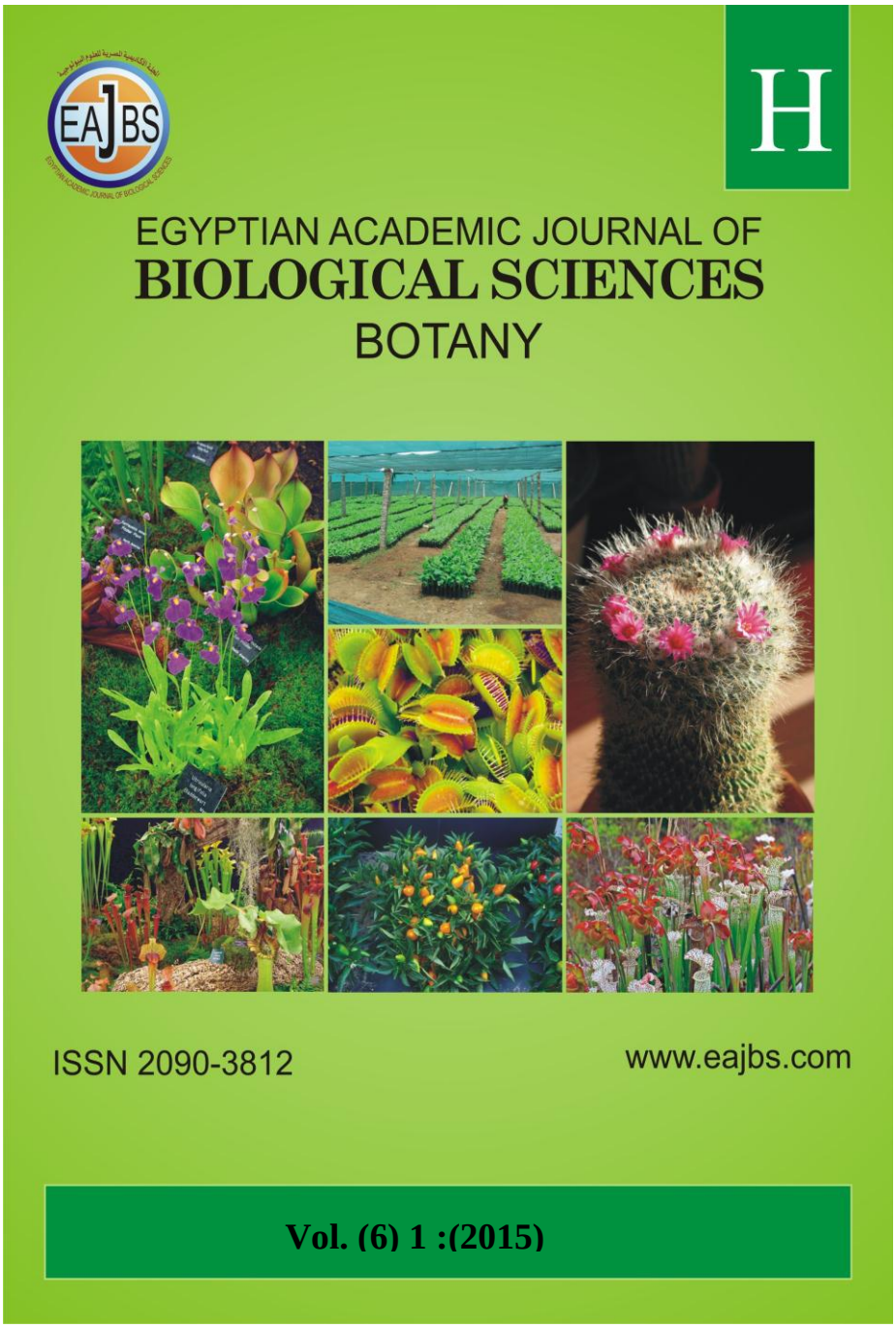

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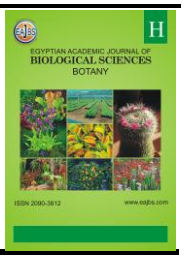

Detection of the Presence of Aflatoxins Type (B_1) in Three Types of Stored Grain: Maize, Wheat, and Rice in Silos of Baghdad, Iraq

