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Detection of aflatoxin B1 from fodder crops in ruler area around Baghdad city in Iraq and studied the toxicity effect of *A. flavus* in mice

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Abstract

The present study was aimed to detection of aflatoxin B1 from Fodder crops which included maize and sesame in rural area around Baghdad city and studied the toxicity effect of *A. flavus* in mice fed on diet contaminated with spores of toxigenic *A. flavus* after 30 days of experiment, fifty (50) sample from maize and sesame were collected from the ruler areas around Baghdad city which were (Al Tarmia, Abu Ghraib, Mahmoudiya, Alabegi and Taji), so ten (10) sample from maize and ten (10) sample from sesame were obtained from each of the areas above in one (1) kilogram from each sample during four months begging from (November/2016 till march 2017)., the detection percentages of aflatoxin B1 from maize and sesame were 50% and 24% respectively, this study was conducted with experimental infection included diet of mice on feed contaminated with *A. flavus* in three different doses, the histopathological examination had been done on spleen, lung, small intestine and liver of mice in experiment, so the spleen appeared with different types of inflammatory cells and lymphocytes with highly infiltration with macrophages which appeared in diffuse and nodular forms, the lung characterized by infiltration with inflammatory cells and macrophages as well as breaking of epithelial lining the bronchiole, an addition to intestine revealed highly infiltration with inflammatory cells with irregular in intestinal villi while liver showed sever degenerative in the hepatocytes with payknosis in their nuclei in addition to presence of lymphocytes and kupffer cell.

Keywords: Aflatoxin B1, mice, contamination, detection, *A. flavus*

1. Introduction

Mycotoxins are poisonous fungal metabolite. Which represents assortment of byproduct secondary metabolites. Those able of eliciting harmful effects on other organisms [1]. There are thousands of fungal classes but comparatively few of these grow on agrarian products and only a part is able of producing mycotoxins. It is known that around 100 fungi which cultivate on standing crops or stored feeds, produce poisonous substances and about 20 of these have been connected with naturally occurring sicknesses. aflatoxins are a group of chemically connected mycotoxins and are divided into B1, B2, G1, G2 [2]. The chiefly and toxic of the aflatoxin class is B1. Aflatoxin was first identified in stored grain, as a product of the fungus *Aspergillus flavus*. Aflatoxin has been found in wheat, corn, oats, coconut oil and meal, cottonseed, dry peameal and other ore food products. The molds which produce aflatoxin usually do not grow in silage, but aflatoxins already present can survive the acids produced during the ensiling process [3]. *Aspergillus flavus* is a yellow - green mold found on many types of crops in the field or in storage and subsequent production of aflatoxins is considered to be one of the most serious food safety problems worldwide [4]. The hyphae of *A. flavus* are septate and hyaline. Conidial heads are radiate to loosely columnar with age and Conidiophores are coarsely roughened, uncolored [5]. Pollution of many dietary dietetic crops with *Aspergillus* section Flavi [6] and the subsequent production of aflatoxins is regarded to be one of the most dangerous food safety problems worldwide. Aflatoxins cause the most cancer referable to their carcinogenic, immune-inhibition and growth-retardant effects in both humans and animals [5]. They also cause economic losses in international trade when toxin contamination augments allowable levels [7]. Aflatoxins are notedness to be mutagenic, teratogenic, carcinogenic and immunosuppressive in animals and perhaps in humans [8, 9, 10, 11, 12]. So the aims of this study was detection of aflatoxin B1 from maize and sesame in ruler areas around Baghdad city with experiment infection to study the most important pathological and toxigenic effects of *A. flavus* in mice.

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2. Materials and Methods

2.1 Study area and design

During the period of November 2016 till March 2017 a survey was conducted in ruler areas around Baghdad city, hundred sample from maize and sesame were collected to determine contaminated of sample with aflatoxin B1.

2.2 Samples collection

The samples of maize and sesames may have been collected from fields of a rural area which surround of Baghdad city (Abu Ghraib, Tagi, Tarmiya, Mahmoudiyah and Alabegi.). 50 sample of maize and sesame were taken, the amount of each sample was one kilogramme, in about 10 samples from each of areas above from maize and from sesame.

2.3 Detection of aflatoxin B1 by aflatoxin B1 Rapid Test.

The N.K. Biotech Aflatoxin Rapid Test Device is used to qualitative detect Aflatoxin B1 in feed and grain at the sensitivity of 5ppb (5µg/kg).

