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Vol. 9(46), pp. 2275-2282, 21 November, 2015
DOI: 10.5897/AJMR2015.7782
Article Number: CA7692856324
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Fungi and aflatoxins associated with wheat grains in Gaza governorates

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Received 27 September, 2015; Accepted 3 November, 2015

Cereal and its products are susceptible to mold damage during pre-and post-harvesting stages of the production. This study was carried out to determine the extent of fungal contamination and the aflatoxins AFB_1, AFB_2, AFG_1, AFG_2 levels of fifty wheat grain samples were collected randomly from different markets in the five Gaza governorates. The obtained results indicated that the most common molds isolated from different wheat grain samples were *Aspergillus flavus* 84%, *Aspergillus parasiticus* 72%, *Fusarium oxysporum* 64%, *Aspergillus niger* 48%, *Alternaria alternate* 36%, *Penicillium* 22%, *Aspergillus ochraceus* 20% and *Aspergillus versicolor* 4%. Forty one wheat grain samples which represented (82%) were contaminated with aflatoxins. Considering the high incidence of contamination by AFB_1 (80%) in Gaza city and 70% in both Khan Younis and Mid Zone governorates. The level of total aflatoxin AFs in North Gaza, Rafah, Khan Younis, Mid Zone, Gaza City were 8.62, 6.361, 4.187, 3.134 and 2.33 (ng/g), respectively. These levels of AFs are higher than the standard levels in North Gaza, Rafah and Khan Younis. The highest amount of aflatoxin B_1 were found in Mid Zone and North Gaza and their aflatoxins contamination were 2.51 and 2.31 ng/g, respectively.

Key words: Wheat, fungal contamination, aflatoxin.

INTRODUCTION

Fungal contamination is one of the major causes of food spoilage. It not only brings about great economic losses, but also represents a high risk for human and animal health through the synthesis of mycotoxin (MacDonald et al., 2004; Tutelyan, 2004; Pitt and Hocking, 1997). Mycotoxins are fungal secondary metabolites produced by some phytopathogenic spoilage fungi such as *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* species that are hazardous to consumers' health, and lead to economic losses of the commercial value of food products (Moss, 1998). Wheat is susceptible to these fungus infections through its growth, harvest, transport, and storage (Giray et al*.,* 2007).

Toxicogenic *Alternaria* and *Fusarium* species are often classified as field fungi, while *Aspergillus* and *Penicillium* species are considered as storage fungi (Logrieco et al., 2003). Mycotoxin production depends on various factors such as the presence of toxic fungi, chemical

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composition of the substrate, moisture, temperature, and time course of fungal growth (Placinta et al., 1999). Food products with high fungal contamination and higher humidity rates are susceptible toearly spoilage if inappropriately stocked (Abdullah et al., 1989). Climatic diversity of Gaza governorates, with its uniform high temperature and high relative humidity, may be a conducive factor for the growth of aflatoxin-producing fungi.

MATERIALS AND METHODS

Sample collection

A total of fifty samples (1 kg) of wheat grains from different markets in the five Gaza governorates (North Gaza, Gaza City, Mid Zone, Khan Younis and Rafah) were collected randomly and kept in polyethylene bags at -18°C. Wheat in Palestine is planted from mid-October through November, and is harvested from mid-May through June for season 2013.

Sample preparation and analysis

According to IGAFN (1980) guide about 10 g of the wheat grains were surface sterilized using 2.5% sodium hypochlorite solution for 3 min. and washed with ten successive 100 ml volume of sterile distilled water. Five grains were placed at random in each of the Petri-dishes containing potato dextrose agar (PDA) and chloramphenicol (500 mg per liter) in triplicate. The dishes were incubated at 25°C and examined daily for five days. Fungi from plated grains were transferred to Potato Dextrose Agar (PDA) slant medium for identification. Identification of isolates was carried out according to Nelson et al. *(*1983).Each pure culture was characterized and identified based on their morphology and microscopic characteristics using the keys of Pitt and Hockings (1997) and Raper and Fennel (1965).

