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Original Research Article

Occurrence of moulds, toxicogenic capability of *Aspergillus flavus* and levels of aflatoxins in maize, wheat, rice and peanut from markets in central delta provinces, Egypt

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A B S T R A C T

This study aimed to survey the incidence and load of fungi and aflatoxins in cereal grains and peanut, collected from some markets in central delta provinces, Egypt. The levels of aflatoxins produced by isolated strains of *Aspergillus flavus* were also evaluated. Forty food grains including maize, wheat, rice and peanut seeds were analyzed for fungal contamination. Eight fungal genera belonged to *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor*, *Cladosporium*, *Trichoderma*, *Rhizopus* and *Alternaria* were isolated and identified. Total fungal loads as CFU and percentages of fungi in the analyzed samples ranged between 21.7-33.2x10^3 CFU/g and 1.6-36.7%, respectively. Aflatoxins were analyzed using TLC plates and quantified fluorodensitometry at 366 nm. Percentage of contamination with aflatoxin B1 (AFB1), aflatoxin B2 (AFB2) and aflatoxin G1 (AFG1) differed according to the toxin and the food stuff. AFB1 was more predominant than AFG1. The former was present in 54-100% of the samples and the latter was present in 15-40% of the samples, while AFB2 was not detected. Average contents of AFB1, AFG1 ranged between 427- 466 and 337-540 µg/kg grain dry weights, respectively. Contamination of grains with aflatoxins was in the following order; rice > peanut > wheat > maize. In the cultures of *A. flavus* Link isolates, AFB1, AFB2 and AFG1 were detected in 78%, 71%, and 36% of the isolates. The average contents were 205, 100, and 107 µg/g cell dry weights, respectively. The mycoflora counts and aflatoxin contamination of food samples surveyed in this study are acceptable for food manufacturers. The natural occurrences and recent approaches on the fate and decontamination of mycotoxins and related fungi were discussed.

Keywords

Zea mays; *Triticum vulgaris*; *Oryza sativa*; *Arachis hypogea*; *Aspergillus flavus*; Aflatoxins

Introduction

All of us are concerned with the quality and safety of food. Harmful components in plant derived foods may be a manmade sources or from the activities of

microorganisms. Mycotoxins occurring in food commodities are secondary metabolites of filamentous fungi. Mycotoxins contaminate many types of food crops throughout the food chain (Reddy *et al.*, 2010). They are produced by the three main genera *Aspergillus*, *Fusarium* and *Penicillium* during crop growth, harvesting or storage. Among these toxins, the aflatoxins synthesized by the filamentous fungus *Aspergillus flavus* and related aspergilla are of concern of many investigators. These fungi affect cereals notably peanuts, corn, wheat and rice. In this context, *Aspergillus* and *Penicillium* were reported as the most dominant genera in Egyptian peanut and Brazilian peanut seeds (Gonçales *et al.*, 2008; Rustemeyer *et al.*, 2010; Yassin *et al.*, 2011). These commodities are more liable to fungal infection particularly *Aspergillus*, *Pencillium* and *Fusarium* species in tropical and subtropical regions, dependent on high levels of moisture and temperature (Creepy, 2002). The degree of mould contamination in stored grains can be used as a measure of their quality (Karunaratne and Bullerman, 1990).