2.4 Test Principle

Smart Rapid test technology is based upon colloidal gold immunochromatographic (GICA) technologies, with high sensitivity and specificity. Colloidal gold is a kind of hydrophobic colloid with a negative charge, which maintains a steady colloidal system depending on their mutual repulsive reactions. The diameter of colloidal gold varies from several nanometers to tens of nanometers. Moreover, for colloidal gold could absorb kinds of substances such as SPA, IgG, toxin, glycoprotein, enzyme, antibiotics and hormone, etc, based on its strong absorption function. Colloidal gold has become a good marker used in immunoreaction.

2.5 Samples preparation

- Weigh 1g of ground sample into a 5ml centrifuge tube.
- Add 4ml PBS A, shake vigorously for 3 minutes.
- Centrifuge for 5 minutes at 4000r/minutes
- Suck 100 µL supernatant liquid into another 1.5ml eppendroff tube.
- Add 100µL AFL PBST Buffer and shake for mixing fully.
- Suck 100µL mixed samples for test.

2.6 Test procedure

- 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 100µL) 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.

2.7 Reading Results

- 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.

To acquire the exact sensitivity, the reduplicative experiment has been done on the sample containing 5ppb Aflatoxin B1.

There is no cross reaction with Zearalenone, Fumonisin, Ochratoxin, or Deoxynivalenol and etc.

2.8 Experimental study

2.8.1 Spore suspension

Plates containing sabouraud dextrose agar were inoculated with the isolates of *Aspergillus flavus* which is proved to secrete aflatoxin B1 by using aflatoxin B1 rapid test. The plates were incubated at 25 C for (7 days). Spore suspensions were prepared according to [13], spores were harvested by adding (10ml) of sterilised PBS containing 0.1% Tween 80 to aid wetting and separation of the spores; then the fungal growth was separated by the harvester. The suspension was filtered through sterile gauze, the filtrate was transferred to centrifuge tubes and centrifuged at 3000 rpm and the precipitated spores were taken and washed with PBS then centrifuged for two times at 3000 rpm for (5) minutes, then (5 ml) of sterile PBS was added to the precipitated spores and mixed by the vortex mixer for (1) minute.

0.1 ml of the suspension was put into haemocytometer chamber, spores were calculated under high power (40X) of the light microscope using the following equation to determine dose which were given to mice in the experiment.

Concentration = $(Z \times 4 \times 10^6) / N$ spores / ml of suspension

$$\frac{Z \times 4 \times 10^6}{N}$$

2.8.2 Experimental Design

Eighty mice were divided randomly into four groups (Twenty mice for each groups. The three different doses which were given to three groups of mice with twenty mice for each group and for 30 days. The first group was given 11×10^6 spores / ml / 10 g of feed, the second group dose was 22×10^6 spores / ml / 10 g of feed, while the third group was given a dose of 44×10^6 spores / ml / 10 g of feed, while the fourth group were given diet free from *A. flavus* spores, which represented the control group.

2.8.3 General histopathological preparations

The specimens (liver, spleen, intestine, lung) were fixed in 10% neutral formalin buffer solution, till the preparation of histological sections.

2.8.4 Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to study the effect of difference factors in study parameters (percentage). Chi-square test was used to significant compared between percentages in this study.

3. Results

3.1 Detection of aflatoxin B1 by using aflatoxin B1 rapid test

The detection percentages of aflatoxin B1 from maize in rural areas around Baghdad city revealed high occurrence of aflatoxin B1 in Al Tarmia and Alabegi with 60% whereas Abu Ghraib and Mahmoudiya appear 50% of detection for both of them while Taji appeared lower percentage of detection in 30% so the aflatoxin B1 appeared in 50% from examined samples as appear in Table (1).

Table 1: The result of aflatoxin B1 detection by rapid test in maize samples.

Area (location)	No of sample	Positive		Negative		P-value
		No	%	No	%	
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 **
Mean	50	25	50.00	25	50.00	
Chi-Square	---	9.373 **		9.373 **		---
P-value	---	0.001		0.001		---

** (P<0.01), NS: Non-significant.

The detection percentages from sesame from the areas around Baghdad city appeared high percentage in Mahmoudiya with 40% followed by Taji in 30% while Abu Ghraib and Alabegi revealed 20% for both of them whereas Al Tarmia appeared

the lowest detection percentage with 10% and so the detection percentage from the rural areas around Baghdad city were 24% as appeared in Table (2).

Table 2: The result of aflatoxin B1 detection by rapid test in sesame samples.

