Isolation of Aspergillus flavus from maize and sesame with detection of aflatoxin B1 in rural area around Baghdad

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Abstract

The results of this investigation have demonstrated the isolation and diagnosis of toxigenic *Aspergillus flavus* with detection of the aflatoxin B1 from maize and sesame samples in the rural area around Baghdad. A hundred sample of maize and sesame were collected from Abu Ghraib, Tagi, Tarmiya, Mahmoudiyah and Alabegi. Maize and sesames seeds were collected and mixed uniformly, After being mixed, 5seeds were randomly selected and surface sterilized in a 2.5% NaOCl solution for 1 min followed by washing two times in sterile distilled water, then dried by sterilized filter paper, Five complete grains were added via sterilized forceps into a previously prepared plate, containing sabouraud dextrose agar, Incubation of all plates for (7) days at 25 C° with intermittent observation of the fungal growth, plug of mycelium was aseptically removed from the orange medium below each infected seed and sub-cultured on Rose Bengal Agar (RBA) and incubation at 25°C for 5 days. *Aspergillus flavus* was isolated from maize sample in area around Baghdad, that obtained *Aspergillus flavus* in 5 samples in the total of 10 samples in Abu Ghraib, 3 samples from the total of 10 samples in Tagi, 6 samples of the total of 10 samples in Al Tarmia, 5 samples from the total of 10 samples in Mahmoudiya and 4 samples from the total of 10 samples in Alabegi while *Aspergillus flavus* was isolated from sesame sample in area around Baghdad, that obtained *Aspergillus flavus* in 2 samples in the total of 10 samples in Abu Ghraib, 3 samples from the total of 10 samples in Tagi, 1sample of the total of 10 samples in Al Tarmia, 4 samples from the total of 10 samples in Mahmoudiya and 2 samples from the total of 10 samples in Alabegi.detected of aflatoxin B1in maize sample in the area around Baghdad, whereby was obtained on 5 a contaminated samples with aflatoxin B1 from the total of 10 samples in Abu Ghraib, 3 contaminated samples with aflatoxin B1from the total of 10 samples in Tagi, 6 contaminated samples with aflatoxin B1from the total of 10 samples in Al Tarmia, 5 contaminated samples with aflatoxin B1from the total of 10 samples in Mahmoudiya and 6 contaminated samples with aflatoxin B1from the total of 10 samples in Alabegi while detected of aflatoxin B1in sesame sample in the area around Baghdad, whereby was obtained on 2 a contaminated samples with aflatoxin B1 from the total of 10 samples in Abu Ghraib, 2contaminated samples with aflatoxin B1from the total of 10 samples in Tagi, 1contaminated samples with aflatoxin B1from the total of 10 samples in Al Tarmia,4 contaminated samples with aflatoxin B1from the total of 10 samples in Mahmoudiya and 2 contaminated samples with aflatoxin B1from the total of 10 samples in Alabegi.

Key words: Isolation, Aspergillus flavus, maize and sesame, detection of aflatoxin B1.
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عزل فطر فلافوس من عينات الذرة والسمسم مع الكشف عن الفلاتوكسين B1 من المناطق الريفية حول بغداد

