

## Aspergillus and Aflatoxin B<sub>1</sub> Contamination of Stored Corn Grains in Western Iran

<sup>1</sup>Khosrow Chehri, <sup>2</sup>Ehsan Azami and <sup>1</sup>Ali Mosaber

<sup>1</sup>Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran

<sup>2</sup>Department of Biology, Faculty of Science, Payame Noor University, Eslamabad-e-Gharb, Iran

**Abstract:** Maize (*Zea mays* L.) is one of the most important primary food crops and is grown in all the continents of the world. Mycotoxin contamination of stored corn grains has long been a major problem in different regions of Iran, which mostly are produced by toxigenic fungal species. *Aspergillus* species are major causes of pre- and post-harvest degradation of corn grains. This study was aimed to identify morphologically *Aspergillus* species isolated from stored corn grains in Kermanshah province during 2006-2013 growing seasons. One-hundred samples, mostly from diseased corn grains for human and animal consumptions were collected from different geographic regions of the province. One-hundred *Aspergillus* isolates were collected and identified into six species *i.e.* *A. niger* (40%) followed by *A. flavus* (27%), *A. ochraceus* (15%), *A. fumigatus* (10%), *A. japonicus* (5%) and *A. sclerotiorum* (3%). We also determined the Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) contamination status in the samples by enzyme-linked immune sorbent assay (ELISA). Natural occurrence of AFB<sub>1</sub> could be detected in 66 samples ranging from 0.046-10.776 µg/kg. The highest AFB<sub>1</sub> levels were detected in samples from Bisetoon and Sarpol Zehab (up to 10.000 µg/kg).

**Key words:** *Aspergillus* • Aflatoxin B<sub>1</sub> • Corn • Iran

### INTRODUCTION

Maize (*Zea mays* L.) is one of the most widely grown cereal crop and is serves as human food, animal feed and industrial uses [1]. Like any other stored agricultural crops, corn grains are also infected by several types of diseases caused by toxigenic fungi. *Aspergillus* spp. is often associated with diseases on maize that can reduce the yield and quality of the crops which lead to heavy losses to the farmers [1, 2]. Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and Ochratoxin A (OTA) are the major mycotoxin produced by *Aspergillus* spp.. AFB<sub>1</sub> is one of the most potent naturally occurring animal carcinogens [3]. Many studies have shown that these mycotoxins are frequently present in a series of products especially corn grains [4, 5]. Hence various researches demonstrated the possibility of mycotoxigenic fungal contamination during storage of corn grains, but, until today, no attempt has been made to identify members of the *Aspergillus* spp. in Western Iran. Therefore, the objectives of this study were (1) to isolate and identify *Aspergillus* spp. and (2) to determine natural occurrence of AFB<sub>1</sub> in stored corn grains collected from various places in Western Iran.

### MATERIALS AND METHODS

**Isolation and Identification of *Aspergillus* spp.:** During 2006-2013 growing seasons, corn grain samples were collected from 100 commercial markets and warehouses in Western Iran. *Aspergillus* spp. were isolated from the corn grains by the method of Reddy *et al.* [6]. Briefly, 100 seeds were placed on one-half strength potato dextrose agar plates with rose bengal (final concentration 50 ppm) and incubated at 25°C for 7 days. The resulting single-spore *Aspergillus* colonies were transferred to fresh potato dextrose agar (PDA) plates and maintained at 4°C for further studies. The fungal species were identified into species level according to the methods of Klich [7]. For growth rates and pigmentations, the colonies were transferred onto CYA (Czapeks yeast agar), CY 20S (Czapeks yeast agar with 20% sucrose), MEA (malt extract agar) and CZ (Czapeks Dox agar) and incubated at 25°C. For macroscopic and microscopic observations, all isolates of *Aspergillus* were assessed when they were 3, 7 and 14 days old. Colony colour were observed by naked eye and compared with the colour charts of Methuen Handbook of Colour [8].