Area (location)	No of sample	Positive		Negative		P-value
		No	%	No	%	
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 **
Mean	50	12	24.00	38	76.00	
Chi-Square	---	9.281 **		9.281 **		---
P-value	---	0.001		0.001		---

** (P<0.01).

3.2 Histopathological result.

The histopathological examination had been done on spleen, lung, small intestine and liver of mice in experiment given the contaminated diet, so the spleen appeared with different types of inflammatory cells and lymphocytes with highly infiltration with macrophages which appeared in diffuse and nodular forms, the lung characterized by infiltration with inflammatory cells and macrophages as well as breaking of epithelial lining the bronchiole, an addition to intestine revealed highly infiltration with inflammatory cells with irregular in intestinal villi while liver showed sever degenerative in the hepatocytes with payknosis in their nuclei in addition to presence of lymphocytes and kupffer cell.

4. Discussion

4.1 Detection of aflatoxin B1 by aflatoxin B1 rapid test.

The results of the present study showed (50%) from maize sample contaminated by aflatoxin B1 and (24%) from sesame samples contaminated by aflatoxin B1 in rural area around Baghdad city.

The N.K. Biotech Aflatoxin Rapid Test Device is use to qualitative detect Aflatoxin B1 in feed and grain at the sensitivity of 5ppb (5µg/kg).

Levels of *A. flavus* infection and aflatoxin B1 contamination are related primarily to environmental conditions especially to drought stress during pod maturation. Hence, the levels of *A. flavus* seed infection cannot be directly correlated to the aflatoxin B1 production which is agree with [14].

The contamination of many dietary staple crops with *Aspergillus flavus* and the subsequent production of aflatoxins B1.

Aflatoxin B1 found in maize and sesame which is agree with [15]. Aflatoxins B1 often occurs in crops in the field prior to harvest. Post-harvest contamination can occur if crop drying is delayed and during storage of the crop if water is allowed to

exceed critical values for the mould growth.

Food contamination with aflatoxins B1 depends on environmental conditions, particularly temperature and water activities, as well as *Aspergillus* strain composition. *Aspergillus flavus* represent the most common aflatoxin B1 producing species which agree with [16, 17].

Agricultural commodities that are susceptible to aflatoxin B1 contamination in nature, such as corn, sesame, peanuts, and rice, are excellent substrates for aflatoxin B1 production because composition of these crops promote growth of *A. flavus* and subsequent production of aflatoxin B1 which agree with [18].

Moisture and temperature are two factors that have a crucial effect on fungal proliferation and toxin biosynthesis which is agree with [19].

The incidence and level of aflatoxin B1 contamination are closely related to the geographic position and to seasonal factors as well as to the cultivation, harvesting, stocking, and transport conditions which agree with [20].

Studies focusing on aflatoxin B1 contamination in foodstuffs have in fact been reported in many countries, especially those in tropical and subtropical regions, such as Asia and Africa [20]. Aflatoxin B1 contamination can develop both in the pre- and post-harvest periods, but the highest levels are usually associated with post-harvest spoilage of food commodities, stored under inappropriate high moisture content and high temperature conditions which facilitate the rapid growth of moulds which is identical to environmental conditions appeared in ruler areas around Baghdad city; so the level of contamination depends on the plant stress, temperature, water activity, genotype, culture and storage conditions which agree with [21].

Aspergillus flavus growth may occur, without the production of aflatoxin B1 and aflatoxin B1 may present in sample in absent of *Aspergillus flavus*. The absence of aflatoxin B1 in

some samples in this study may not suggest that the fungus *A. flavus* did not produce aflatoxin B1. On the other hand it may be possible that the level of aflatoxin B1 produced was too small to be detected which agree with [22].

In Iraq, Hassan *et al.*, (2014) were reported Correlation between the presence of aflatoxin B1 and *A. flavus* in maize samples which is agree with recent study not all isolated *A. flavus* production aflatoxin B1.

In addition, to the diversity of ingredients in sesame and corn which were play important roles in aflatoxins productions. we can conclude from the obtained results, that the apparently healthy grains may possible be infected in the field before harvest, and some fungi lose their vitality after seeds drought, but their secondary metabolites (mycotoxins) still remain in those seeds. While some times the grains contain fungi but free of mycotoxins, and this may be due to that the condition not available for fungi to produce mycotoxins or the reason may be genetically which is agree with [23].

The results of the present study showed highly maize samples contaminated with aflatoxin B1 more than sesame samples this due to that high carbohydrate substrate such as maize give larger yields of aflatoxin B1 than oil seeds such as sesame that are not immediately metabolized by *A. flavus* which agree with [24].

In Saudi Arabia, Bokhari (2002) were reported (20%) of sesame samples contaminated with aflatoxin B1 which was approach to results in this study related with sesame contaminated with aflatoxin B1 and (12.5) of maize samples contaminated with aflatoxin B1 by thin layer HPLC chromatogram (quantitative assay for aflatoxin).