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الخلاصة

هدفت هذه الدراسة الى عزل وتشخيص فطر *Aspergillus flavus* السام والمنتج لسم الأفل توكسين B1 من المحاصيل العلفية والتي شملت الذرة والسمسم من المناطق الريفية المحيطة بمدينة بغداد، وتم الحصول على النتائج التالية: تم عزل فطر *Aspergillus flavus* من عينات الذرة من المناطق الريفية المحيطة بمحافظة بغداد، حيث تم الحصول على فطر *Aspergillus flavus* في 5 عينة من مجموع 10 عينة ذرة في قضاء أبو غريب و3 عينة من مجموع 10 عينة ذرة في قضاء التاجي و6 عينة من مجموع 10 عينة ذرة في قضاء الطارمية و5 عينة من مجموع 10 عينة ذرة في قضاء المحمودية و4 عينة من مجموع 10 عينة ذرة في قضاء العبايجي. بينما تم عزل فطر *Aspergillus flavus* من عينات السمسم من المناطق الريفية المحيطة بمحافظة بغداد، حيث تم الحصول على فطر *Aspergillus flavus* في 2 عينة من مجموع 10 عينة سمسم في قضاء أبو غريب و3 عينة من مجموع 10 عينة سمسم في قضاء التاجي و1 عينة من مجموع 10 عينة سمسم في قضاء الطارمية و4 عينة من مجموع 10 عينة سمسم في قضاء المحمودية و2 عينة من مجموع 10 عينة سمسم في قضاء العبايجي. تم الكشف عن سم الأفلاتوكسين B1 بواسطة Aflatoxin B1 rapid test في عينات الذرة من المناطق الريفية المحيطة بمحافظة بغداد، حيث تم الحصول على 5 عينة ملوثة بسم الأفل توكسين B1 من مجموع 10 عينة ذرة في قضاء أبي غريب و3 عينة ملوثة بسم الأفلاتوكسين B1 من مجموع 10 عينة ذرة في قضاء التاجي و6 عينة ملوثة بسم الأفلاتوكسين B1 من مجموع 10 عينة سمسم في قضاء الطارمية و5 عينة ملوثة بسم الأفلاتوكسين B1 من مجموع 10 عينة سمسم قضاء المحمودية و6 عينة ملوثة ملوثة بسم الأفلاتوكسين B1 من مجموع 10 عينة سمسم في قضاء العبايجي. بينما تم الكشف عن سم الأفلاتوكسين B1 بواسطة Aflatoxin B1 rapid test في عينات السمسم من المناطق الريفية المحيطة بمحافظة بغداد، حيث تم الحصول على 2 عينة ملوثة بسم الأفلاتوكسين B1 من مجموع 10 عينة سمسم في قضاء أبي غريب و3 عينة ملوثة بسم الأفلاتوكسين B1 من مجموع 10 عينة سمسم في قضاء التاجي و1 عينة ملوثة بسم الأفلاتوكسين B1 من مجموع 10 عينة سمسم في قضاء الطارمية و4 عينة ملوثة بسم الأفلاتوكسين B1 من مجموع 10 عينة سمسم قضاء المحمودية و2 عينة ملوثة ملوثة بسم الأفلاتوكسين B1 من مجموع 10 عينة سمسم في قضاء العبايجي.

الكلمات المفتاحية: عزل، فطر فلافوس، الذرة والسمسم، الكشف عن الفلاتوكسين B1.

Introduction

Simply, a mycotoxin is any toxic fungal metabolite. Fungi produce a variety of secondary metabolites as a byproduct of their metabolism,those capable of eliciting deleterious effects on other organisms are classified as mycotoxins (1). There are thousands of species of fungi but relatively few of these grow on agricultural products and only a fraction are capable of producing mycotoxins. It is known that about 100 fungi which grow on standing crops or stored feeds, produce toxic substances and approximately 20 of these have been associated with naturally occurring sicknesses. Aflatoxins are a group of chemically related mycotoxins and are classified as B1, B2, G1, and G2. The most common and toxic of the aflatoxin group is B1. Aflatoxin was first identified in stored grain, as a product of the fungus Aspergillus flavus, or A.

flavus, hence the prefix afla-. It was later found that similar toxins were produced by toxigenic strains of Aspergillus parasiticus. Aflatoxin has been found in wheat, corn, barley, oats, coconut oil and meal, cottonseed, cassava, dry pea meal and other raw food products. The molds which produce aflatoxin usually do not grow in silage, but aflatoxin already present can survive the acids produced during the ensiling process (2). Contamination of many dietary staple crops with Aspergillus section Flavi (3) and the subsequent production of aflatoxin are considered to be one of the most serious food safety problems worldwide (4). Aflatoxins cause the most concern due to their carcinogenic, immune-suppressing and growth retardation effects in both humans and animal (4). They also cause economic losses in international trade when toxin contamination exceeds permissible levels (5). Food contamination with aflatoxin depends on environmental conditions, particularly temperature and water activity (6), as well as Aspergillus strain composition (7, 8, 9, 10, 11, 12, 13, 14, 15). Aflatoxins are known to be mutagenic, teratogenic, carcinogenic, and immunosuppressive in animals and possibly in human(16). Many sickness outbreaks of aflatoxicosis in humans and animals have been recorded due to the ingestion of aflatoxin contaminated food and feed (17). Toxigenic fungi can attack maize prior to harvest and further decay the crop during storage. As a result, mycotoxins may form both during crop development and in storage. *Aspergillus*, Fusarium, *Penicillium* and *Cladosporium* are the predominant fungal genera associated with grain in storage. Of all mycotoxins, aflatoxin probably cause the most concern.This is due to their carcinogenic and immunosuppressive effects in both humans and domestic animals (18) and economic losses due to significant reductions in export value (5). Aflatoxin contamination is one of the most serious food safety problems worldwide (19) and regulatory limits on the quantity of aflatoxin permitted in food and feed exist inseveralcountries (20).

