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Mycoflora of Fungal Contamination in Wheat Storage (Silos) in Golestan Province, North of Iran

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A B S T R A C T

Background: Cereal products are susceptible to mould damage during pre- and post-harvesting stages of the production. The regional specificity of Golestan province in the northern region of of Iran, with its high temperature and high relative humidity, acts as a leading factor for the growth of aflatoxin-producing fungi. It is well known that contamination of starch-based ingredients with mycotoxigenic fungi is a risk factor among the consumers due to its aflatoxins.
Objectives: This survey was carried out to determine the extent of fungal contamination of wheat in three silos of Golestan province in Iran.
Materials and Methods: 34 samples from three active silos were collected in order to clean the polyethylene bags. Wheat analyzed for fungal contamination and aflatoxins extracted by immunoaffinity column chromatography, and measured by HPLC method.
Results: The most common moulds isolated were *Alternaria* spp. 26.7%, *Aspergillus niger* 21.4%, *Fusarium* spp. 17.8%, *Aspergillus flavus* 10.7%, *Cladosporium* spp. 10.7%, *Penicillium* spp. 8.9%, and *Rhizopus* spp. 3.5%. The screening of aflatoxin, B1, B2, G1 and G2 was carried out. 10(29.4%) samples of wheat had traces of aflatoxin, but in a level lower than the standard levels [Institute of Standards and Industrial Research of Iran (ISIR< 15 ng/g)].
Conclusions: Despite the lower detected aflatoxin levels (lower than the ISIR level), the fungal contamination rate could not be neglected. Since the isolated mycotoxigenic fungi such as *Aspergillus* spp. and *Fusarium* spp. are important in food industry, it would be possible that the increased retention time of samples might have raised the detected contamination rate.

Keywords: Wheat; Fungal Contamination; Aflatoxin

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Implication for health policy/practice/research/medical education:
The present study on wheat silo in Golestan province is the first survey of fungal contamination of cereal in Golestan Province and may be useful for further mycotoxin evaluation.

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1. Background

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 (1-3). Mycotoxins are fungal secondary metabolites produced by some phytopathogenic spoilage fungi such as *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* species that are hazardous for consumers' health, and lead to economic losses of commercial value of food products (4). Toxicogenic *Alternaria* and *Fusarium* species are often classified as field fungi, while *Aspergillus* and *Penicillium* species are considered as storage fungi (5).

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 (6). Cereal products are at risk to mould damage through pre- or post-harvesting stages of agriculture. Food products with high fungal contamination and higher humidity rates are susceptible to early spoilage if inappropriately stocked (7). Contamination of starch-based ingredients with mycotoxigenic fungi parallels the risk of mycotoxicosis among the consumers. Climate diversity of Golestan province, located in north of Iran, with its uniform high temperature and high relative humidity, may be a conducive factor for the growth of aflatoxin-producing fungi.

2. Objectives

Due to the importance of the contamination of starch-based ingredients with mycotoxigenic fungi and existence of any data on the natural occurrence of mycotoxigenic fungi and also the levels of wheat's aflatoxin in Golestan province, the present survey was conducted in order to determine the extent of fungal contamination of wheat in three silos of Golestan province in Iran.

3. Materials and Methods

3.1. Sample Collection

During 2008 and 2009, a total of 34 samples (500 g) of wheat from 3 active silos in Golestan province, North of Iran, including 14 reservoirs in Gorgan (Latitude36°50´ N; Longitude: 54°44´E), 11 reservoirs in Gonband (Latitude: 34°33´N; Longitude: 48°71´S), 17 reservoirs in Galikesh (Latitude: 37°25´N; Longitude: 55°48´E) were collected in order to clean the polyethylene bags. Samples were submitted to the mycology laboratory at the University's mycology department, by the aim of assessing any possible fungal contaminations, aflatoxin determination, and fungal identification. The samples were stored at 4°C until the beginning of laboratory analysis.

3.2. Sample Preparation and Analysis

Each sample (20 g) was surface disinfected for 2 minutes with 0.2% sodium hypochlorite solution and rinsed three times with sterile distilled water. From each sample, 40 grains were randomly selected and then put in Petri plates (90 mm diameter, 10 grains/dish) containing Sabouraud's dextrose agar (Merck, Darmstadt, Germany) with 5% chloramphenicol in duplicate. Petri plates were incubated at 25°C for 6 to 10 days. Each pure culture was characterized and identified based on their morphological and microscopic characteristics using the keys of Pitt and Hockings (2) and Raper and Fennel (8).

