2009). Decreasing fungal growth and Mycotoxins formation in food and feed are essential since it is consumed by both human and animals. *Fusarium* and *Aspegillus* are found in food storage places which produce mycotoxin at suitable moisture and temperature. (Saini et al., 2011; Tanaka et al., 2007). Selouane et al. (2009) detected the prevalence of Ochratoxin in grapes by 59% with 0.08-4 ppb concentration and reported the highest rate of Ochratoxin A production by *A. niger*. Moreover, Abadias et al. (2008) in the highest microorganism isolated fresh, fruit and vegetables. *Aspegillus carbonarius, A. niger* and

Table 2. Distribution of mycoflora in Grapes and Raisin samples in spring 2010, in Mazanderan province, Iran.

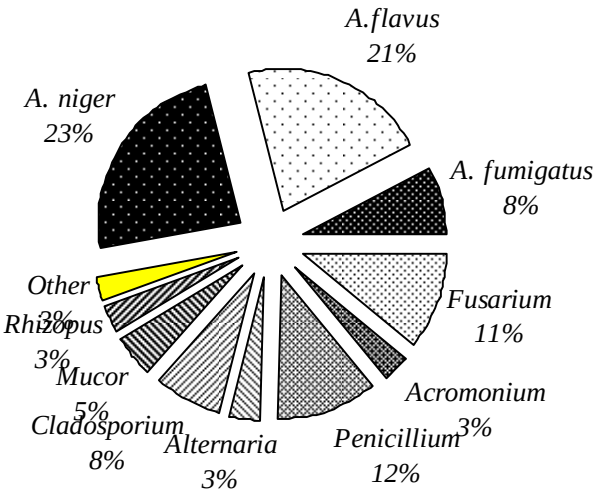
Samples	Grape (N=100)		Raisin (N=100)		Sum (N=200)		SD	Se
Fungi	**F**	**%**	**F**	**%**	**F**	**%**		
A .niger	83	53.5	72	46.5	155	100	0.50	0.04
A. flavus	47	67.1	23	32.9	70	100	0.47	0.6
A. fumigatus	21	52.5	19	47.5	40	100	0.51	0.8
Mucor spp.	41	47.7	45	52.3	86	100	0.50	0.5
Rhizopus sp.	38	62.3	23	37.7	61	100	0.50	0.54
Mortierella sp.	14	56	11	44	25	100	0.51	0.10
Penicillium spp.	61	46	52	46	113	100	0.50	0.05
Fusarium spp.	22	53.2	25	53.2	47	100	0.50	0.07
Acremonium sp.	9	60	6	40	15	100	0.51	0.13
Alternaria sp.	23	56.1	18	43.9	41	100	0.50	0.08
Cladosporium spp.	23	46.9	26	53.1	49	100	0.50	0.07
Unknown	3	60	2	40	5	100	-	-



Graph 1. The percent contaminations fungi in human food.

Table 3. Distribution of mycoflora in cattle farms and Poultry feed samples in winter 2009, in Mazanderan province, Iran.

Samples	Cattle farm feed (N=135)		Poultry feed (N=45)		Sum (N=180)		SD	Se
Fungi	**F**	**%**	**F**	**%**	**F**	**%**		
A .niger	91	84.3	17	15.7	108	100	0.37	0.35
A. flavus	77	81.9	17	18.1	94	100	0.39	0.04
A. fumigatus	25	69.4	11	30.6	36	100	0.47	0.08
Mucor spp.	17	77.3	5	32.7	22	100	0.43	0.09
Rhizopus sp.	8	61.5	5	38.5	13	100	0.51	0.14
Mortierella sp.	2	100	0	0	2	100	-	-
Penicillium spp.	41	78.8	11	21.2	52	100	0.41	0.06
Fusarium spp.	35	72.9	13	27.1	48	100	0.45	0.07
Acremonium sp.	11	78.6	3	21.4	14	100	0.43	0.11
Alternaria sp.	9	60	6	40	15	100	0.51	0.13
Cladosporium spp.	28	80	7	20	35	100	0.41	0.07
Geotrichum sp.	7	77.8	2	22.2	9	100	0.44	0.15
Absidia sp.	1	100	0	0	1	100	-	-
Unknown	1	100	0	0	1	100	-	-



Graph 2. The percent contaminations fungi in cattle farm and poultry feed.

