FUNGI ASSOCIATED WITH FRESHLY HARVESTED CORN GRAINS IN DUHOK GOVERNORATE

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ABSTRACT

Freshly harvested grains of five corn hybrids collected from Duhok governorate were screened for their associated mycoflora using agar plate and blotter methods. The percentage occurrence of the isolated fungi was varied with respect to corn hybrid. *Aspergillus niger, A. flavus* and *Rhizopus spp* were the most frequently detected species. From 4 to 70% of grains were infected with fungi and their percentage germination ranged from 53 to 98%. The species composition and percentage germination of grains differed among corn hybrids. A total of 48 strains of *A. flavus* obtained from different hybrids were tested for their aflatoxigenic potential. The positive strains ranged from 70 to 100%

KEY WORD: Corn grains, fungi, aflatoxin, Iraq.

INTRODUCTION

Corn (Zea *mays* L.) is one of the most important dietary staple foods and feedstuffs in different regions of the world (FAO, 2002) This due to its high yields per hectare, its ease of cultivation and adaptability to different agro-ecological zones, versatile food uses and storage characteristics (Asiedu, 1989). Its economical importance is also relevant for its use as feed stuff, mainly in the economically developed countries (Munkvold and Desjardins, 1997). Reports indicate that maize is prone to fungal infection during the pre and post harvest period (Hussein and Brasel, 2001; Abarca et *al.,* 2001).

It is now becoming clear that fungal infection of seeds before and after harvest remains a major problem of food safety. Problems associated with this infection include deterioration of seeds, reduced germ inability and the production of mycotoxins that are toxic to man and animals (Godika *et al,* 1996; Bhutta *et al*. 1997; Bokhari 2002; Abdullah and Al-Mousawy, 2006,2009).

Among the several secondary metabolites produced by filamentous fungi harboring corn grains, aflatoxins are the most important. Aflatoxins are well documented as highly carcinogenic, teratogenic and immunosuppressive (Hocking, 1997; Hedayati *et al*.2007). Aflatoxins are produced by some strains of *Aspergillus* section *Flavi* (Hedayati *et al*.2007).

Investigation on the mycoflora of corn grains and their significance in Iraq was limited to few reports on fungi associated with stored corn and to the incidence of mycotoxins (Al-Heeti *et al.* 1980; Sulaiman and Hassan, 1985; Abdullah and Al-Mousawi, 2006, 2009; Al-Rawi *et al.*2011). However, to our knowledge this study may represents the first survey for fungi associated with freshly harvested corn grains.

This work was conducted to obtain data on the mycobiota associated with freshly harvested corn grains in Duhok governorate and to asses aflatoxin-producing potentials of strains from *Aspergillus* section *Flavi* using simple and rapid method.

Materials and Methods:

Grains of five hybrids of *Corn* (*Zea mays*L.) Zp. 707× Un 44052, Un 44052 × Dk 17, D17 × H5, IK 8 × Zp. 707 and Ik 58 × H5 were obtained from Department of Field Crops, Faculty of Agriculture and Forestry, University of Duhok. The Grains of these hybrids were collected immediately after harvest in 2012.

One hundred fresh harvested grains from each hybrid were surface disinfected with 1% sodium hypochlorite solution in a beaker for 10 min. Two trails were used to isolate fungi, agar plate method and blotter method. For the agar plate method, the surface disinfected grains were placed on two culture media, potato dextrose agar (PDA) and malt extract agar (MEA) (Himedia laboratories, India). Both media were supplemented with 50mg/L chloramphenicol. Ten seeds per Petri plate (10 Petri Plates) were incubated at 24±2 ºC in alternating cycle of 12 hr light and 12 hr darkness for 7 days.

In blotter method, Surface disinfected grains were placed on water soaked blotter in sterilized trays as described by Abdullah and Kadhum (1987).The trays were covered by autoclavable cellophane sheets and fresh grains in the trays were incubated for 7-10 days at 25 ºC. Grains were examined individually under a dissecting microscope. The percentage frequency of occurrence for each fungus and the percentage of both contamination and germination for seeds were calculated. The individual isolates were transferred to potato dextrose agar (PDA) and malt extract agar (MEA) plates with chloramphenicol.

The detected fungi were identified based on morphological and cultural characteristics according to manuals of Pitt and Hocking (1997); Klich (2002).

Detection for aflatoxin-producing strains of *Aspergillus* Section *Flavi*

A rapid detection method for identification of aflatoxin-producing strains of *Aspergillus flavus* depending on the colour change with ammonia vapor was adopted as described by Saito and Machida (1999). Tested strains of *Aspergillu* section *Flavi* were grown in Petri plates of coconut agar (COA). The medium was prepared according to Davis *et al*. (1987). Each strain was inoculated at the center of solidified COA medium in 9-cm Petri dishes and incubated at 25 °C.