Chemical and reagents

Aflatoxins

Aflatoxins B_1, B_2, G_1 and G_2 standards were purchased from Sigma Chemical Co. (St. Luis, MO63118, U.S.A.).

The immunoaffinity column

The immunoaffinity column AflaTes® HPLC were obtained from VICAM (Watertown, MA, USA). Methanol, trifluoroacetic acid, and sodium chloride, were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Solvents

All solvents were of HPLC grade. The water was double distilled with Millipore water purification system (Bedford, M A, USA).

Sample extraction

1. Weigh 50 g ground sample with 5 g salt (NaCl) and place in blender jar.
2. Add to jar 100 ml methanol: water (80:20).
3. Cover blender jar and blend at high speed for 1 min.
4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

Extract dilution

1. Pipet or pour 10 ml filtered extract into a clean vessel.
2. Dilute extract with 40 ml of purified water. Mix well.
3. Filter dilute extract through glass microfibre filter into a clean vessel or directly into glass syringe barrel using markings on barrel to measure 10 ml.

Column chromatography

1. Pass 10 ml filtered diluted extract (10 ml = 1 g sample equivalent) completely through AflaTest ®-P affinity column at a rate of about 1 to 2 drops/second until air comes through column.
2. Pass 10 ml of purified water through the column at a rate of about 2 drops/second.
3. Elute affinity column by passing 1.0 ml HPLC grade methanol through column at a rate of 1 to 2 drops/second and collecting all of the sample eluate (1 ml) in a glass cuvette.
4. Dryness under a nitrogen stream, then determination with HPLC.

High performance liquid chromatography (HPLC)

Aflatoxin concentrations were reported in ng/g of wheat by immune affinity column chromatography method (Aflaclean, LCTech, Germany) and evaluated by HPLC system, consisting of a fluorescence detector (Knauer, Germany). Aflatoxins were separated in HPLC column with a mobile phase of water: methanol: acetonitrile (60:30:15, v/v/v), , injected volume 10 µl, excitation and emission wavelengths of 365 and 440 mm, respectively, flow rate of 1.2 ml/min, and retention times of 25 min.

Statistical analysis

The data were then analyzed using statistical package for social sciences (SPSS) software (Version 15). The significance of the differences among treatment groups was determined by Waller-Duncan k-ratio. All statements of significance were based on probability of $P<0.05$.

RESULTS

Fungi associated with wheat grain samples collected from five different Gaza governorates: *Aspergillus flavus, Aspergillus parasiticus, Fusarium oxysporum, Aspergillusniger, Alternaria alternate, Penicillium, Aspergillus ochraceus and Avicularia versicolor* were isolated from wheat grain samples and identified based on cultural and morphological characteristics. The most common molds isolated were *A. flavus* 84%, *A. parasiticus* 72% and *F. oxysporum*64% (Table 1). The total fungi count of wheat grains, which collected from five different Gaza governorates were tabulated in Tables 2 and 3 and Figure 1. Figure 2, illustrated the frequency (%) of fungi contaminated wheat grain samples which were collected from five different Gaza governorates.

Table 1. The percentage of fungi associated with wheat grain samples collected from five different Gaza governorates

Fungal Isolates	No.[a] (%)
A. flavus	42 (84)
A. parasiticus	36 (72)
F. oxysporum	32 (64)
A. niger	24 (48)
A.alternate	18 (36)
Penicillium	11 (22)
A. ochraceus	10 (20)
A. versicolor	2 (04)

[a]Wheat samples contamination.

Frequency (%) of *A. flavus* was 100% in North Gaza, Gaza City and Mid Zone while, 80% of *F. oxysporum* inNorth Gaza and Mid Zone. Frequency (%) of both *F. oxysporum* and *A. niger* was 80% in Mid Zone.