#### Enzyme-Linked Immune Sorbent Assay (Elisa) Analysis:

According to the protocol of the manufacturer, Aflatoxin B<sub>1</sub> content in the samples was analyzed using the Quantitative Aflatoxin Test Kit (EuroProxima, Netherland). One-hundred subsamples of the whole grain were ground and 3 g of corn powder extracted with 9 ml methanol: water (80:20 v/v) and blended 10 minutes. The mixture was filtered through filter paper (Whatman No. 1, England). The AFB<sub>1</sub> standard solution was placed in microtiter plate. Fifty µl of filtrate samples were mixed with 150 µl of dilution buffer before ELISA analysis and 50 µl of the mixture placed into the each mixing well of the microtiter plate (H<sub>1</sub> to G<sub>11</sub>). Then Microtitre plates were coated by adding 25 µl of antibody solution and 25 µl of conjugate (Aflatoxin-HRP) to each well and incubated for 1 hr in the dark at 37°C in a moist chamber. The plates were emptied, washed thrice with rinsing buffer and 100 µl of substrate was added to each well and incubated for 30 minutes and then added 100 µl of stop solution to each well and mixed prior to absorbance reading at 450 nm for a few seconds.

#### RESULTS AND DISCUSSION

In this study, 100 *Aspergillus* isolates were obtained from corn grain samples and based on morphological characteristics these isolates were identified into six species *i.e.* *A. niger*, *A. flavus*, *A. ochraceus*, *A. fumigatus*, *A. japonicus* and *A. sclerotiorum*. *Aspergillus niger* was the most prevalent with a frequency of 40%, followed by *A. flavus* (27%), *A. ochraceus* (15%), *A. fumigatus* (10%), *A. japonicus* (5%) and *A. sclerotiorum* (3%).

*Aspergillus niger* strains produced black to dark brown colonies. Cultures grew nearly fast, the growth rates (mm/day) on PDA at 25°C in intermittent light ranged from 6.3-7.2 mm/day. Conidial heads produced biserial phialides with metulae covering the entire vesicle's surface. Conidia were globose and sometimes subglobose in shape with finely rough to very rough surfaces. *Aspergillus flavus* strains produced green colonies. Cultures grew fast, the growth rates (mm/day) on PDA at 25°C in intermittent light ranged from 7.5-8.4 mm/day. All isolates produced black shiny sclerotia in oval shape. Conidial heads produced biserial phialides on CYA, PDA and CZ media and almost all isolates were uniseriate on MEA. Conidia were globose to elongate in shape with nearly rough surfaces. *Aspergillus ochraceus* strains produced yellow to pale brown colonies. Cultures grew slowly, the growth rates (mm/day) on PDA at 25°C in intermittent light ranged from 3.8-4.9 mm/day.

Conidial heads produced biserial phialides with metulae tightly packed three quarters to the entire surface of the vesicles. Conidia were globose in shape with smooth to finely roughen. All isolates produced sclerotia with purplish in colours and crowded at the centre of colonies. *Aspergillus fumigatus* strains produced greyish to dark green colonies. Cultures grew nearly fast, the growth rates (mm/day) on PDA at 25°C in intermittent light ranged from 8.3 - 9.2 mm/day. Conidial heads produced uniseriate phialides. Conidia were globose and ellipsoidal in shape with finely rough surfaces that are produced on pyriform vesicles. *Aspergillus japonicus* strains produced brown to dark brown colonies. Cultures grew nearly fast, the growth rates (mm/day) on PDA at 25°C in intermittent light ranged from 8.5-9.4 mm/day. Conidial heads produced uniseriate phialides with metulae covering the entire vesicle's surface. Vesicles were globose to elongate. Conidia were globose and ellipsoidal in shape with finely rough surfaces. *Aspergillus sclerotiorum* strains produced yellow to light brown colonies. Cultures grew slowly, the growth rates (mm/day) on PDA at 25°C in intermittent light ranged from 4.8-5.9 mm/day. Conidial heads produced biserial phialides with metulae covering the entire vesicle's surface. Conidia were globose in shape with smooth to finely roughen. Some isolates produced sclerotia with white to buff in colours in MEA.

