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Research Article

Identification of *Aspergillus flavus* and aflatoxins contamination in inflorescences of wild grasses in Iran

Khosrow Chehri[1*] and Seyyed Meqdad Hasani[2]

1. Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran.
2. Department of Biology, Faculty of Science, Islamic Azad University, Yasuj Branch, Iran.

Abstract: Wild grasses are the most important primary feedstuffs which are susceptible to contamination with toxigenic fungi belonging to *Aspergillus* spp. In order to explore diversity of *Aspergillus* species associated with the inflorescences of gramineous weeds, infected inflorescences were collected from wild grasses in western parts of Iran. Fifty-six *Aspergillus* isolates were obtained from all diseased spikes and based on morphological features identified as 4 species i.e. *Aspergillus niger* (26) followed by *Aspergillus flavus* (24), *Aspergillus fumigatus* (4), and *Aspergillus japonicus* (2). The identification of *A. flavus* was confirmed using species specific primers of AFLA-F/AFLA-R by producing amplicons about 413 bp. In this study, aflatoxins (AFs) contamination of wild grasses was evaluated by enzyme linked immunosorbent assay (ELISA). Natural occurrence of AFs could be detected in 24 samples ranging from 0.63-134.86 µg/kg. The highest AFT levels were detected in samples from Ravansar, Bisetoon, Mahidasht, and Sarpol Zehab (up to 50 µg/kg), which is more than the recommended limits by European Union standard and National Standard of Iran (20 µg/kg for animal feed).

Keywords: Aflatoxins, species specific primers, ELISA

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Introduction

Wild grasses convert the sun's energy into carbohydrates for their own food and are also feed for animals. So, wild grasses are of major importance in animal nutrition throughout the world and in Iran. Also, Fibrous roots of wild grasses build up soil fertility and improve soil texture (Badaeva *et al*., 2002; Yazdanseta *et al*., 2004). Like any other agricultural and non-agricultural plants, wild grasses are also infected by several types of diseases caused by toxigenic fungi. Various fungi are capable of producing toxins. These mycotoxins are produced by fungi during growth or storage of grains and nuts (Liu *et al*., 2006; Iqbal *et al*., 2006; Karami-Osboo *et al*., 2012). One of the most frequently reported mycotoxins in cereals is aflatoxin (Rasti *et al*., 2000; Reddy *et al*., 2009), because cereals represent a very good substrate for *Aspergillus* growth and toxinogenesis. Aflatoxins are a family of compounds produced primarily by toxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* (Yu *et al*., 2004; Karami-Osboo *et al*., 2012). Although other fungi, such as *Penicillium* spp., *Rhizopus* spp., and *Fusarium* spp. are capable of producing toxins, their relevance to livestock production (mainly ruminants) has not been established (CAST, 2003; FAO, 2004; Speijers and Speijers, 2004). Aflatoxin is the major mycotoxin contaminant of food and feeds worldwide, although other compounds such as ochratoxin A and trichothecenes can be locally important in

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* **Corresponding author**, e-mail: khchehri@gmail.com
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some years (Wiatrak *et al*., 2005; Khayoon *et al*., 2010). AFs are most toxic to mammals, causing dissimilar harmful effects including hepatotoxic, carcinogenic, teratogenic and mutagenic, causing toxic hepatitis, immunosuppression, equine leukoencephalomalacia, and oesophageal cancer (Li *et al*., 2001; Ardic *et al*., 2008). In many countries, to safeguard the health of humans and animals, AFs effect on animal health was investigated. The European Union has a maximum tolerance level of 20 ng/g for AFT in animal feed. However, the Iranian standard institute has set a maximum tolerance level of 15 ng/g for AFT in animal feed (CAST, 2003; FAO, 2004). Aflatoxins contamination of gramineous weeds in animal feed could later pose human health hazards even at low levels because of the production of animals' milk being consumed by humans (Li *et al*., 2001; CAST, 2003; FAO, 2004; Ardic *et al.,* 2008). The accurate information about maximum level of AFs in grassy weeds is therefore necessary in order to prevent its mal-effects.