Aflatoxin concentrations were reported in ng/g of wheat by immunoaffinity column chromatography method (Aflaclean, LCTech, Germany) and evaluated by HPLC system, consisting of a fluorescence detector (Knauer, Germany). Aflatoxins were separated on HPLC column with a mobile phase of water: methanol: acetonitrile (60:30:15, v/v/v), excitation and emission wavelengths of 365 and 440 nm respectively, flow rate of 1.2 ml/min, and retention times of 25 minutes. The data were then analyzed using SPSS software (Version 15) and the P value less than 0.05 was considered significant.

4. Results

4.1. Fungal Isolation and Identification

The most common moulds isolated were *Alternaria* spp. 26.7%and *A. niger* 21.4 % (*Table 1*). The screening of aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2 was carefully performed. 10 (29.4%) samples of wheat had traces of aflatoxin, but in a level lower than that of the Institute of Standards and Industrial Research of Iran (ISIR, < 15 ng/g). No significant differences were observed between the contamination fungal isolates, and the aflatoxin in the grains ($P > 0.05$).

Table1 . The Percentage of Wheat and Samples Containing Identified Fungal Genera

Fungal Isolates	No[a]. (%)
***Alternaria* spp.**	15 (26.7)
Aspergillus niger	11 (21.4)
***Fusarium* spp.**	11 (17.8)
Aspergillus flavus	6 (10.7)%
***Cladosporium* spp.**	6 (6)
***Penicillium* spp.**	5 (8.9)
***Rhizopus* spp.**	2 (3.5)
Other	3 (0.3)

[a] wheat samples contamination

5. Discussion

The purpose of the present study was to assess the fungal contamination of the selected wheat samples, and to subsequently determine the possible contaminations of these samples by aflatoxins. The contamination of wheat 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 (9). 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 (10).

The fungal growth cannot only change the chemical and physical properties of the food products, but also the nutrient content of the grains. Beatriz Roigé in his research showed that *Penicillium* (42%), *Fusarium* (27%) and *Alternaria* (25%) were the most frequently genera recovered from wheat (11). In the present study, members of the genus *Alternaria* spp. and *A. niger* were highly prevalent, while *Fusarium* spp. was the third more frequent fungus isolated from the wheat grains. High incidence rates of *Alternaria* spp. from different geographical areas of the Argentina have been observed in freshly harvested wheat, which is in close agreement with the findings of this research (12, 13).

The high frequency and abundance of *Aspergillus* spp. in the present study's findings could be due to failures in food production and conservation. *Fusarium* was isolated in 17.8 % silage samples. Pelhate reported that *Fusarium* was present at harvesting as a result of field infection, and can no longer stay alive once the oxygen level reduces (14). Similar to the findings of our study, Abdel-Hafez *et al*. showed in their research that *Alternaria* spp. was the most prevalent fungi in harvested wheat and, sorghum dusts from Egypt and *Aspergillus* spp. ranked as the second place (15). *Fusarium*, *Penicillium*, *Alternaria*, Mucor and especially *Aspergillus* (belonging to section Flavi and Nigri) were the most important fungal species, and are commonly isolated from Algerian wheat species (16).

Aflatoxin B1 was detected in only 10 out of 34 wheat samples, and its abundance in only one sample was 6.91 ng/g, which is more than the ISIR level (< 5 ng/g). Although aflatoxigenic fungi were found at high levels in our study, total aflatoxins were found in levels lower than the ISIR level (< 15 ng/g). Therefore, it can be hypothesized that our strains were nontoxic or were not capable of producing aflatoxin up to dangerous levels of toxicity, and that toxin development needs a longer period of time.

Our results were consistent with the findings another research in Argentina that introduces most predominant fungal species of *Alternaria* and *Fusarium* as endogenous mycoflora (17); and another study, confirming that the most commonly isolated fungi from Algerian wheat are *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., *Alternaria* spp. and *Mucor* spp. (16). 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. (57.1–89.2%) and *Alternaria* spp. (82.4–100%) species were the predominant fungal species as endogenous mycoflora (18).

According to our findings, *A. niger* with 21.4% ranked second in fungi isolated from wheat. This may be indicated that air fungal flora is variable in different areas. Although the number of analyzed samples was limited, our 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, even a low 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.

Despite the fact that the total aflatoxin estimated in the samples was lower than the ISIR limits, the fungal contamination rate could not be neglected. Isolation of mycotoxigenic fungi such as *Aspergillus* spp. and *Fusarium* spp. Is of 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.

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Authors' Contribution

None declared.

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