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ABSTRACT

Aflatoxins is one of the dangerous mycotoxins, especially aflatoxin B_1, where it is one of the causes of cancer disease to humans and animals, also leads to a large perdition. The current study was conducted on the investigation of environmental conditions in the local stores and their impact on contamination of aflatoxin B_1. Three models of economic crops: maize, wheat, and rice samples were collected from Baghdad and Wasit silos for six consecutive months. Sixty samples of the three grain crops were collected from the stores, (12) samples of cornand (24) samples each of wheat and rice, all samples with three replicates planted on the (PDA) medium. Then, Agricultural and ELISA tests were done. The results showed that the concentration of aflatoxin B_1 by ELISA technique in corn crop was more affected by the pollution compared with other crops. The concentration of aflatoxin B_1 in cornmeasured 46.2ppb, while rice crops scored less concentration of toxin reached to1.8 ppb.

INTRODUCTION

Food contamination with fungi which produce toxin is consider one of the important problems especially at the present time. The Food and Agriculture Organization (FAO) reports indicated that at least 25% of the world's food is contaminated with mycotoxins (Bohoot, 2003). The mycotoxins are known as chemical compounds that cause a lot of pathological cases to various organisms including plants (Ciegler, *et al.* 1971; Ciegler, 1975). The famous mycotoxins and the most serious is the Aflatoxin (AFS), since its discovery, it made way for extensive study to mycotoxins in food. The designation of aflatoxin taken from the first letters of the genus and species and fungus *Aspergillus flavu*s, and there are four types of Aflatoxins (B2, B1, G1, G2) (Essa, 1996). Aflatoxin B_1 (AFB1) affects human food and cattle feed because it's direct consumption in food or as metabolic remain in livestock tissues. Even little contamination is significant, hence there is a need to determine their rate in feed also, appropriate analytical models for detection, and the quantum must be permitted for effective food and feed safety programs (Biermann and Terplan, 1980; Morgan, *et al*. 1986; Park, *et al*. 1989; Fukal, 1990). The World

Health Organization (WHO) classification of AFB1 suggests that there is no safe potion (Anklam, *et al*. 2002). The colonies of *Aspergillus flavus* and *Aspergillusp-arasiticus* have wide spread in food crops, such as oil seed, maize, nut, milk, and wheat (Strosnider, *et al*. 2006). Viability of these fungi secretion of AFB1 depended on moisture content, dryness, climate, insect infection, and agriculture enforcement (Wu and Khlangwiset, 2010). The contamination with AFB1 is a certain problem in oil seed, corn, peanuts, and milk (Shephard, 2008).

MATERIALS AND METHODS

Samples Collection

Samples were collected from silos of Baghdad city (Aldora silo, Jorof Alnadaf silo, and Alatefea silo) and Wasitcity (Alsuera silo) on monthly basis from September 2014 to February 2015. Grains samples were maize, wheat, and rice. Samples were collected from sites randomly and placed in clean plastic bags then transferred to the laboratory on the same day. One kilogram of each commodity was taken and stored at room temperature until analysis.

Isolation and Detection of *Aspergillu* fungi

Cereal sample were surface-sterilized by immersion in 3% sodium hypochlorite solution for 1 to 2 minutes, then rinsed three times by sterilized distilled water, dried with sterilized filter paper in a laminar and cultured in Potato- Dextrose Agar (PDA) medium, using three Petri plates for each sample (5 grain/each plate), and incubated for 5 to 7 days at 25°C (Michael and Beder, 1982). The fungi were identified morphologically under light microscope. The diagnosis of fungus depending on the taxonomic characteristics was mentioned by all of (Davise, 1995; Pitt and Hocking, 1997; Williams-Woodward, 2001).

Detection of Aflatoxin B_1 by Indirect Competitive ELISA Test

Typical samples (5 g) were grinded and then mixed with 25 ml of 70% methanol. The mixtures were shacked for 20 minutes with a shaker. The extracts were centrifuged for ten minutes at 4000 rpm. The supernatants were used for determination of AFB1 content by indirect competitive ELISA kit which was supplied by Bioo scientific Corporation (USA). Aspectrophotometric microtiterreader provided with a 450 nm filter was used for absorbance measurements.