Many studies have demonstrated that AFs are frequently present in a series of products especially cereals in different countries including Iran (Rahimi *et al*., 2007; Levin, 2012). Unfortunately, until today, not enough attempts have been made to identify members of the *Aspergillus* spp. associated with wild grasses in Iran and elsewhere (Pacin *et al*., 2002; Hadian *et al*., 2013). Therefore, the objectives of this study were (1) to isolate and identify *Aspergillus* spp. and (2) to determine natural occurrence of AFs in inflorescences of wild grasses in parts of Western Iran.

Materials and Methods

Sample collection and identification of poaceous weeds

A survey was carried out during two seasons (2012 and 2014) in four agro-ecological zones in western Iran including Mahidasht, Sarpole-Zahab, Bisetoon, and Kermanshah districts. In each agro-ecological zone, 14 farms were randomly selected, giving a total of 56 farms. In each farm infected heads and inflorescences of wild grasses were collected. All native grasses of Poaceae family were identified based on pollen morphology using light microscopy (LM) and scanning electron microscope (SEM). To identify Poaceous weeds, plant specimens were transferred to the herbarium at Razi University. For LM observations, pollens were acetolysed following the technique of the Erdtman (1960) and mounted in glycerine jelly. Slides were prepared for LM by mounting pollen in glycerin jelly. Size measurements were taken based on 25 pollen grains; the values of P (polar axis length) and E (equatorial diameter) were measured and the P/E ratios were calculated. Measurements were recorded using both a 40 × objective, and a crossed micrometer eyepiece graticule. For SEM studies unacetolysed pollen grains were examined. The SEM studies were carried out at Razi Metallurgical Research Center (RMRC).

Isolation and Identification of *Aspergillus* spp.

Infected heads and inflorescences were randomly hand-collected and plated onto potato dextrose agar (PDA) 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 PDA medium and maintained at 4 °C for further studies. The fungal species were identified according to the methods of Klich (2002). For growth rates and pigmentations, the colonies were transferred onto CY 20S (Czapeks yeast agar with 20% sucrose), CYA (Czapeks yeast agar), CZ (Czapeks Dox agar), and MEA (malt extract 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 colours were observed by naked eye and compared with the colour charts of Methuen Handbook of Colour (Kornerup and Wanscher, 1978).

Molecular identification of *Aspergillus flavus* using species-specific PCR

Potato dextrose broth (PDB) was used to grow the *A. flavus* strains to produce mycelium for DNA extraction. Studied isolates belonging to *A. flavus* were grown on PDB with shaking at

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100 rpm at room temperature for 5 days. Mycelia were harvested by filtration through Whatman paper No. 1 and freeze-dried for 20 h and DNA was extracted using a DNeasy® Plant Mini Kit (Qiagen) according to the manufacturer's protocol. All *A. flavus* isolates were identified with species-specific PCR assay using previously published primer pairs for *A. flavus* with the sequences 5'-GGTGGTGA-AGAAGTCTATCTAAGG-3 for AFLAF and 5'-AAGGCATAAAGGGTGTGGAG-3' for AFLA-R (Hue *et al.,* 2013). Amplification reactions were performed in a total volume of 25 µl, by mixing12.5 µl PCR master mix 2x (Promega), 12.5 pmol of each primer, ±100 ng/µl DNA template and nuclease free water. PCR amplification was carried out in the Peltier Thermal Cycler, PTC-100® (MJ Research, Inc. USA) with the following programs: an initial denaturation step at 94 °C for 5 min, 35 cycles of 94 °C (30s)/58 °C (60s) /72 °C (90s), and a final extension step at 72 °C for 7 min. The PCR products were visualized by 1 × TBE electrophoresis in ethidium-bromide-stained 1% agarose gel.