There are reports of creating a large economical loss of aflatoxins in the developed and developing countries (Bulatao-Jayme *et al.*, 1982; Dawlatana *et al*., 2002; Kumar *et al.*, 2008; Xu *et al.*, 2008; Njobeh *et al.*, 2009; Bhat *et al.*, 2010). A number of countries have conducted surveys on the incidence of mycotoxins in their agricultural products. Most of these surveys were concerned with occurrence of aflatoxins (Sinha and Sinha, 1991; Yoshizawa, 1991; Azimahtol and Tey, 1992; Refai *et al.*, 1993; Reddy *et al.*, 2009; Moghadam and Hokmabadi, 2010; Sun *et al.*, 2011). Although hundreds of fungal toxins are known, a limited number of toxins have important roles in food safety (Shephard, 2008; Reddy *et al.*, 2010). Among these toxins, aflatoxins are highly toxic secondary metabolites predominantly produced by the filamentous fungi *A. flavus* and *A. parasiticus* (Deiner *et al.*, 1987; Kurtzman *et al.*, 1987; Reddy *et al.*, 2009), in addition to *A. nomius* and *A. tamarii* (Goto *et al.*, 1997), *A. pseudotamarii* (Ito *et al.*, 2001) and *A. bombycis* (Peterson *et al.*, 2001). Among the major known types of aflatoxins are aflatoxin B1; aflatoxin B2, aflatoxin G1 and aflatoxin G2 (Nesbitt *et al.*, 1962; Betina, 1989; Abbas *et al.*, 2010). AFB1 is the most important and toxic to human beings from the public health point of view (Stark and Demain, 1980). It is the most toxic and potent carcinogen, teratogen and mutagen to humans and animals (Sweeney and Dobson, 1998; Shahidi, 2004; Seo *et al.*, 2011), causing damage such as toxic hepatitis, hemorrhage, edema, immunosuppression and hepatic carcinoma (Speijers and Speijers, 2004; Peng and Chen, 2009; Woo *et al.*, 2011). Aflatoxin M1 and M2 were also isolated and identified as mammalian metabolites of aflatoxin B1 and B2 (Allcroft *et al.*, 1966; Saad *et al.*, 1995). The Food and Agricultural Organization of the United Nations (FAO) estimated that at least 25% of the world′s cereal grains are contaminated by mycotoxins, including aflatoxins (FAO, 2004). Aflatoxins can contaminate agricultural commodities including corn, wheat, rice, peanut, and many other crops (Sinha and Sinha, 1991; Aly, 2002, Reddy *et al.*, 2009; Yassin *et al.*, 2011). Aflatoxins, primarily B1 have been found in most staple foods, e.g., cereal grains such as maize, wheat, oats, rice, etc., ground nuts, peanut butter, beans, Brazilian nuts, almonds, cottonseed and meal, cayenne pepper, Indian chili powder, bread, eggs and meat (Stoloff,

1976; Tseng, 1994; Halt, 1994; Hafez, 1996; Idris *et al.*, 2010). Some researchers have investigated the natural occurrence of aflatoxins in range of human foods including peanuts (El-Maghraby and El-Maraghy, 1987; El-Gohary, 1996; Idris *et al.*, 2010), wheat, rice and barley (El-Gohary, 1996; Abdulkadar *et al.*, 2004) and maize (El-Tahan *et al.*, 2000; Ghiasian *et al.*, 2011).

This study aims to investigate the incidence of fungi and aflatoxins B1, B2 and G1, contaminating cereal grains and peanut, collected from some markets in central delta provinces, Egypt. This was done in order to ascertain the safety of such grains for human consumption.

Materials and Methods

Samples collection

A total of 40 food samples, 250 g each, including 13 of maize (*Zea mays* Giza 310), 10 of wheat (*Triticum vulgaris* Giza 168), 9 of rice (*Oryza sativa* Sakha 105), and 8 of peanut (*Arachis hypogea* Giza 4) were collected from some Egyptian local markets in three cities; Tanta, El-Mansoura and Sheben El-Kom belonging to El-Gharbia, El-Dakahlia and El-Menofia provinces, respectively. The samples were labeled, packaged in sterile polyethylene bags, transferred to the laboratory and kept in a cool place (3-5°C) till fungal isolation, identification and aflatoxins analysis.