A standard curve was constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/ml (ppb) on a logarithmic curve.

$$\text{Relative absorbance (\%)} = \frac{\text{Absorbance standard (or sample)} \times 100}{\text{Absorbance zero standard}}$$

RESULTS AND DISCUSSION

Survey and Collection of *Aspergillus flavus* Isolates

The results shown in Table (1) indicate that *Aspergillus* contamination in three cereal crop samples was observed in Baghdad silos and markets. Atotal of 65 isolates of *A. flavus* were identified from (180) samples for six months. This study showed that maize crop was more infected with fungi than other crops under study, where the infection seeds gave (29) isolates from 36 from total cultured. While wheat crop gave 23 isolates from 72 totals cultured, whereas the rice crop showed the least infection compared with others crops, as was noted that 13 isolates of *A. flavus* from 72 total

cultures. In addition, the higher growth proportion of *A. flavus* for six months was achieved in maize crop (80.5%), while growth proportion in wheat crop was (32%), and finally the rice crop recorded the less of growth proportion (18%),Table (1).

Table 1: Type and number of crop samples analyzed for aflatoxins *A. flavus*.

Crops	Sample Number	No. of *A. flavus*isolated	Mean proportion of *A. flavus* growth for six months
Maize	36	29	80.5%
Wheat	72	23	32%
Rice	**72**	**13**	18%
Total	**180**	**65**	

The result shows that these crop items displayed in Baghdad silos and markets contain aflatoxins fungi. It is therefore recommended to investigate, constantly, food especially grains for mycotoxin contents and their store conditions. Globally, high levels of *Aspergillus* contamination of dietary staples have been reported (Pitt and Hocking, 1985; Abbas *et al.,* 2004) from various crops including corn, peanut, rice, and cotton.

The result is consistent with many studies that component of grain is appropriate for fungi growth (Eaton and Groopman, 1994). Also *A. flavus* have the ability to secrete a large number of enzymes to decompose the foodstuff to learn from them the growth and spread capacity in wide range of crops like corn, peanuts, and rice (Pitt *et al*. 1993: Eaton and Groopman, 1994). *A. flavus* appears as a high percentage of grains which were infected because of the helping factors such as insects, spiders, quality of crop, and mixing with contaminated material during harvesting and storage (Abbas, 1983).

These results were in accordance with Talal, *et al*. (2009) who reported that *A. flavus* appeared in three types of crop (maize, wheat, and rice) more than other fungi and showed the high percentage of *A. flavus* growth in maize compared with wheat and rice. Domsch, *et al.* (1980) who isolated *A. parasiticus*from rice, groundnuts, and pecans in the rhizosphere of wilted pineapple plants. Jugenhelmr, (1976) reported that corn crop was more infected with *A. flavus*because of its high content of moisture in its seeds and that it contains large number of essential nutrients like lipid, proteins, carbohydrates, and starches.

The Effects of Environmental Factors on Aflatoxin B_1 Production

Enzyme Linked Immune Sorbent Assay (ELISA) technique for detection of aflatoxin B_1 in (maize, wheat, and rice) samples were done during six months of time; the results were illustrated in Table (2). Twelve samples of maize which were collected from Alsuera Silo and Baghdad markets, the lowest mean of AFB1content in maize crop was done on September (21.95ppb), where superiority of mean was recorded on February (43.04ppb). Twenty four samples for each of wheat and rice crops were collected from three silos in Baghdad (Jorof Alnadaf, Aldora, Alatefea) and from Baghdad markets.

In general, aflatoxin B_1contamination in all cereal crops was dissimilar between six different months, especially with wheat and rice crop. The amount of AFB1 in wheat samples ranged between the least value (2.4ppb) to the highest value (45.26ppb). Furthermore, the highest mean of AFB1concentration in wheat crop was on February 2015 (30.04 ppb), while the low means were recorded on September and October 2014 (1.27-1.08ppb),respectively. On the other hand, rice crop with the same condition formed smaller results. Where the highest mean of AFB1 concentration in rice samples was (19.34 ppb)on February 2015 and the lowest mean was (0.67-0.45ppb)on September and October 2014, respectively.

The results in Table (2) indicated that AFB1 concentration was significantly influenced by both months and stations. As for the stores, the results show that Jorof Alnadaf silo gave high concentration of AFB1 with both of wheat and rice, this is may be due to another helping factors which can promote fungal growth like insects, spiders , quality of crop, and mixing with contaminated material during harvesting and storage (Abaas,1983).So, these results affirm that AFB1range was higher than AFB1 limits (1-20 ng/g) set by world wide range in majority of analysis sampling (FAO, 2004).