Enzyme-linked immunosorbent assay (ELISA) analysis

According to the protocol of the manufacturer, Aflatoxins content in the samples was analyzed using the Quantitative Aflatoxins Test Kit (Neogen Technical Services). After fungal isolation, the whole infected heads of wild grasses were transferred immediately to the incubator with high humidity (85%) at 33 °C for 7 days. All samples were ground and 5g of infected heads extracted with 250 ml methanol: water (70: 30 v/v) and blended 5 minutes. The mixture was filtered through filter paper (Whatman No. 3, England). All reagents were mixed by swirling the reagent bottle prior to use. The AFs levels were detected by manufacturers' protocol; briefly, 100 µl of each conjugate and AFs standard solution (0, 5, 15, and 50) were transferred from the blue-labelled bottle to the red-marked mixing wells and mixed the liquid in the wells by pipetting it up and down 3 times. Then 100 µl of the samples were transferred to the antibody-coated wells and mixed by sliding the microwell holder back and forth on a flat surface for 10-20 seconds without splashing reagents from the wells. All samples were incubated for 2 min at room temperature 25 ± 2 °C and then discarded the red-marked mixing wells Each antibody well was filled with deionized or distilled water using a wash bottle and then dumped the water out. This step was repeated 5 times, then turned the wells upside down and taps out on a paper towel until the remaining water was removed. The needed volume of substrate were transferred from the green-labeled bottle into the green-labeled reagent boat and, with new tips added 100 µl of substrate into the wells and mixed by sliding back and forth on a flat surface for 10-20 seconds, then incubated for 3 minutes. The remaining substrates were discarded and the reagent boat was rinsed with water. The red stop solution was transferred from the red-labelled bottle (same volume as prepared for substrate) into the red-labelled reagent boat. Using the same pipette tips as were used to dispense substrate, added 100 µl red stop to each well and mixed by sliding back and forth on a flat surface. The bottom of micro wells were wiped with a dry cloth or towel and read in a microwell reader using a 650 nm filter. Air bubbles should be eliminated, as they could affect analytical results. Results were read within 20 minutes of completion of the test and data were calculated using Neogen's Log/Logit Software.

Results

Fifty-six samples, mostly from infected spikes were collected from different sites of western Iran. A total of 56 *Aspergillus* isolates were recovered from diseased inflorescences belonging to 9 tribes of Poaceae family (Table 1). All isolates were identified into four species i.e. *A. niger*, *A. flavus, A. fumigatus,* and *A. japonicas*. *Aspergillus niger* was the most prevalent with a frequency of 46%, followed by *A. flavus* (43%), *A. fumigatus* (7%), and *A. japonicus* (4%) (Table 1).

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Twenty-four out of 56 total *Aspergillus* strains showed deep green to olive green in colour, reverse side of culture plates were yellow and exudates were rarely present. Colonies always floccose centrally while on CYA, fluffy, wrinkles colonies were formed (Table 2). *Aspergillus flavus* produced dark brown to black shiny sclerotia oval in shape, deep green conidial heads and spherical to elongate vesicles. Conidia were globose to elongate in shape with rough surfaces. Some isolates were biseriate on CYA and almost all isolates were uniseriate on MEA (Fig. 1). Microscopic and macroscopic characters of *A. flavus* are presented in Table 2. Further identification using molecular analysis by species specific primer (AFLA-F/AFLA-R) demonstrated that all isolates identified as A. flavus based on morphological characters, produced amplicon size of about 413 bp (Fig. 2) and molecular analysis confirmed that all 24 isolates were *A. flavus* species.

Table 1 Overall frequencies and percentage of various *Aspergillus* species and AFs detected in inflorescences of different species of Poaceae family in Iran.