Materials and Methods

- A hundred sample of maize and sesame were collected from Abu Ghraib, Tagi, Tarmiya, Mahmoudiyah and Alabegi.
- maize and sesame ears were shelled and dried, Maize seeds were collected and mixed uniformly.
- After being mixed, 5seeds were randomly selected and surface sterilised in a 2.5% NaOCl solution for 1 min followed by washing two times in sterile distilled water.
- Then dried by sterilised filter paper.
- Five complete grains were added via sterilised forceps into a previously prepared plate, containing sabouraud dextrose agar. Three plates were used for each sample.
- Incubation of all plates for 7 days at 25 C° with the intermittent observation of the fungal growth.
- A plug of mycelium was aseptically removed from the orange medium below each infected seed and sub-cultured on Rose Bengal Agar(RBA) AND incubation at 25°C for 5 days.
- Fungal diagnosis: The diagnosis of different fungi was done according to(21). The identification of the fungus which belongs to Aspergillus species has been done, depending on the shape, colour of fungus on the plate. And examined under the microscope for the microscopical appearance of the fungus. A small part of the fungal growth was taken and mixed with one drop of lactophenol cotton blue and covered with a coverslip and examined under the microscope by using (40 X)Lens.
- **Detection of aflatoxin B1 by aflatoxin B1 Rapid Test:-**

- Weigh 1g of ground sample into a 5 ml centrifuge tube.
- Add 4 ml PBS A, shake vigorously for 3 minutes.

- Centrifuge for 5 minutes at 4000 r/minutes (in case of no Centrifuge, then, keep still for 5 minutes)
- Suck 100 µL supernatant liquid into another 1.5ml centrifuge tube.
- Add 100 µL AFL PBST Buffer and shake for mixing fully.
- Suck 100 µL mixed samples for test.
- Remove aflatoxin rapid test from sealed pouch.
- Suck at least 3 drops of prepared sample, hold the dropper vertically and transfer 3 full drops (around 100 µL) of the solution to the specimen well (S) of the test device, and then start the timer.
- Wait for red bands to appear.
- The result should be read in approximately 3-5 minutes. Do not interpret results after 5 minutes.
- Negative: test line (T) is the same as or darker than control line (C).
- Positive: test line (T) is lighter than control line (C), or there is no test line.

– **Statistical Analysis:** The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters (percentage). Chi-square test was used to significant compare between percentage in this study(22).

Results

The results of isolation of *A. flavus* from maize and sesame by the surface disinfected grain with 2% sodium hypochloride solution (NaOCl) shows:

Table (1) The *A. flavus* isolated from maize with difference of Area

Area (location)	No of sample	Positive (%)	Negative (%)	P-value
Abu Ghraib	10	5 (50.00)	5 (50.00)	1.00 NS
Taji	10	3 (30.00)	7 (70.00)	0.001 **
Al Tarmia	10	6 (60.00)	4 (40.00)	0.0083 **
Mahmoudiya	10	5 (50.00)	5 (50.00)	1.00 NS
Alabegi	10	4 (40.00)	6 (60.00)	0.0083 **
Chi-Square	---	9.227 **	9.227 **	---
P-value	---	0.0027	0.0027	---
** (P<0.01), NS: Non-significant.				

Table (2) The *A. flavus* isolated from sesame with difference of Area

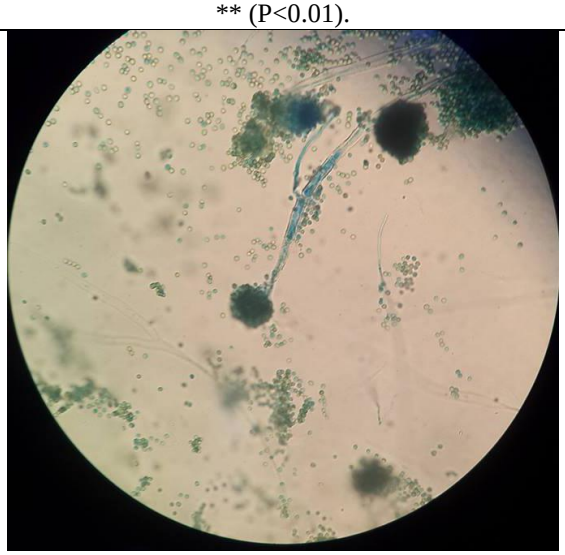
Area (location)	No of sample	Positive (%)	Negative (%)	P-value
Abu Ghraib	10	2 (20.00)	8 (80.00)	0.001 **
Taji	10	3 (30.00)	7 (70.00)	0.001 **
Al Tarmia	10	1 (10.00)	9 (90.00)	0.001 **
Mahmoudiya	10	4 (40.00)	6 (60.00)	0.0083 **
Alabegi	10	2 (20.00)	8 (80.00)	0.001 **
Chi-Square	---	9.018 **	9.018 **	---
P-value	---	0.001	0.001	---
** (P<0.01).				