In this study, aflatoxin levels were monitored in fifty wheat grain samples using HPLC. In the results, forty onesample (82%) was contaminated with aflatoxin. Considering the high incidence of contamination by AFB_1 (80%) in Gaza city and 70% in both Khan Younis and MidZone governorates (Figure 3). Among the groups of aflatoxin, AFB_1 was found to be one of the most potent environmental carcinogens. The European Union regulation is 2 ppb for aflatoxin B_1 and 4 ppb total aflatoxin in foods intended for direct human consumption (EU*,* 2010). The results indicated that AFB_1 levels in wheat grains in Northern Gaza and Mid Zone were 2.31 and 2.51(ng/g), respectively (Table 4 and Figure 4). The order of total AFs concentration in wheat samples collected from five different governorates in the Gaza strip was: North Gaza, Rafah, Khan Younis, Mid Zone and Gaza City, 8.62, 6.361, 4.187, 3.134 and 2.33 (µg/Kg) (Table 4).

DISCUSSION

The main goal of the present study was to assess the fungal contamination of wheat grain samples in different Gaza gov and to subsequently determine the possible contaminations of these samples by aflatoxins. The contamination of wheat grains with microscopic filamentous fungi does not necessarily result in the presence of mycotoxins. The emergence of mycotoxins depends on several factors such as relative humidity, temperature, the properties of the substrate composition, and the degree of contamination (Gallo et al., 2008). The optimal conditions for the growth and emergence of aflatoxins by fungi are different; and fungi optimally grow at about 30°C and 0.95 aw, while mycotoxins' growth is optimal at about 25 to 30°C and 0.99 aw (Alam et al*.,* 2009).

The fungal growth cannot only change the chemical and physical properties of the food products, but also the nutrient content of the grains. Roigé et al. (2009) in his research, showed that *Penicillium* (42%), *Fusarium* (27%) and *Alternaria* (25%) were the most frequently genera recovered from wheat. In the present study, members of the genus *A. flavus, A. parasiticus and F. oxysporum* were highly prevalent, while *A. niger*, *A. alternate*, *Penicillium* and *A. ochraceus* were the second more frequent fungus isolated from the wheat grains. High incidence rates of *A. flavus* and *F. oxysporum* from different geographical areas of Gaza Governorates (North Gaza, Gaza City, Mid Zone, Khan Younis and Rafah) for season 2013 have been observed in wheat grain samples, which were consistent with the findings another study, confirming that the most commonly isolated fungi from Algerian wheat are *Aspergillus spp., Fusarium spp., Penicillium spp., Alternaria spp. and Mucor spp.* (Riba et al*.,* 2008).

Joshaghani et al. (2013), showed that the most common moulds isolated were *Alternaria* spp. *(*26.7%), *A. niger* (21.4%), *Fusarium* spp. (17.8%), *A. flavus*(10.7%), *Cladosporium* spp. (10.7%), *Penicillium spp.* (8.9%) and *Rhizopus* spp. (3.5%). From the finding data of other researcher, it was concluded that the type of growth fungi on the agriculture commodities depends on the environmental condition, and it is known the Gaza weather differ than other countries.

A mycological survey on the stored wheat samples in Iran showed that 46 species belonging to 23 different genera fungi were isolated; and that *Cladosporium spp.* (57.1 to 89.2%) and *Alternaria spp.* (82.4 to 100%) species were the predominant fungal species as endogenous mycoflora (Kachuei et al*.,* 2009). While mycological survey carried out by Embabyet al. (2012) on freshly harvested wheat grains from the main production regions in Egypt resulted in eight fungal genera isolates and some identified as: *Alternaria* (36.9%) and *Penicillium* (18.3%) were in agreement with the results of this study.

The high frequency and abundance of *Aspergillus spp.* in the present study's findings could be due to failures in food storage and conservation. *Fusarium* was isolated in 64% wheat samples. Pelhate (1977) reported that *Fusarium* was present at harvesting as a result of field infection, and can no longer stay alive once the oxygen level reduces. According to these study findings, *A. niger* with 48% ranked second in fungi isolated from wheat. This may be indicated that air fungal flora is variable in different areas.