Microbiological analysis

The dilution-plate method described by (Johnson and Curl, 1972) was applied for fungal isolation. One ml of suitable dilution was used to inoculate Petri dishes containing 15 ml Czapek's Dox agar medium (Oxoid Manual, 1981). Chloramphenicol (0.5 mg/ml) as bacteriostatic agent and rose Bengal (30 ppm) to restrict wide spreading of fungi were added to the medium as recommended by (Al-Doory, 1980). Plates were incubated at 28±2 ºC for 3-5 days and examined for the growth of moulds. The developing fungi were counted and calculated as total colony-forming units (CFU) per gram. The obtained fungi were subjected to identification using macro and microscopic characteristics, and by the aid of the following keys and references (Gilman, 1959; Booth, 1971; Ellis, 1976; Raper and Fennell, 1977; Pitt, 1979; Domsch *et al.*, 1980; Kozakiewicz, 1989; Moubasher, 1993; Samson *et al.*, 1995; Klich, 2002; Samson *et al.*, 2002).

Aflatoxin analysis in food samples

Aflatoxins were extracted from food samples according to the method described by (Schuller *et al.*, 1983). 25 g of each ground sample were added to a 250-ml conical flask containing 25 ml distilled water and 50 ml chloroform. The flasks were shaken for 30 min on a rotator shaker and the suspensions were filtered. The resulting chloroform extracts were purified according to (Takeda *et al.*, 1979) with some modification. Elutes were evaporated to dryness on steam bath. Each residue was re-dissolved in 1 ml chloroform. Aflatoxins were analyzed on 20 x 20 cm TLC-plastic sheets, precoated with silica gel plate type 60 F254 (Merck). Each sample extract containing aflatoxins was loaded on the silica gel plates. Standard aflatoxins B1, B2, and G1, supplied from Sigma Chemical Company, USA were used for comparison with the unknown samples. The plates were developed in a glass jar containing chloroform-acetone (9:1, v/v) as

developing solvent. Aflatoxins were quantified as described by (Shannon *et al.*, 1983; FAO , 1990; FAO. 2004) using fluorodensitometer (TLD-100 vitatron). Measurements were performed through fluorescence at 366 nm.

Aflatoxins in cultures of *Aspergillus flavus* isolates

Selective forty isolates of *Aspergillus flavus* isolated from foodstuffs used in the current study were examined. The method adopted in the present investigation was carried out according to (A.O.A.C., 1984). One ml aliquot of spore suspension; about 10^6 spores/ml was used to inoculate 250 ml conical flask containing 50 ml Czapek's-Dox Medium (Oxoid Manual, 1981). The cultures were incubated for 10 days in the dark at 28°C. At the end of the incubation period, each culture was filtered and the filtrate was extracted with chloroform. The extract was treated as described before, as for analysis of food samples. Mycelia growth was expressed as the dry weight of the mycelia mass collected after extraction of aflatoxins and drying at 70°C for approximately 24 h (Kane and Mullins, 1973).

Results and Discussion

Fungal contamination of foodstuffs

The count forming unit of different fungal colony was determined as a parameter for contamination of food samples. Data shown in Table (1) illustrated the presence of different fungi in the forty food samples, collected from the three selected localities (Tanta, El-Mansoura and Sheben El-Kom). Different fungi were isolated with different frequencies and percentages that differed according to the food stuff and the fungus. Among the presented fungi; *Aspergillus flavus*, *A. niger*, *Penicillium* spp. and *Fusarium* spp. were the most common fungi in the investigated samples. The data presented isolation and identification of *A. flavus*, *A. niger*, *A. terrus*, *Penicillium* spp., *Fusarium* spp. and *Mucor* spp. from maize samples. The counts of fungi ranged between 2.6-8.8x10^3 CFU/g dry wt., total counts of 30.1x10^3 CFU/g dry wt. and the percentages ranged from 10.2 to 36.0%. *A. flavus*, *A. niger*, *Penicillium* spp., *Rhizopus* spp., *Fusarium* spp., *Alternaria* spp. and *Cladosporium* spp were isolated from wheat. The counts of fungi ranged between 1.6-6.3x10^3 CFU/g dry wt., total counts of 21.7x10^3 CFU/g dry wt. and the percentages ranged from 11.7 to 36.0%. *A. flavus*, *A. niger*, *Penicillium* spp., *Fusarium* spp., *Alternaria* spp., *Mucor* spp. and *Rhizopus* spp. were isolated from rice. The counts of fungi ranged between 2.8-9.4x10^3 CFU/g dry wt., total counts of 33.2x10^3 CFU/g dry wt. and the percentages ranged from 8.4 to 28.2%. Fungi contaminated peanut samples were identified as *A. flavus*, *A. niger*, *Penicillium* spp., and *Fusarium* spp.. The counts of fungi ranged between 4.9 to 10.4x10^3 CFU/g dry wt and the percentages ranged from 17.3 to 36.7%.