Place of sample collection	Isolation sources	n	No of spikes (%)[1]	AFs levels (µg/kg)[2]	*Aspergillus* spp. (No)
Sarpole-Zahab districts					
Sarpole-Zahab	*Avena wiestii, Bromus sericeus, Dactylis glumerata*	6	6 (100)	103.00 µg/kg, 1.14 µg/kg, 0.63 µg/kg	*A. niger* (2), *A. flavus* (3), *A. fumigates* (1), *A. japonicas* (1)
Gilan Gharb	*Avena wiestii, Bromus sericeus, Dactylis glumerata, Ermopoa persica*	4	4 (100)	71.03 µg/kg	*A. niger, A. flavus* (1)
Qasre-Shirin	*Avena wiestii, Lolium perenne, Melica jacquemontii, Aegilops cylindrical*	2	2 (100)	0.66 µg/kg	*A. niger* (2), *A. flavus* (1)
Eilam	*Ermopoa persica, Aegilops cylindrical, Stipa barbata, Agropyron repens*	2	2 (100)	0.68 µg/kg	*A. niger, A. flavus* (1)
Kermanshah districts					
Ravansar	*Avena wiestii, Aegilops cylindrical, Stipa barbata, Agropyron repens*	6	6 (100)	134.86 µg/kg, 1.05 µg/kg, 1.15 µg/kg	*A. niger* (2), *A. flavus* (3), *A. fumigates* (1), *A. japonicas* (1)
Kermanshah	*Lolium perenne, Melica Jacquemontii, Aegilops cylindrical, Stipa barbata*	6	6 (100)	127.65 µg/kg, 1.36 µg/kg, 1.62 µg/kg	*A. niger* (2), *A. flavus* (3), *A. fumigates* (1)
Kamyaran	*Lolium perenne, Melica Jacquemontii, Aegilops cylindrical, Stipa barbata*	2	2 (100)	80.22 µg/kg	*A. niger* (2), *A. flavus* (1)
Bisetoon districts					
Sahneh	*Avena wiestii, Bromus sericeus, Dactylis glumerata, Agropyron repens*	2	2 (100)	98.48 µg/kg	*A. niger* (2), *A. flavus* (1)
Bisotun	*Bromus sericeus, Dactylis glumerata Melica Jacquemontii, Agropyron repens*	6	6 (100)	117.05 µg/kg, 1.24 µg/kg, 1.39 µg/kg	*A. niger* (2), *A. flavus* (3), *A. fumigates* (1)
Kangavar	*Dactylis glumerata, Ermopoa persica, Lolium perenne, Stipa barbata*	4	4 (100)	1.04 µg/kg	*A. niger* (2), *A. flavus* (1)
Harsin	*Avena wiestii, Lolium perenne, Melica Jacquemontii, Aegilops cylindrical*	2	2 (100)	1.07 µg/kg	*A. niger* (2), *A. flavus* (1)
Mahidasht districts					
Mahidasht	*Ermopoa persica, Lolium perenne, Aegilops cylindrical, Agropyron repens*	6	6 (100)	103.00 µg/kg	*A. niger* (2), *A. flavus* (3),
Kouzaran	*Avena wiestii, Bromus sericeus, Aegilops cylindrical, Agropyron repens*	4	4 (100)	95.64 µg/kg	*A. niger* (2), *A. flavus* (1)
Eslam Abad	*Avena wiestii, Lolium perenne, Melica Jacquemontii, Aegilops cylindrical*	4	4 (100)	1.16 µg/kg	*A. niger* (2), *A. flavus* (1)

n: Total No of spike samples.
[2] No of spike samples infected with *Aspergillus* spp. (percentage).
[3] AFs levels Positive Samples/Range (µg/kg).

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Table 2 Microscopic and macroscopic characters of *Aspergillus flavus* isolates.