Aspegillus tubingensis find on the grapes when surface of the grapes to sustain an injury (Khoury et al., 2008). Begum and Samajpati (2000) studied rice for mycotoxigenic fungi and mycotoxin at Calcutta sopermarkets. They isolated *A. flavus, Aspegillus japonicus, A. ochraceus, A. parasiticus and Penicillium citrinium*. AFB_1 produces *A. flavus* and *A. parasiticus* but AFG_1 just produces *A. flavus. A. ochraceus, A. japonicas* and *P. citrinium* produce ochratoxin, stergmatocystin and citrinin, respectively. Reddy et al. (2009) considered 1200 samples of rice in 20 Indian provinces for AFB_1 67% of samples contamination range 0.1 to 308 µg/kg and the *A. flavus* was isolated from total samples. The other species were isolated *A. niger, A. ochraceus, A. parasiticus.* Also, Makun et al. (2007) identified. *Penicillium* spp.*, A. flavus, A. parasiticus, A. niger, Mucor* spp.*, Rhizopus* spp. and *Alternaria* spp. from rice samples that were the most prevalent fungal species. Fakhrunnisa et al. (2006) identified *Absidia* sp.*, Alternaria alternata, A. candidus, A. flavus, A. niger, Aspegillus sulphureus, Cephalosprium* sp.*, Chaetomium globosum, Cladosporium herbarum, Curvularia lunata, Drechslera dematioidea, Drechslera halodes, Drechslera hawaiiensis, D. tetramera, F. moniliforme, Fusarium oxysporum, Fusarium pallidoroseum, Fusarium subglutinans, Nigrospora oryzae, Penicillium* spp.*, Piptocephalis* sp., *R. solani, Rhizopus* sp., *Stemphylium* sp.*, Syncephalastrum racemosum, Trichoderma hamatum, Trichothecium roseum* and *Ulocladium sp*. in 19 samples of wheat, 27 samples of sorghum and 14 samples of barley in Pakistan. El-Maraghy (1996) isolated fungi in 25 samples of sheep, cattle and camel feedstuffs collected in Libya. *Aspergillus, Penicillium and Fusarium* was the most common genera in the three substrates tested. Also, Simas et al. (2006) in brewers grain used to feed dairy cattle reported *Aspergillus* with the most frequently isolate (42.5%), followed by *Penicillium, Mucor, Rhizopus* and *Fusarium.* But in our study the most prevalence mycotoxigenic fungi were detected such as, A. *flavus, Penicillium* spp., *Fusarium* spp. and *Acremonium* sp. Penugonda et al. (2010) of the many species of *Aspergillus* elaborated aflatoxins, patulin, terreic acid and sterigmocystin, while species of *Fusarium* elaborated zearalenone, fusarinone-X, deoxynivalenol, nivalenol, diacetoxyscripenol, neosolanil and HT-2 toxins also *Penicillium griseofulvum* elaborated cyclopiazonic acid from finger millet. Amadi and Adeniyi (2009) determined the fungi in maize, rice and millet sach as *A. terreus, A. flavus, A. niger, A. oryzae, Penicillium italicum, Penicillium spinulosum, Rhizopus stolonifer* and *Fusarium* sp. to which were showed these were produced aflatoxin B_1, fumonisin B_1, and zearalenone. In the study Mngadi et al. (2008) were found co-occurring mycotoxins and fungal contamination (the similar our study) in animal feeds.

As there is no reduction in the level of mycotoxin after cooking (Tanaka et al., 2007), it would be feasible to pass some regulations to decrease mycotoxigenic moulds in food and feed. There should be some standards for suitable storage of food because these products may get contaminated which endanger human health; therefore, it is crucial to pass some regulations to reduce mould contamination (Hell and Mutegi, 2011). In any case, products may be contaminated and jeopardize human health therefore, the following recommendations are given: 1) Not using molded food; 2) Preventing in production, storage and consumption; 3) On time harvesting and preventing damage to the product; 4) Cultivating and producing plant strains resistant to mould growth, or toxic formation (Brown et al., 2003); 5) Applying chemical measures to stop fungal growth in food; 6) Appling drying of food before storage; 7) Providing suitable physical condition in storage; 8) Stopping prolonged storage of food in unsuitable conditions; 9) Determining standard regulations for moulds and mycotoxins in food; 10) Measuring mycotoxin in suspected food and feed products in production and shopping centers. 11) Doing constant control on imported food and feed in ports; 12) Establishing laboratories to detect toxins in the capital of provinces; 13) Enhancing producers and officials care to achieve mycotoxin standard level in food and feed.

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