The results indicated that the overall average of AFB_1 levels in wheat grains in North Gaza and Mid Zone were 2.31 and 2.51 (ng/g), respectively and AFB_1 was detected in 28 out of 50 wheat grain samples, and its abundance in five samples (6.116, 5.935 and 5.097 ng/g) in Mid Zone and (4.52 and 2.064 ng/g) in North Gaza and Rafah, respectively, which is higher than the EU level (2 ng/g). Although aflatoxigenic fungi were found at high

Table 2. Fungal contamination levels of wheat grains collected from three different Gaza governorates.

Fungi	Wheat grains (10 samples from each governorate) *								
	Khan Younis			Mid zone			North Gaza		
	No. of contaminated samples	No. of isolates	R.P (%)	No. of contaminated samples	No. of isolates	R.P (%)	No. of contaminated samples	No. of isolates	R.P (%)
A. alternate	4	12	21.8	4	5	7.8	4	9	13.23
A. ochraceus	2	2	3.64	2	3	4.7	-	-	0
Penicillium	2	2	3.6	2	2	3.13	2	3	4.41
F. oxysporum	7	13	23.6	8	21	32.8	8	21	30.88
A. flavus	4	6	10.9	10	17	26.6	10	18	26.47
A. versicolor	-	-	0	1	1	1.56	-	-	0
A. niger	6	11	20.0	8	10	15.62	4	10	14.7
A. parasiticus	8	9	16.4	5	5	7.81	7	7	10.29
Total fungi count	55			64			68		

*using PDA media R.P = relative percentage (relative percentage (%) = Number of fungal species isolated / Total Number of fungi isolated × 100).

Table 3. Fungal contamination levels ofwheat grains collected from twodifferent Gaza governorates.

Fungi	Wheat grains (10 samples from each governorate)*					
	Rafah			Gaza city		
	No. of contaminated samples	No. of isolates	R.P (%)	No. of contaminated samples	No. of isolates	R.P (%)
A. alternate	2	5	10.64	4	10	14.28
A. ochraceus	4	7	14.89	2	3	4.28
Penicillium	3	7	14.89	2	2	2.85
F. oxysporum	4	5	10.64	5	13	18.57
A. flavus	8	10	21.27	10	16	22.85
A. versicolor	-	-	-	1	1	1.43
A. niger	2	4	8.51	4	12	17.14
A. parasiticus	8	9	19.15	8	13	18.57
Total fungi count	47			70		

*Using PDA media. R.P= relative percentage (relative percentage (%) = (Number of fungal species isolated / Total Number of fungi isolated) x 100).

levels in this study, total aflatoxins were found in levels higher than the EU level (4 ng/g) in three governorates of Gaza strip: North Gaza, Rafah and Khan Younis (8.62, 6.361 and 4.187). Trombete et al. (2014), showed that Aflatoxin B_1 had the highest prevalence in Brazilian wheat grain samples and the total aflatoxins levels higher than the limit established by Brazilian legislation for cereals in general (5 ng/g).