Characters	MEA	PDA	CYA	CZ	CZ 37 °C
Colony diameter (mm)	70	80	80	68	90
Colony color	Deep green	Deep green to olive green	Yellow to bright yellow	Deep green	Light green to deep green
Conidia color	Deep green	Deep green	Light yellow	Deep green	Deep green
Colony texture	Floccose especially centrally, light concentric rings formed	Floccose especially centrally, light concentric rings formed	Fluffy, radially furrowed	Granular	Low, plane, not deep
Mycelium	White to parrot green	White to parrot green	White	White to parrot green	White to parrot green
Reverse	Yellow to pale brown, wrinkled mycelial growth	Light white, normal mycelial growth	Yellowish, wrinkled mycelial growth	Uncolored to light white	Uncolored to light white
Sclerotia	Present	Absent	Present	Present	Absent
Sclerotia shape	Oval	-	Oval	Oval	-
Sclerotia color	Dark brown	-	Dark brown	Dark brown	-
Conidiophore length (µm)	430-520	600-700	560-660	670-760	Not tested
Conidiophore breadth (µm)	10.2-11.00	10.4-11.00	9.0-12.2	9.8-10.8	Not tested
Conidiophore color	Pale brown	Pale brown	Pale brown	Pale brown	Not tested
Surface texture	Finely rough	Finely rough	Finely rough	Finely rough	Not tested
Conidiosphore size (µm)	2.5-3.6	3.0-3.8	3.2-4.2	3.6-4.4	Not tested
Conidiospore shape	Globose to ellipsoidal	Globose to ellipsoidal	Globose to ellipsoidal	Globose to ellipsoidal	Not tested
Surface texture	smooth to finely roughened	smooth to finely roughened	smooth to finely roughened	smooth to finely roughened	Not tested
Vesicle diameter (µm)	22.0-44.0	30.0-34.8	23.2-29.0	25.0-35.0	Not tested
Vesicle shape	Spherical to elongate	Spherical to elongate	Spherical to elongate	Spherical to elongate	Not tested
Phialides	Uniseriate	Biseriate	Biseriate	Biseriate	Not tested

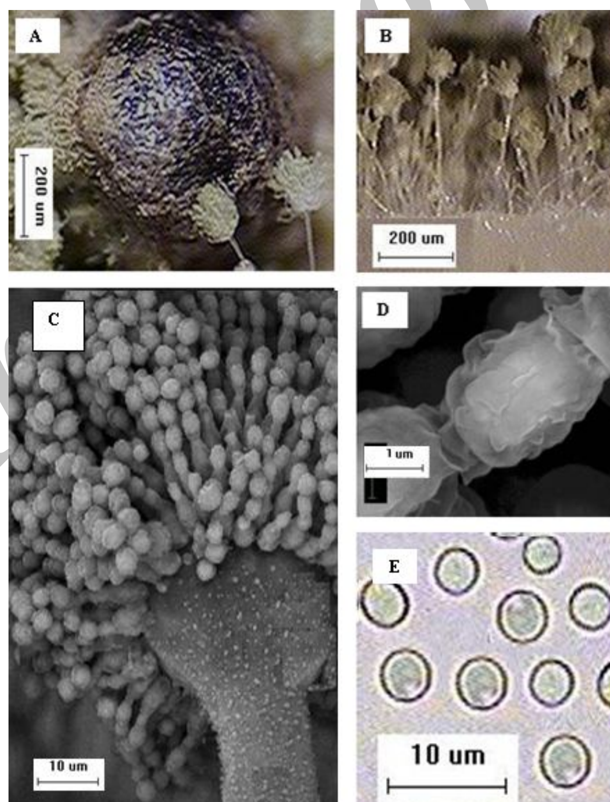

Figure 1 Microscopic characteristics of *Aspergillus flavus* isolated from inflorescences of wild grasses in Iran. (A) Black shiny sclerotia, (B) Radiate to columnar conidial heads, (C) Uniseriate phialides and finely rough-walled conidiophores, (D) Elongate conidia, (E) Globose conidia.