To observe of the colour change of colony reverse after incubation, dishes were placed upside down and a small strip of filter paper saturated with ammonia solution was put into the lid of the Petri dishes. The colony reverse of the aflatoxin- producing strains turned pink.

Results and discussion

Fungi associated with the corn grains recorded through agar plate method are presented in Table (1).The occurrence of isolated fungi were varied significantly with respect to corn hybrid. *Aspergillus flavus and A.niger* was found associated with grains of all corn hybrids. These results are in line with several surveys carried out on the crops in several tropical and subtropical regions of the world (Lillehoj and Zuber, 1988; Adebajo *et al.* 1994; Etcheverry *et al.* 1999; Abdullah and Al-Mousawi, 2006; 2009). The highest prevalence of *Aspergillus niger* was recorded in corn hybrids Un 44052×Dk 17 (47%) followed by Ik 58×H5 (27%) and Ik8×Zp.707 (25%) grown on PDA. *A. flavus* was recorded in Zp.707×Un 44052 (19%) followed by the hybrid of Un44052×Dk17 (7%). *A. parasiticus* was isolated by 8% from Ik58×H5 hybrid. These fungi invade maize grains in the field while they are developing on the plants. *Penicillium* spp. was isolated in low frequency in rest of the tested corn hybrids. Mostafa and Kazem (2011) found only 2% of maize grains to be contaminated by *Penicillium* species in Iran. The Zygomycete, *Rhizopus* spp. was found in high frequency (22%) from Zp.707×UN 44052 hybrids grown in PDA media.

Table (1): Frequency of detected fungi from five hybrids on two types of media.

Corn hybrids	Fungi	% Frequency	
		MEA	PDA
Zp. 707× Un 44052	*Aspergillus niger*	18	19
	A.flavus	19	-
	A.candidus	1	25
	A.japonicus	2	3
	Alternaria chlamydospora	1	-
	Penicillium spp.	-	2
	Rhizopus spp.	13	22
Un 44052 × Dk 17	*Aspergillus niger*	15	47
	A.flavus	7	9
	A.japonicus	5	5
	A.terres	4	3
	Penicillium spp.	1	-
	Chaetonium sp.	-	1
	Rhizopus spp.	13	-
D17 × H5	*Aspergillus niger*	15	1
	A.flavus	3	2
	Penicillium spp.	3	1
	Rhizopus spp.	10	1

IK 8 × Zp. 707	*Aspergillus niger*	12	25
	A.flavus	6	5
	A.japonicus	4	-
	A.terres	2	-
	A.parasiticus	-	3
	Verticillium sp.	1	-
	Penicillium spp.	-	3
	Rhizopus spp.	8	17
Ik 58 × H5	*Aspergillus niger*	8	27
	A.flavus	-	5
	A.japonicus	-	7
	A.parasiticus	8	-
	A.candidus	5	3
	Penicillium spp.	1	-

In blotter method (Table 2), the most frequent isolated fungus from the five tested hybrids was *A. niger*, particularly in Un44052 × Dk17 (59%) and Ik8×Kp.707 (56%). *Rhizopus spp.* was the second predominant fungus isolated from Zp.707×Un 44052 (35%) and Ik8×Kp.707 hybrid (20%).
The blotter method was found most suitable for the isolation of *Aspergillus* species this was in contrast with Nizar and Dawar (2009) who reported that Agar plate method was more superior for the detection saprophytic fungi.

Table (2): Detected fungi from five corn hybrids by blotter method .

Corn hybrids	Fungi	% Frequency
Zp. 707× Un 44052	*Aspergillus niger*	33
	A. flavus	6
	A.japonicus	8
	Penicillium spp.	2
	Rhizopus sp.	35
Un 44052 × Dk 17	*Aspergillus niger*	59
	A. flavus	2
	Rhizopus spp.	15
D17 × H5	*Aspergillus niger*	42
	A.flavus	11
	A.japonicus	12
	A.candidus	8
	Rhizopus sp.	9
IK 8 × Zp. 707	*Aspergillus niger*	56
	A.flavus	9
	A.japonicus	11
	Rhizopus sp.	20
Ik 58 × H5	*Aspergillus niger*	31
	A.flavus	15
	Penicillium spp.	22
	Rhizopus sp.	5

The degree of fungal contamination ranged between 22 to 65% on MEA and 4 to 71% on PDA of infected grains and the germination percentage of the seeds ranged from 53 to 84% and 80 to 94 on MEA and PDA respectively (Table 3). The highest fungal contamination was found in Zp.707×Un44052 hybrid (71%) ,whereas the least fungal contamination(4%) was found on D17xH5 hybrid.