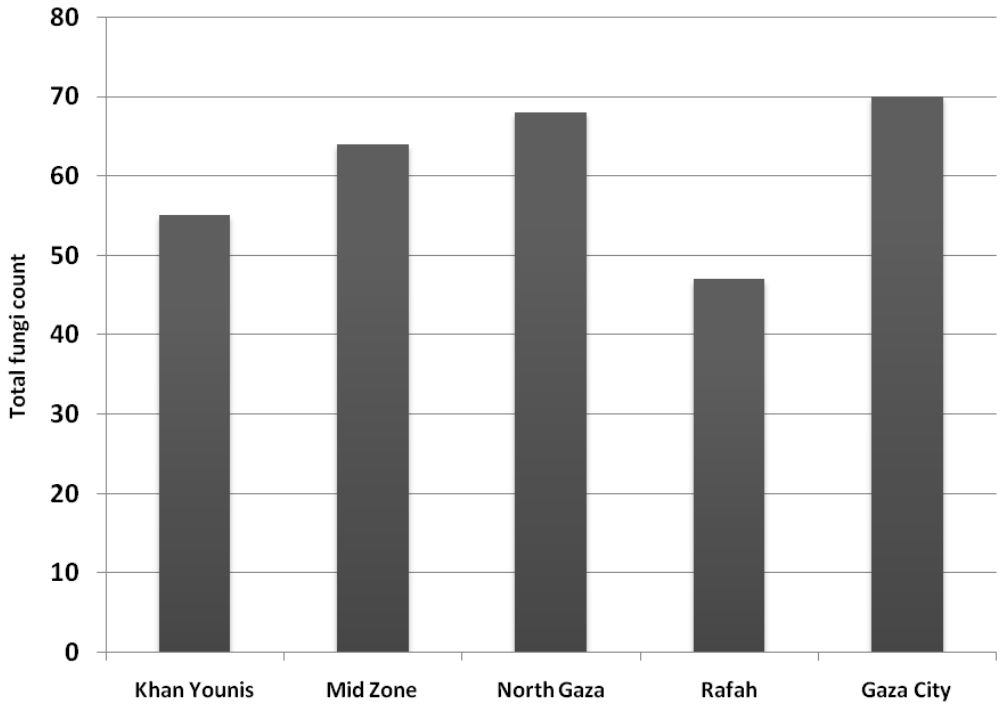

Figure 1. Total fungal count of wheat grains collected from five different Gaza governorates.

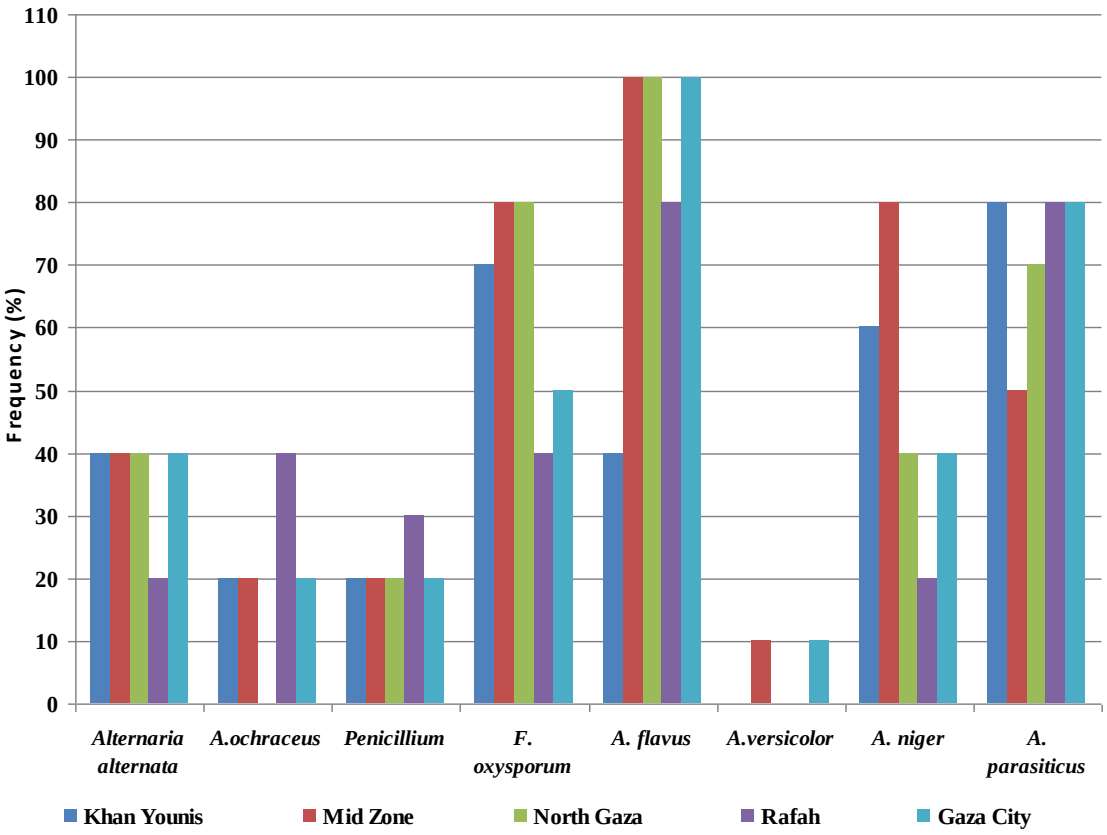

Figure 2. The frequency (%) of fungi contaminated wheat grain samples collected from five different Gazagovernorates (frequency (%) = number of samples infected with fungi /total number of sample analysis x 100).