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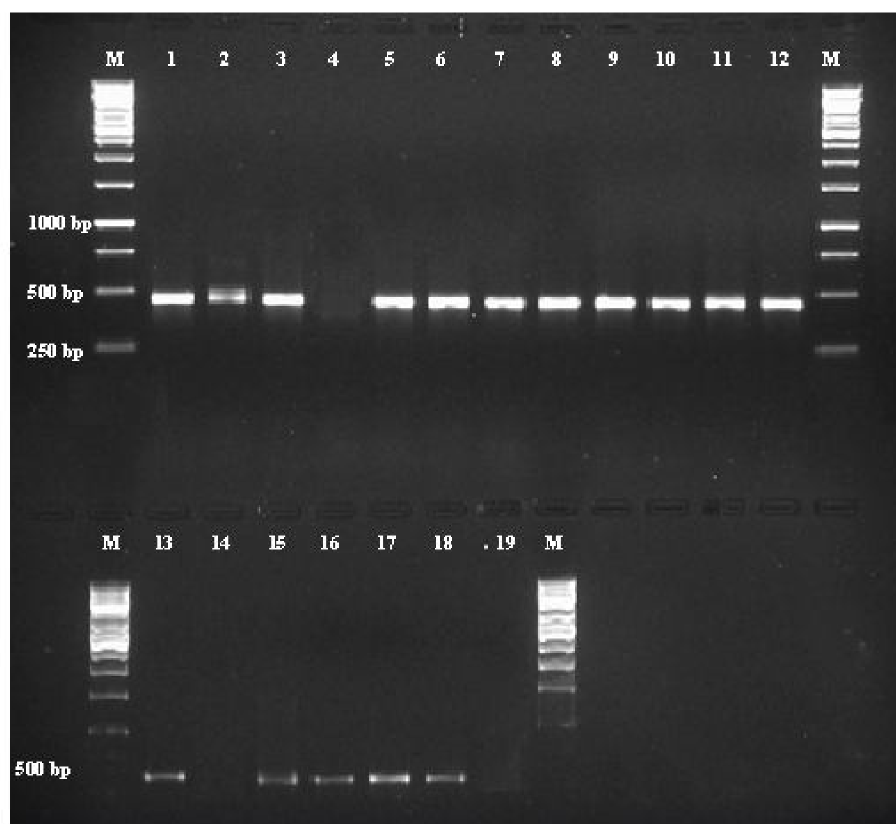

Figure 2 PCR products obtained from specific primer pair AFLA-F/AFLA-R (band, 413 bp) for 16 isolates of *Aspergillus flavus*. Lane M: GeneRuler 1 kb DNA Ladder. (1 = Asperflav33weed, 2 = Asperflav34weed, 3 = Asperflav134weed, 4 = *A. fumigates*10weed, 5 = Asperflav35weed, 6= Asperflav36weed, 7 = Asperflav37weed, 8 = Asperflav38weed, 9 = Asperflav39weed, 10 = sperflav40weed, 11 = Asperflav41weed, 12 = Asperflav42weed, 13 = Asperflav43weed, 14 = *A. niger*12weed, 15 = Asperflav44weed, 16 = Asperflav45weed, 17 = Asperflav46weed, 18 = Asperflav47weed, 19 = *A. japonicus*48weed isolates.

Of the 56 samples analysed, 43% (24) were found positive for AFs contamination ranging from 0.63–134.86 µg/kg (Tables 1 and 3). All samples from Mahidasht, Sarpole-Zahab, Bisetoon, and Kermanshah districts were contaminated with AFs. The highest AFs levels were detected in samples from Kermanshah district (134.86 µg/kg, 127.65 µg/kg and 80.22 µg/kg) followed by Bisetoon (117.05 µg/kg and 98.48 µg/kg), Mahidasht (103.00 µg/kg and 95.64 µg/kg), and Sarpol Zehab (103.00 µg/kg and 71.03 µg/kg) districts (Table 3). Results in this research showed, 9 out of 56 (16%) samples had contamination levels higher than the maximum level of AFs Quantitative Test kit (range of quantitation 5-50 µg/kg) and 13 samples (23%) had contamination levels lower than the minimum level of AFs Quantitative Test kit (Fig. 3).

Discussion

Mycotoxins can enter food chains as contaminated plant products, or as carry-through in other products, *e. g.*, milk or meat, from animals that have consumed contaminated feed (Accensi *et al*., 2004; Frenich *et al*., 2009). Wild grasses in Iran are one of the main feeds used for domestic animal. So, the establishment of a maximum permissible level of the most potent and frequently found mycotoxin in wild grasses, AFs, should be defined in all countries where wild grasses are the staple feeds (Brown *et al*., 1981).