Table (3) : Percentage of germination and contamination of Corn hybrids

Corn hybrids	% germination		%contamination	
	MEA	PDA	MEA	PDA
Zp. 707× Un 44052	69	94	65	71
Un 44052 × Dk 17	84	84	50	62
D17 × H5	70	80	31	4
IK 8 × Zp. 707	53	86	34	69
Ik 58 × H5	83	90	22	42

Table (4) showed the result of screening *Aspergillus* section *Flavi* strains for aflatoxigenic production abilities using coconut agar medium. Out of 48 strains of *Aspergillus flavus* obtained from corn grains. The highest percentage of positive strains (100%) showed a positive color change (pink color) on their reverse after exposure to ammonia vapor, were obtained from Zp.707×Un 44052, Ik8×Zp.707 hybrids , whereas the percentage of positive strains in D17×H5 hybrid was 70%. This was expected since not all strains of *Aspergillus flavus* produce aflatoxins as demonstrated previously by several workers (Schroder and Boiler, 1973; Abdel-Malek *et al*. 1993; Abdullah and Al-Mousawi, 2009; Mohamed *et al*.2010; Al-Rawi *et el.* 2011).

Table (4): Isolates of *Asperigillus flavus* produced aflatoxin detected from five corn hybrids

Corn hybrids	No. of tested isolates	% positive isolates
Zp. 707× Un 44052	6	100
Un 44052 × Dk 17	9	80
D17 × H5	9	70
IK 8 × Zp. 707	12	100
Ik 58 × H5	12	80

The recent widely used method in several food safety labrotaries for direct visual determination of aflatoxins production by isolates of *Aspergillus* section *Flavi* is color change on coconut agar medium (Davis *et al.* 1987; Abbas *et al*. 2004). This rapid and simple method enable us for inexpensively identification *of Aspergillus* section *Flavi* strains with potential aflatoxins production in food and feeds. In recent studies on the aflatoxigenic potential of *Aspergillus* section *Flavi*, strains detected in corn grains in Iraq, 62.5% of *A. flavus* isolates and 100% of *A. parasiticus* isolates proved aflatoxigenic (Abdullah and Al-Mousawi 2009),whereas Mohamed *et al*.(2010) showed that 81.85% and 100% of isolates of *A.flavus* and *A.parasiticus* respectively isolated from soil and other agricultural commodities in Duhok were able to produce aflatoxin.

In conclusion, based on our results that the freshly harvested corn grains contaminated by aflatoxigenic strains of *A.flavus* and contamination with other fungi particularly black aspergilli will represents a special hazard to consumers health. Therefore, monitoring for survey for contaminated food should be done before used.

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كورتى

ديفچوونەك هاتە ئەنجامدان ل سەر دەست نيشانكرنا گەرووێن توشى پێنج جورێن گەنم شاميێ دبن ، دوو ريك هاتنە بكارئينان، ريكا پەروكێت هشك كرى و ريكا ميديا يێت جێكرى. ريژا توژبوونێ هاتە گوهرين ل دويف گوهرينا جورێ گەنم شاميا. بەرزترين كەرويت هاتينە ديتن *Aspergillus niger* ، *A. flavus* و *Rhizopus* spp بوو. ريژا توژبوونێ دناڤبەرا 4–70٪ بوو و ريژا شينبونا گەنم شاميا 53 – 98٪ بوو. جورێ كەروا و ريژا شينبونێ هاتە گوهرين ل دويف گوهرينا جورێ گەنم شاميا. جوريت كەروا يێت ئەرينى بيكئينانا افلاتوكسينى دناڤبەرا ريژا 70 – 100 ٪ بوون ل دويف جورێ گەنم شاميا

الخلاصة

تم التحرى عن الفطريات المصاحبة لحبوب خمسة هجن من الذرة الصفراء الطرية وعزلت الفطريات بطريقتي الورق النشاف والوسط الزرعي. سجل اختلافا فى النسبة المئوية لتردد الفطريات تبعا لنوع الهجين . الانواع الاكثر ترددا هي *Aspergillus niger* ، *A. flavus* و*Rhizopus* spp . تراوحت النسبة المئوية لتلوث الحبوب بالفطريات ما بين 4–70% والنسبة المئوية للانبات تراوحت بين 53–98% . وجد اختلافا بين تركيبة الانواع الفطرية وكذلك النسبة المئوية للانبات تبعا لنوع الهجين. العزلات الموجبة بانتاج الافلاتوكسين توزعت بنسبة 70–100% على مختلف الهجن