In Senegal, Diedhiou *et al* (2011) were reported contaminated of maize samples with aflatoxin B1 at (40%) and contaminated of sesame samples with aflatoxin B1 at (25%) by using thin layer HPLC chromatogram (quantitative assay for aflatoxin).3

In Iraq, Tikrit, Thalij *et al.* (2015) were reported contaminated of maize samples with aflatoxin B1 at concentration (7.0-260 ng/g) by using thin layer HPLC chromatogram (quantitative assay for aflatoxin).

In Iraq, Hassan *et al.*, (2014) were reported not all *A. flavus* isolates were aflatoxin B1 producers and vice versa which agree with results in this study so that isolation of *A. flavus* recorded 46% whereas the detection of aflatoxin B1 was 50% in maize samples.

In Iraq, Maysan, Ali, (2009) founds all *A. flavus* that isolated from maize samples have the ability to produce aflatoxin B1. While In sudan, Suliman *et al.*, (2015) were reported (90%) of sesame samples contaminated with aflatoxin B1 by ELISA Ram *et al.*, (1986) in sudan detected aflatoxin B1 in maize by ELISA and found that the toxin is in the range of 7 to 422 µg/kg.

4.2 Histopathological studies

During experiment when contamination of feed with *A. flavus* and prepare suitable conditions for aflatoxin B1 production (high temperature and humidity) which lead to production of aflatoxin B1 by *A. flavus*, had been detected by rapid aflatoxin B1 Kit used to examine samples of feed which were given to experimental groups of mice and this is approach to environmental conditions when experiment was done by [25].

The results of histological section in the liver of mice given feed contaminated with *A. flavus* showed necrosis and degenerative of most liver cell, the central vein in each lobule empty from blood and vacuoles in the cytoplasm which appeared as cavities these changes due to production of

aflatoxin B1 by *A. flavus*, which causes hepatic necrosis through free-radical production, lipid peroxidation, as well as inhibition of RNA and protein synthesis have been well documented in several livestock and poultry species as mentioned by [26].

As well as formation of proteins essential for the integrity of cellular membranes that has been completely blocked by the action of aflatoxin B1 which lead to cavities in cytoplasm which is agree with [27].

The small intestine of mice given feed contaminated with *A. flavus* showed the villi of mucosa were not well arranged, desquamated and sloughing in the epithelium of intestine due to the aflatoxin B1 produce by *A. flavus* have concentration effect and causes reduction in alkaline phosphatase activity in the intestine and also aflatoxin B1 stimulate inflammatory response which lead to highly infiltrated of lamina propria with WBCs, which agree with Tomková *et al.*, 2001, as well as aflatoxins B1 interfere with intestinal morphology, sialic acid production and apparent digestible energy which is comparable to [28].

In the spleen of mice given feed contaminated with *A. flavus* showed highly infiltration in the parenchyma of spleen with lymphocytes and other WBCs, the blood sinuses of red pulp appeared empty from blood and red pulp is rarely recognized because the overcrowding of lymphocyte in the tissue this due to unusually high concentration of inorganic iron and debris from the circulation caused by aflatoxin B1 produce by *A. flavus* which agree with [29].

In the lung of mice given feed contaminated with *A. flavus* showed: the infiltration of inflammatory WBCs in the interstitial connective tissue, even in the lumen of the bronchiole as well as thickening of the alveolar walls was noted and the epithelium of the bronchiole were breaking down and desquamated all these change were due to *A. flavus* and it's toxin which agree with [30].

5. Conclusions

1. Aflatoxins B1 are not one a great problem at crop production level, but also it has become a worldwide health issue due to consumption of this poison generates diseases in animals and human.
2. The isolation percentage of *Aspergillus flavus* from maize and sesame in rural areas around Baghdad city were 46% and 24% respectively.
3. The detection percentage of aflatoxin B1 from maize and sesame in rural areas around Baghdad city were 50% and 24% respectively.
4. Absence of *A. flavus* isolates compained by detection of aflatoxin B1 in maize crop whereas the isolation percentage of *A. flavus* was comparable to detection percentage of aflatoxin B1 in sesame samples.
5. The maize more susceptible to contamination by *Aspergillus flavus* than sesame.
6. High temperature and humidity play important role in growth of *Aspergillus flavus* and production of aflatoxin B1 in fodder crops.
7. Histopathological changes in mice group given contaminated diet with spores of toxicogenic *A. flavus* causes changes in the internal organs like (liver, intestine, spleen and lung) characterized by degeneration, necrosis and highly infiltration with WBCs.

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