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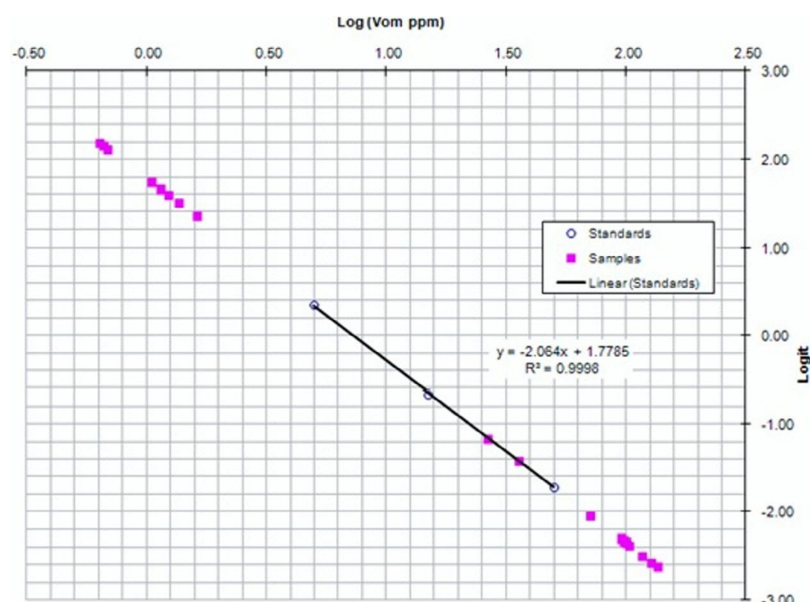

Figure 3 Concentrations of aflatoxins recovered from inflorescences of wild grasses in Iran by AFs Quantitative Test kit (range of quantitation 5-50 µg/kg).

Table 3 AFs levels detected by ELISA method in inflorescences of wild grasses in Iran.

Conc. (ppb)	O.D.(@ 650nm)	(ppb)	Conc. (ppb)	O.D.(@ 650nm)	(ppb)
0	0.941	0.00	Sample 11	0.072	117.05
5	0.551	4.95	Sample 12	0.770	1.36
15	0.319	15.32	Sample 13	0.080	103.00
50	0.143	49.50	Sample 14	0.064	134.86
Sample 1	0.791	1.14	Sample 15	0.067	127.65
Sample 2	0.085	95.64	Sample 16	0.224	26.63
Sample 3	0.182	35.77	Sample 17	0.747	1.62
Sample 4	0.083	98.48	Sample 18	0.108	71.03
Sample 5	0.846	0.63	Sample 19	0.781	1.24
Sample 6	0.843	0.66	Sample 20	0.767	1.39
Sample 7	0.080	103.00	Sample 21	0.098	80.22
Sample 8	0.840	0.68	Sample 22	0.801	1.04
Sample 9	0.800	1.05	Sample 23	0.798	1.07
Sample 10	0.790	1.15	Sample 24	0.789	1.16

Samples 1,5,13 = Afl.Sarpol.1, Afl.Sarpol.5, Afl.Sarpol.13; Samples 9,10,14= Afl.Ravansar.9,10,14; Samples 12,15,17= Afl.Kermanshah.12,15,17; Samples 11,19,20 = Afl.Bisotun.11,19,20; Sample 18 = Afl.GilanGarb.18; Sample 6 = Afl.QasreShirin.6; Sample 8 = Afl.Eilam.8; Sample 21 = Afl.Kamyaran.21; Sample 4 = Afl.Sahneh.4; Sample 22 = Afl.Kangavar.22; Sample 23 = Afl.Harsin.23; Sample 7 = Afl. Mahidasht.7; Sample 2 = Afl. Kouzaran.2; Sample 24 = Afl.EslamAbad.24.

