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 alternata* 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
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 to early 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 Hocking (1997) and Raper and Fennel (1965).

**Chemical and reagents**

**Aflatoxins**

Aflatoxins B₁, B₂, G₁ and G₂ standards were purchased from Sigma Chemical Co. (St. Louis, MO63118, U.S.A.).

**The immunoaffinity column**

The immunoaffinity column AflaTest® 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. oxysporum64% (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.
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 of another study, confirming that the most commonly isolated fungi from Algerian wheat are *Aspergillus spp.*, *Fusarium spp.*, *Penicillium 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 with 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 AFB1 levels in wheat grains in North Gaza and Mid Zone were 2.31 and 2.51 (ng/g), respectively and AFB1 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 1.** The percentage of fungi associated with wheat grain samples collected from five different Gaza governorates

| Fungal Isolates | No. (%) |
|-----------------|--------|
| *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)  |

*Wheat samples contamination.

---

**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).
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 of wheat grains collected from two different 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) × 100).

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). Trombele et al. (2014) showed that Aflatoxin B1 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 five different Gaza governorates (n=10).

| Concentrations of AFs (µg/Kg)* | Khan Younis | Mid zone | North Gaza | Rafah | Gaza city |
|-------------------------------|------------|----------|------------|-------|-----------|
| AFG<sub>1</sub>               | 1.49       | 0.242    | 2.88       | 3.79  | 1.16      |
| AFB<sub>1</sub>               | 0.523      | 2.51     | 2.31       | 0.807 | 0.305     |
| AFG<sub>2</sub>               | 0.594      | 0.234    | 2.14       | 1.57  | 0.788     |
| AFB<sub>2</sub>               | 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...
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.

**REFERENCES**

Abdullah N, Nawawi A, Othman I (1998). Survey of fungal counts and natural occurrence of aflatoxins in Malaysian starch-based foods. Mycopathologia 143(1):53-58.

Alam S, Shah HU, Magan N (2009). Water availability affects extracellular hydrolytic enzyme production by *Aspergillus flavus* and *Aspergillus parasiticus*. World Mycotoxin J. 2(3):313-322.

Embaby EM, Ayaat NM, Abd El-Hamid NH, Abdel-Galil MM, Younos MA (2012). Detection of fungi and mycotoxins affected wheat quality. J. Appl. Sci. Res. 7:3382-3392.

EU (2010). European Union Commission Regulation (EU) No. 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. Off J. Eur. Union L 50:8-12.

Gallo G, Lo Bianco M, Bognanni R, Saimbene G (2008). Mycotoxins in durum wheat grain: hygienic-health quality of sicilian production. J. Food Sci. 73(4):T42-T47.

Giray B, Girgin G, Engin AB, Aydin S, Sahin G (2007). Aflatoxin levels in wheat samples consumed in some regions of Turkey. Food control 18:23-29.

IGAFN (1980). International groundnut *Aspergillus flavus*. Nursery ICRISAT J. pp. 1-25.

Joshaghani H, Namjoo M, Rostami M, Kohsar F, Niknejad F (2013). Mycoflora of Fungal Contamination in Wheat storage (silos) in Golestan Province, North of Iran. Jundishapur J. Microbiol. 6(4):e6334.

Kachuei R, Yadegari MH, Rezaie S, Allameh A, Safaie N, Zaini F, Khanezad YF (2009). Investigation of stored wheat mycoflora, reporting the Fusarium cf. langsethiae in three provinces of Iran during 2007. Ann Microbiol. 59(2):383-390.

Logrieco A, Bottalico A, Mulé G, Moretti A, Perrone G (2003). Epidemiology of Toxigenic Fungi and their Associated Mycotoxins for Some Mediterranean Crops. Eur. J. Plant Pathol. 109(7):645-667.
Macdonald S, Prickett TJ, Wildey KB, Chan D (2004). Survey of ochratoxin A and deoxynivalenol in stored grains from the 1999 harvest in the UK. Food Addit. Contamin. 21(2):172-181.

Moss MO (1998). Recent studies of mycotoxins. J. Appl. Microbiol. Symp. 27(Suppl 84):62S-76S.

Nelson PE, Toussoun TA, Marasas WFO (1983). Fusarium Species-An Illustrated Manual for Fusarium Research. The Pennsylvania State University Press, University Park and London.

Pelhate J (1977). Maize silage: Incidence of moulds during conservation. Folia Vet. Lat. 7(1):1-16.

Pitt JI, Hocking AD (1997). Fungi and food spoilage, 2nd edn, London: Blackie Academic and Professional.

Placinta CM, D'Mello JPF, Macdonald AMC (1999). A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. Anim. Feed Sci. Technol. 78(1-2):21-37.

Raper KB, Fennell DI (1965). The genus Aspergillus. pp. ix+686.

Riba A, Mokrane S, Mathieu F, Lebrihi A, Sabaou N (2008). Mycoflora and ochratoxin A producing strains of Aspergillus in Algerian wheat. Int. J. Food Microbiol. 122(1-2):85-92.

Roigé MB, Aranguren SM, Riccio MB, Pereyra S, Soraci AL, Tapia MO (2009). Mycobiota and mycotoxins in fermented feed, wheat grains and corn grains in Southeastern Buenos Aires Province, Argentina. Rev. Iberoam Micol. 26(4):233-237.

Trombete FM, Moraes DD, Porto YD, SantosTB, Direito GM, Fraga ME, Saldanha T (2014). Determination of Aflatoxins in Wheat and Wheat by-products Intended for Human Consumption, Marketed in Rio de Janeiro, Brazil. J. Food Nutr. Res. 2(10):671-674.

Tutelyan VA (2004). Deoxynivalenol in cereals in Russia. Toxicol. Lett. 153(1):173-179.