Aflatoxins in foodstuffs

The contamination of the tested samples with aflatoxins is recorded in Table 2. The forty foodstuff samples were examined for aflatoxin contamination and content. Thirty four samples (85%) were contaminated with aflatoxins. Aflatoxin B1 (AFB1) was detected in all crops, with the exception of wheat. Although 100% contamination with AFB1were detected in *Arachis hypogea* Giza 4, as shown in Table 2, the highest average concentration of AFB1 was recorded in *Oryza sativa*

Sakha 105 (466 µg/kg dry wt.), as shown in Table 3. Contamination with aflatoxin B2 (AFB2) was nil in all the tested samples. Aflatoxin G1 (AFG1) was detected in all crops tested. Although the highest number of AFG1-contaminated samples were 4 and percentage of 40 were detected in *O. sativa* Sakha 105 (Table 2), the highest average concentration (540 µg/kg dry wt.) was detected in *Triticum vulgaris* Giza 168 (Table 3) .

Aflatoxins produced in cultures of *Aspergillus flavus* isolates

Thirty three isolates of *A. flavus* were screened for aflatoxin(s) production. Results in Table 4 indicated that twenty six isolates were able to produce all the tested aflatoxins (AFB1, AFB2, AFG1 and total aflatoxins). Among the toxigenic isolates of *A. flavus*, there was a variation in toxin type and quantities produced by each isolate (data not shown). The number and percentage of isolates producing the toxins differed according to the type of toxin; with AFB1 being the highest detected (78.5%). The levels of average aflatoxins in the cultures of toxigenic fungi varied according to the type of toxin (Table 5). The average concentrations detected were 205, 100, 107 and 236 µg/g dry wt. of the fungus, for AFB1, AFB2, AFG1 and total aflatoxins, respectively.

Maize, rice and wheat are the staple food in Egypt and are grown there; peanut is also consumed in large quantities. So, the present investigation was established to study the incidence of natural fungi and aflatoxins contaminating forty food samples. The samples were collected from the Egyptian local markets in three provinces (El-Gharbia, El-Dakahlia and El-Menofia) in order to ascertain the extent of aflatoxin exposure for human consumption in the studied regions. In this study, the total counts of fungi fluctuated and the highest counts were present in rice and maize. The lower numbers were found in wheat and peanut. These results are in agreement with The International Commission on Microbiological Specifications for Foods (ICMSF) (ICMSF, 1986), which recommended that fungal tolerances for flour and cereal products in the range 10^2-10^4/ gram and rejected products with $>10^5$ colonies/gram. The fungal contamination of the investigated samples was detected and identified; the greater numbers of species which contaminate food stuffs and produce aflatoxins belong to *Aspergillus*, *Penicillium* and *Fusarium* species. Similar data were reported by (Udagawa, 1976; Bullerman, 1986; El-Magraby *et al.*, 1988; Kpodo *et al.*, 2000; CAST, 2003; Gonzáslez *et al.*, 2003; Reddy *et al.*, 2009; Rustemeyer *et al.*, 2010). The degree of mould contamination in stored grains can be used as a measure of their quality, i.e., grains with low mould counts (10^1-10^3 colonies/g dry weight of samples) are of higher quality and safer than those having higher mould count (10^6 colonies/g dry weights of samples), in a study by Karunaratne and Bullerman (1990). Thus, the mycoflora counts on food samples surveyed in this study are acceptable for food manufacturers.