The presence of fungi such as *A. flavus* in wild grasses in Iran can lead to the presence of mycotoxins produced by this fungus that may cause serious toxicity and illness in domesticated animals. In this research, of the 56 samples analysed, 43% were found positive for AFs contamination ranging from 00.63–134.86 µg/kg. Predominance of *A. flavus* as an aflatoxigenic species in feed commodities has been reported in most other important cereals-producing regions worldwide (Pacin *et al.*, 2002; Frenich *et al.*, 2009; Ghiasian *et al.*, 2011; Khayoon *et al.*, 2010) and our results are in harmony with these researchers. In this research the highest AFs levels

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in wild grasses were detected in samples from Ravansar, Bisetoon, Mahidasht, and Sarpole-Zahab. Our results were in agreement with the findings reported by Chehri *et al.* (2015) and Ghiasian *et al*. (2011) who demonstrated that aflatoxin contamination in corn grains were at higher level than in other districts of western Iran. To confirm morphological identification of *A. flavus* from other *Aspergillus* spp., further identification using molecular analysis by species specific primer was applied and results showed that all *A. flavus* isolates produced amplicon size of about 413 bp for AFLA-F/AFLA-R primer pair (Fig. 2). Our results were in agreement with the finding reported by Hue *et al.* (2013) and Nagur *et al.* (2014) who tested the same species specific primer of *A. flavus* to distinguish *A. flavus* from other *Aspergillus* spp. especially *A. parasiticus* that are morphologically very similar. Our studies revealed the high frequency of AFs contamination in the wild grasses in Western Iran which can be used as a guide for better management strategies towards reduction of mycotoxin contamination.

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شناسایی گونه آسپرژیلوس فلاووس (*Aspergillus flavus*) و ردیابی افلاتوکسین در گونه‌های غلات وحشی در غرب ایران

خسرو چهری[1*] و سیدمقداد حسنی[2]

۱- استادیار گروه زیست‌شناسی، دانشکده علوم دانشگاه رازی، کرمانشاه، ایران.
۲- دانشجوی کارشناسی ارشد میکروبیولوژی دانشگاه آزاد اسلامی واحد علوم تحقیقات یاسوج، ایران.
* پست الکترونیکی نویسنده مسئول مکاتبه: khchehri@gmail.com
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چکیده: غلات وحشی از جمله خوراک اولیه احشام محسوب می‌شوند که قابلیت فراوانی به آلودگی‌های قارچی به‌ویژه گونه‌های قارچی تولیدکننده زهرابه نظیر گونه‌های آسپرژیلوس دارند. به‌منظور بررسی تنوع زیستی گونه‌های آسپرژیلوس مرتبط با سنبله علف‌های هرز متعلق به خانواده غلات، سنبله‌های آلوده این گیاهان از مناطق مختلف غرب ایران جمع‌آوری شدند. پنجاه و شش جدایه آسپرژیلوس از تمام خوشه‌های آلوده به‌دست آمد و براساس ویژگی‌های مورفولوژیکی چهار گونه (۲۶) *Aspergillus niger*، (۲۴) *Aspergillus flavus*، (۴) *Aspergillus fumigatus* و (۲) *Aspergillus japonicus* شناسایی شد. شناسایی گونه *A. flavus* با استفاده از آغازگرهای اختصاصی AFLA-F/AFLA-R با تولید باند اختصاصی ۴۱۳ جفت بازی تأیید شد. هم‌چنین در این مطالعه، آلودگی غلات وحشی به آفلاتوکسین (AFs) با استفاده از روش الیزا مورد بررسی قرار گرفت. نتایج ردیابی افلاتوکسین در ۲۴ نمونه مثبت ارزیابی شد و مقدار آن در نمونه‌های مختلف بین ۰/۶۳ تا ۱۳۴/۸۶ میکروگرم بر کیلوگرم تعیین شد. بالاترین سطح آفلاتوکسین در نمونه‌های متعلق به روانسر، بیستون، ماهیدشت، و سرپل ذهاب (بیش از ۵۰ میکروگرم بر کیلوگرم) مشاهده شدکه بالاتر از میزان مجاز استاندارد در اتحادیه اروپا و ایران (۲۰ میکروگرم بر کیلوگرم در غذای حیوانات) می‌باشد.

واژگان کلیدی: افلاتوکسین، پرایمر اختصاصی، الایزا

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