Table (3) The result of aflatoxin B1 detection by rapid test in maize samples

Area (location)	No of sample	Positive (%)	Negative (%)	P-value
Abu Ghraib	10	5 (50.00)	5 (50.00)	1.00 NS
Taji	10	3 (30.00)	7 (70.00)	0.001 **
Al Tarmia	10	6 (60.00)	4 (40.00)	0.0083 **
Mahmoudiya	10	5 (50.00)	5 (50.00)	1.00 NS
Alabegi	10	6 (60.00)	4 (40.00)	0.0083 **
Chi-Square	---	9.373 **	9.373 **	---
P-value	---	0.001	0.001	---
** (P<0.01) , NS: Non-significant.				

Table (4) The result of aflatoxin B1 detection by rapid test in sesame samples

Area (location)	No of sample	Positive (%)	Negative (%)	P-value
Abu Ghraib	10	2 (20.00)	8 (80.00)	0.001 **
Taji	10	3 (30.00)	7 (70.00)	0.001 **
Al Tarmia	10	1 (10.00)	9 (90.00)	0.001 **
Mahmoudiya	10	4 (40.00)	6 (60.00)	0.0083 **
Alabegi	10	2 (20.00)	8 (80.00)	0.001 **
Chi-Square	---	9.281 **	9.281 **	---
P-value	---	0.001	0.001	---
** (P<0.01).				



Fig(1). Microscopic feature of *Aspergillus flavus.*

Discussion

The isolation of Aspergillus flavus from disinfected maizes According to the method by (3, 6) whereas isolation of Aspergillus flavus from disinfected sesame According to (3, 6). Diagnosis of molds were made on the bases of morphological characteristics appeared on the plates including color, texture & reverse color in addition to the microscopic appearance by lactophenol cotton blue mount smear made from the colony appeared & observed under 10x & 40x lenses microscope (3, 20, 21, 23, 24). The frequency of toxigenic Aspergillus flavus of maize 50% in Abu Ghraib, 30% in Tagi, 60% in Al Tarmia, 50% in Mahmoudiya and 40% in Alabegi whereas The frequency of toxigenic Aspergillus flavus of sesame 20% in Abu Ghraib, 30% in Tagi, 10% in Al Tarmia, 40% in Mahmoudiya and 20% in Alabegi.The results of the present study showed high percentage of fungi isolated from maize and sesame due to Several factors influence mold development, including moisture, temperature, aeration and substrate. Moisture is probably the most important of these factors but the type of mold and whether or not a toxin is produced will depend on the interplay of all these factors. Physical damage, such as breakage and stress cracks in grain, will increase the likelihood of fungal growth. The high frequencies of A. flavus in maize grains are linked with the corresponding levels of Aspergillus section Flavi resident in the soil, which is the reservoir for field infections(25). Storage conditions, play an important role in colonization by fungi and sporulation (26). The frequency of aflatoxin B1 contaminated of maize 50% in Abu Ghraib, 30% in Tagi, 60% in Al Tarmia, 50% in Mahmoudiya and 60% in Alabegi whereas The frequency of aflatoxin B1 contaminated of sesame sesame 20% in Abu Ghraib, 30% in Tagi, 10% in Al Tarmia, 40% in Mahmoudiya and 20% in Alabegi. Aflatoxin contamination and A. flavus in maize differed between the different area. These may be due to the prevailing climatic conditions (27), the cultivars grown in each zone, the cultural practices and/ or the

storage methods (28). Land management strategies and, particularly, crop rotation systems and factors such as genotype may influence crop infestation by Aspergillus section Flavi and the aflatoxin content of maize. Sesame was less prone to Aspergillus section Flavi infestation and aflatoxin contamination compared to maize.

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