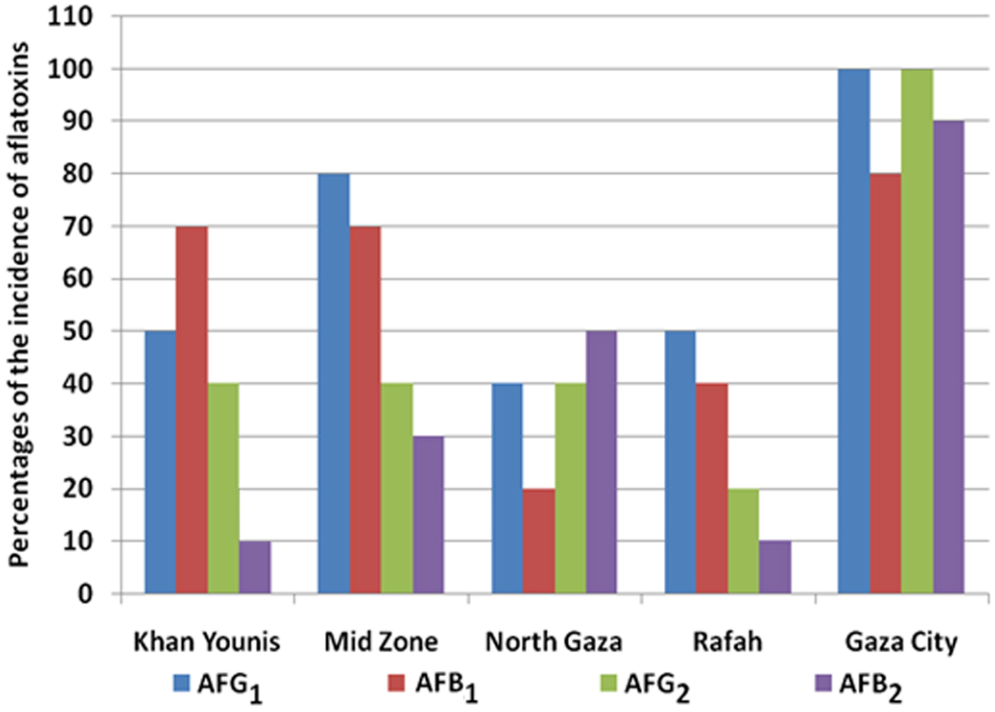

Figure 3. Percentages the occurrence of AFs in wheat grain samples collected from five different Gaza governorates**.**

Table 4. Natural occurrence of aflatoxins in wheat grain samples collected from fivedifferent Gaza governorates (n=10).

Concentrations of AFs (µg/Kg)*	Name of Governorate				
	Khan Younis	Mid zone	North Gaza	Rafah	Gaza city
AFG_1	1.49	0.242	2.88	3.79	1.16
AFB_1	0.523	2.51	2.31	0.807	0.305
AFG_2	0.594	0.234	2.14	1.57	0.788
AFB_2	1.58	0.148	1.29	0.194	0.077
Total AFs	4.187	3.134	8.62	6.361	2.33

*Mean with positive sample only.

Although the number of analyzed samples was limited, the study results revealed a relatively higher contamination of wheat grain. On the other hand, as a result of the continuous use of flour products in the diet, a high level of contamination by aflatoxins may have adverse effects on human health. Moreover, it is feasible to decrease fungal contamination by sufficient education in the field of food industry, and favorable farm management. With high contamination the probable daily intake PDI of Palestinians could be affected by wheat grains consumption. High levels of aflatoxin in food samples emphasise the need for regular surveillance and improved control of aflatoxin levels.