Thin layer chromatography technique was used in this study to evaluate the quantities of aflatoxins and the highest values of AFB1 were present in rice, in agreement with (Niles *et al.*, 1985; Farag *et al.*, 1986) who found that high carbohydrate substrates such as wheat and rice give larger yields of *A. flavus* aflatoxins than oil, that are not immediately metabolized by toxigenic fungi. The level ranged from 431 – 586 µg/kg. The European

Table.1 Fungal contamination levels in cereal grains and peanut collected from some Egyptian markets

Fungus	Grains and peanut (No. of samples analyzed)											
	Maize *(Zea mays* Giza) 310 (13)			Wheat *(Triticum vulgaris* Giza 168) (10)			Rice *(Oryza sativa* Sakha 105) (9)			Peanut *(Arachis hypogea* Giza 4) (8)		
	(n)[a]	(CFU/g dry wt.)[b] x10^3	(%)[c]	(n)[a]	(CFU/g dry wt.)[b] x10^3	(%)[c]	(n)[a]	(CFU/g dry wt.)[b] x10^3	(%)[c]	(n)[a]	(CFU/ g dry wt.)[b] x10^3	(%)[c]
Aspergillus flavus	11	3.5	13.6	6	2.8	13.1	8	4.3	13.0	8	6.3	22.3
A. niger	11	3.0	11.8	6	2.8	12.8	5	2.8	8.4	5	6.7	23.7
A. terrus	7	2.6	10.2	0	0.0	00.0	0	0.0	0.0	0	00.0	00.0
A.candidus	0	0.0	00.0	0	0.0	00.0	4	0.0	0.0	0	00.0	00.0
A. fumigatus	0	0.0	00.0	0	0.0	00.0	5	0.0	0.0	0	00.0	00.0
Penicillium spp.	11	9.2	36.0	6	5.1	23.6	8	9.4	28.2	7	10.4	36.7
Fusarium spp.	8	8.8	34.2	6	6.3	29.0	8	6.7	20.2	4	4.9	17.3
Trichoderma spp.	0	0.0	00.0	0	0.0	00.0	4	0.0	0.0	0	00.0	00.0
Alternaria spp.	0	0.0	00.0	5	3.1	14.1	5	2.9	8.8	0	00.0	00.0
Cladosporium spp.	0	0.0	00.0	4	1.6	7.3	0	0.0	0.0	0	00.0	00.0
Mucor spp.	6	3.0	11.7	0	0.0	00.0	3	3.4	10.4	0	00.0	00.0
Rhizopous spp.	0	0.0	00.0	4	0.0	00.0	5	3.7	11.0	0	00.0	00.0
Total	30.1			21.7			33.2			28.3		

[a]Number of samples showing fungal contamination.
[b]CFU/g grains or seeds (average of positive samples).
[c]Percentage of CFU of each fungus/g of grains or seeds with respect to the total count CFU/g in each grain or seed.

Table.2 Contamination of some grains and peanut collected from Egyptian markets with aflatoxins

Grains and peanut	No. samples					% positive samples			
	Assayed	Positive samples							
		AFB1	AFB2	AFG1		AFB1	AFB2	AFG1	
Maize *(Zea mays* Giza 310)	13	7	0	2		53.8	0	15.3	
Wheat *(Triticum vulgaris* Giza 168)	10	0	0	3		00.0	0	30.0	
Rice *(Oryza sativa* Sakha 105)	9	7	0	4		77.7	0	40.0	
Peanut *(Arachis hypogea* Giza 4)	8	8	0	3		100.0	0	37.5	

Table.3 Aflatoxins detected in some grains and peanut collected from some Egyptian markets