The occurrence of AFB<sub>1</sub> produced by *Aspergillus* spp. in corn grains is of one of the great concern worldwide, because their presence in processed feeds and foods seems unavoidable. Consequently, it has been associated with severe mycotoxicosis in humans and animals [9]. Of the 100 samples analysed, 77 % were found positive for AFB<sub>1</sub> contamination ranging from 0.046-10.776 µg/kg. The greatest AFB<sub>1</sub> contamination was detected in samples collected from Bisetoon ranging from 0.056-10.776 µg/kg followed by samples from Sarpol Zehab ranging from 0.152-10.006 µg/kg (Table 1). All samples (100%) from Bisetoon, Ravansar and Gilan Gharb were contaminated with AFB<sub>1</sub> ranging from 0.056-10.776 µg/kg, 0.480-6.564 µg/kg and 0.880-7.776 µg/kg, respectively (Table 1).

AFB<sub>1</sub> is the mycotoxin most frequently reported in corn grains and is more commonly reported for maize than for many other cereal crops [6, 10], because maize represents a very good substrate for *Aspergillus* growth and toxinogenesis. Corn grains in Iran are chiefly used for domestic animal feeds. Various studies revealed the possibility of AFB<sub>1</sub> contamination during importation and storage of corn grains [11, 12]. Nogaim *et al.* [13]

Table 1: *Aspergillus* spp. and AFB<sub>1</sub> detected in corn grains from Western Iran

Place of sample collection	Total No of samples	Number of samples infected with <i>Aspergillus</i> spp.	Frequency of isolation (Fr) (%)	AFB <sub>1</sub> levels Positive Samples/Range (µg/kg)	<i>Aspergillus</i> spp.
Bisotun	10	10	(100%)	0.056–10.776	<i>A. niger</i> , <i>A. flavus</i> , <i>A. ochraceus</i> , <i>A. fumigatus</i> , <i>A. japonicus</i> and <i>A. sclerotiorum</i>
Gilan Gharb	10	10	(100%)	0.880–7.776	<i>A. niger</i> , <i>A. flavus</i> and <i>A. ochraceus</i>
Kamyaran	10	6	(60%)	0.312–8.446	<i>A. niger</i> , <i>A. flavus</i> and <i>A. ochraceus</i>
Kermanshah	10	7	(70%)	0.408–7.344	<i>A. niger</i> , <i>A. flavus</i> , <i>A. ochraceus</i> , <i>A. fumigatus</i> and <i>A. japonicus</i>
Kangavar	10	7	(70%)	0.396–6.875	<i>A. niger</i> , <i>A. flavus</i> and <i>A. ochraceus</i>
Qasr Shirn	10	6	(60%)	0.160–0.446	<i>A. niger</i> , <i>A. flavus</i> and <i>A. ochraceus</i>
Ravansar	10	10	(100%)	0.480–6.564	<i>A. niger</i> , <i>A. flavus</i> , <i>A. ochraceus</i> , <i>A. fumigatus</i> , <i>A. japonicus</i> and <i>A. sclerotiorum</i>
Sarpol Zohab	10	8	(80%)	0.304–10.006	<i>A. niger</i> , <i>A. flavus</i> , <i>A. ochraceus</i> , <i>A. fumigatus</i> , <i>A. japonicus</i> and <i>A. sclerotiorum</i>
Sahneh	10	5	(50%)	0.384–2.800	<i>A. niger</i> , <i>A. flavus</i> , <i>A. ochraceus</i> , <i>A. fumigatus</i> and <i>A. japonicus</i>
Sonqor	10	8	(80%)	0.046–0.232	<i>A. niger</i> , <i>A. flavus</i> and <i>A. ochraceus</i>
Total	100	77	77%	3.426-61.265	

found that the levels of AFB<sub>1</sub> in the tested corn samples collected from Egypt were 5.8-7.5 µg/kg. Also, AFB<sub>1</sub> were detected in 5 out of the 11 corn samples, at levels ranging from 2.5 to 12.2 µg/kg in Malaysia [14] and our results are in harmony with these researchers. Aflatoxins are produced as metabolites by the *Aspergillus* spp. in Flavi group (*A. flavus* and *A. parasiticus*) and exist in nature worldwide [15, 16, 17, 18, 19, 20, 21] and in our study, *A. flavus* were the most dominant from all samples.

These studies revealed the high frequency of AFB<sub>1</sub> contaminants in the corn grains in Western Iran and hopefully useful in feasible management strategies for reduction of mycotoxin contamination by several means including botanicals and biocontrol agents.

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