Because the total aflatoxin estimated in some samples were higher than the EU limits, the fungal contamination rate could not be neglected. Isolation of mycotoxigenic fungi such as *Aspergillus spp.* and *Fusarium* spp. is vital importance in the food industry. In the year of study due to shortage of wheat storage, the sources of sampling were not long-lasting, and, it is probable that the contamination would be raised with an increase in the retention time of samples.

Conclusions

The results of this study demonstrate that the abundant fungi species detected in Gaza wheat grain samples

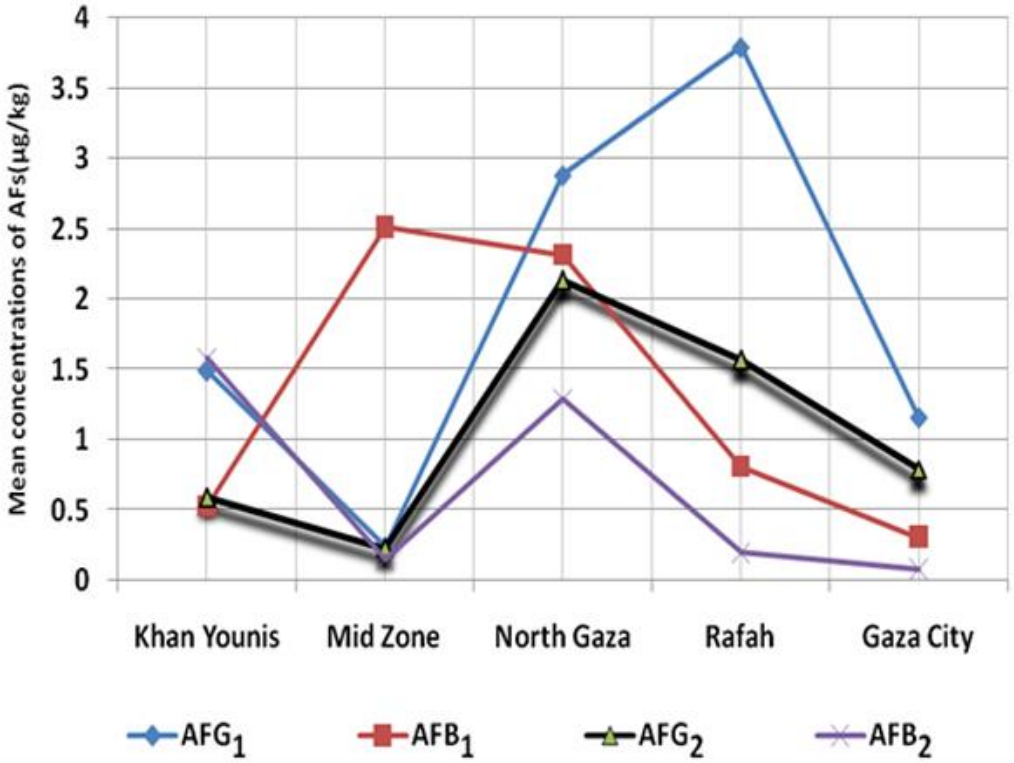

Figure 4. Mean concentrations of AFs contamination of wheat grain samples collected from fivedifferentGaza governorates.

were *A. flavus*, *A. parasiticus*, *F. oxysporum, A. sniger* and *A. alternate,* which mean probability to produce AFs on wheat grains. Twenty-four percent (12 samples) of samples showed AFs contamination higher than the EU permissible limits (>4 ng/g) and ten percent (5 samples) of samples showed AFB1 contamination above the permissible limits (>2 ng/g). The persistent investigation of cereals consumed every day is very important to save human organism against systematic intake of toxic compounds.

Mycotoxin contamination should be monitored routinely for food safety. It is so important to establish the permanent controlling and monitoring program from the production until consumption of cereals in order to minimize the contamination risk of AFs. On the other hand, the training programs on this problem should be developed especially for farmers and agriculturists. Using the optimum techniques for harvesting, handling and storage and selection of proper time for harvesting reduce or eliminate this problem for foods and prevent the threat to human health and the risk of great economic loss.

Conflict of Interests

The authors did not declare any conflict of interest.

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