Grains and peanut	[a]Average aflatoxins (µg/kg dry wt.) (range)		
	AFB1	AFB2	AFG1
Maize (*Zea mays* Giza 310)	440 (280-720)	0.0 (0.0)	400 (360-440)
Wheat *(Triticum vulgaris* Giza 168)	00.0 (0.0-0.0)	0.0 (0.0)	540 (400-640)
Rice *(Oryza sativa* Sakha 105)	466 (220-800)	0.0 (0.0)	348 (330-800)
Peanut *(Arachis hypogea* Giza 4)	427 (210-600)	0.0 (0.0)	337 (250-400)

[a]Average levels of aflatoxins in positive samples

Table.4 Capability of *Aspergillus flavus* isolates from grains and peanut collected from Egyptian markets to produce aflatoxins.

Fungus	No. isolates							
	Assayed	Positive isolates				% positive isolates		
		AFB1	AFB2	AFG1		AFB1	AFB2	AFG1
Aspergillus flavus Link	33	11	10	5		78.5	71.4	35.7

Table.5 Levels of aflatoxins produced in cultures of *Aspergillus flavus* isolates obtained from some grains and peanut collected from Egyptian markets

Fungus	No. isolates assayed	[a]Average aflatoxins (µg/g dry weight) (range)		
		AFB1	AFB2	AFG1
Aspergillus flavus Link	33	205 (80-321)	100 (86-195)	107 (74-146)

[a]Average levels of aflatoxins in the cultures of positive isolates

Commission has set the limits on groundnuts subject to further processing at 15 ppb for total aflatoxins and 8 ppb for aflatoxin B1, and for nuts and dried fruits subject to further processing at 10 ppb for total aflatoxins and 5 ppb for aflatoxin B1. The aflatoxin standards for cereals, dried fruits, and nuts intended for direct human consumption are even more stringent, and the limit for total aflatoxins is 4 ppb and 2 ppb for aflatoxin B1 (Otsuki *et al.*, 2001). The range of aflatoxins concentrations in food samples analyzed in this study were beyond the safe limits for human consumption as recommended by WHO and FDA (Food and Drug Administration, of United States) (ICRISAT, 2000). In this connection, fifty six samples of stored rice were tested and 12 were positive for aflatoxin as concluded by (Prasad *et al.*, 1987) and levels of aflatoxins ranged from 184 to 2830 µg/kg, aflatoxin contamination rate of 65 µg/kg in groundnut samples from Bangladesh was reported by (Dawlatana *et al.*, 2002), an aflatoxin level of 162 µg kg-1 was reported in Gambian ground nut samples (Hudson *et al.*, 1992; Williams *et al.*, 2004) and the highest incidence (1706 mg/kg) of total aflatoxin in foods was found in Brazilian ready to eat peanuts (Caldas *et al.*, 2002). On the other hand, the results obtained by (Reddy *et al.*, 2009) indicated that 2% of tested rice recorded by (Hesseltine *et al.*, 1970; Dorner *et al.*, 1984; Cotty, 1989; Hadwan grains collected from different Indian states showed AFB1 contamination above the permissible limits (>30 mg/kg), while milled rice grains showed below the permissible levels of AFB1 (average 0.5–3.5 mg/kg). No aflatoxin was found in corn meal and flour samples collected from Sao Paulo Market (Bittencourt *et al.*, 2005). A market research of cereals from Qatar revealed no detected levels of aflatoxin contamination in rice and wheat (Abdulkadar *et al.*, 2004). A study on Turkish wheat samples revealed 60% contamination level, in a very low range (Giray *et al.*, 2007).

Toxin production by the genus *Aspergillus* has become now of major importance in human and animal diseases because of the direct toxicity and long term carcinogenic effect of it's certain metabolites such as aflatoxins (Masri *et al.*, 1969). Grains and peanut were reported as a good substance for aflatoxins producing fungi. Thus, it was imperative to investigate the toxicity of *A. flavus* isolates, obtained from the contaminated grains. A wide variation of aflatoxins production was obvious among the tested isolates of *A. flavus*. Using, TLC technique, it was found that twenty six isolates, among thirty three were able to produce aflatoxin(s). There was a variable in the toxin type produced by each isolate. These results are in agreement with those *et al.*, 1995; Abou-Zeid *et al.*, 1997) who found that strains of *A. flavus* grown on

natural and synthetic media had different ability for production of the aflatoxins AFB1, AFB2, AFG1 and AFG2. On the other hand, 65% of the tested isolates had no ability to produce any kind of aflatoxins. A similar finding was recorded by **(**Holtmeyer and Wallin, 1980). The percentage of toxigenic *A. flavus* varies with strain, substrate and geographic origin. In this context, there are many reports of aflatoxin contamination in grains including reports from Egypt (El-Khadem *et al.*, 1975); United State of America (Klich and Pitt, 1988); China (Wang *et al.*, 1993); Turkey (Giray *et al.*, 2007) and India (Reddy *et al.*, 2009).

With increasing awareness of aflatoxin as one of the sources of health hazard to both human and animals, application of advanced methods such as DNA biosensors and infrared spectroscopy for rapid and accurate detection of mycotoxin and related fungi increased. Furthermore, efforts have been made to eliminate the toxin or reduce its content in foods and feedstuffs to significantly lower levels. Although different strategies have been applied for elimination or inactivation of aflatoxins, problems still remain with the efficacy, safety and cost requirements for these methods (Ji *et al.*, 2011). Although preventing mycotoxin production at farm level is the best way to control mycotoxin contamination (Sengun *et al.*, 2008), chemical, biological and physical methods have been tried to inactivate aflatoxins or reduce their content in foodstuffs (Wu, 2004; Rustom, 1997; Allam *et al.*, 2012). Advances in molecular techniques and other decontamination methods such as gamma irradiation and microwave heating could help in this respect (Herzallah *et al.*, 2008). Several protocols usefull to mycotoxin biodegradation have been provided in food and feed, using potential bacteria such as *Lactobacillus* and *Bifidobacterium* (Fuchs *et al.*, 2008; Kabak and Var, 2008; Halasz *et al.*, 2009; Awad *et al.*, 2010; Wei *et al.*, 2010)**.** Successful detoxification rate against mycotoxins has been shown by *Bacillus subtilis* and *Bacillus lichenformis*. The two bacterial strains could be applied for aflatoxin decontamination (Wei *et al.*, 2010). Further studies are needed to help demonstrate and develop more effective decontamination methods.

The mycoflora of the tested cereal grains and peanut presented different fungi, with different frequencies and percentages, some with the ability to produce aflatoxins. A total of 15 species of fungi belonging to 8 genera were isolated and the greater number of species was related to the genus *Aspergillus*; *A. flavus* being the most dominant. The total counts of fungi fluctuated between 21.7 to 33.2×10^3 colonies/g dry weights of samples. The highest counts were detected in rice and maize; the lower were found in wheat and peanut. This indicates that the mycoflora counts on food samples surveyed in this study are acceptable for food manufacturers. Aflatoxins AFB1 and AFB2 were recorded in 85% of the tested samples and AFB1 was more predominant. The highest values of AFB1 were recorded in rice, indicating that high carbohydrate substrates may help larger yields of aflatoxienic fungi than oil. Investigation the toxicity of *A. flavus* isolates, obtained from the contaminated samples indicated a wide variation of aflatoxins production. In cultures, 205, 100 and 107µg/g dry weights of AFB1, AFB2 and AFG1 were detected, respectively. The ranges were beyond the safe limits for human consumption recommended by WHO and FDA. Application of advanced methods for rapid

and accurate detection of mycotoxins and related fungi, in addition to efficient, safe and low cost decontamination methods are needed to deal more effectively with aflatoxin contamination.

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