Table 2: Aflatoxin B_1 content in maize, wheat, and rice samples assessed by indirect Competitive EL

Stations	**Sep. 2014**	**Oct. 2014**	**Nov. 2014**	**Dec. 2014**	**Jan. 2015**	**Feb. 2015**	**Mean**
	AFB1 in maize crop						
Alsuera silo	24.38	28.75	30.53	35.01	41.58	46.23	34.41
Market	19.53	27.13	31.38	22.66	34.36	39.85	29.15
Mean	21.95	27.94	30.95	28.83	37.97	43.04	
LSD ≤ 0.05	station = 1.135,		month = 1.96,		interaction = 2.78		
AFB1 in Wheat crop							
JorofAlnadaf silo	2.4	2.13	25.81	31.58	35.55	45.26	23.78
Aldora silo	0.0	2.2	24.2	32.25	33.95	42.95	22.59
Alatefea silo	0.0	0.0	0.0	27.58	0.0	0.0	4.59
Market	2.7	0.0	0.0	0.0	31.3	31.95	10.99
Mean	1.27	1.08	12.50	22.85	25.2	30.04	
LSD ≤ 0.05	station = 0.575,		month = 0.704,		interaction = 1.409		
AFB1 in Rice crop							
JorofAlnadaf silo	2.7	1.81	12.93	19.21	28.98	40	17.60
Aldora silo	0.0	0.0	9.98	0.0	26.35	37.38	12.28
Alatefea silo	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Market	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	0.67	0.45	5.72	4.80	13.83	19.34	
LSD ≤ 0.05	station = 0.409,		month =0.501,		interaction = 1.003		

Data which illustrated in Figure (1) indicate that AFB1contamination in maize crop was more and higher compared with wheat and rice crops. Moreover, the effect of temperature and relative humidity towards *A. flavus*growth and production of AFB1was observed in Table (2) and Figure (1).

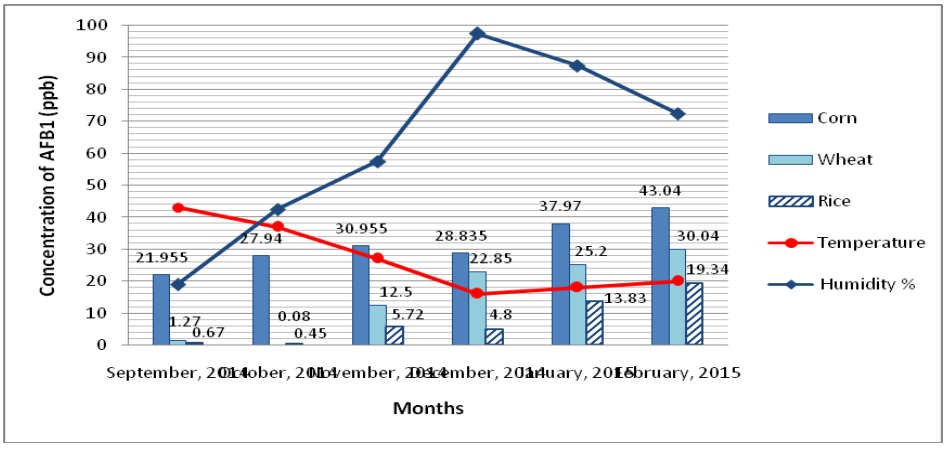

Fig. 1: The relationship between environmental factors and contamination three cereal crops (maize, wheat, and rice) with AFB1 for six months.

These results were concordant with Trenk and Hartman (1970) who reported that percentage of *A. flavus* infection was increased with the increasing humidity and warmth. This result is also in accordance with Al-Juboury (1998) who recorded *A. flavus* was a higher proportion of maize infection. A research result obtained by Hassan, *et al.* (2014) exhibited that AFB1was present in twelve samples of stored maize collected from Iraqi governorate and the concentration of toxin ranged between (2.30 to 30 ppb). Kusumaningrum, *et al.* (2010) stated that relative humidity can affect the growth of *A. flavus*in maize significantly. This result is consistent with the study which conducted that the maize crops are more susceptible to aflatoxin inflation because of the main fungal species which damage the maize (*A. flavus, A. parasiticus*) grow perfect in the range of 19-35°C (Sanchis and Magan, 2004). Atehnkeng, *et al*. (2008) found that corn crops from a moist area contain higher aflatoxin compared to those that come from a dry and warm area. This is consistent with the study which conducted by Talal, *et al*. (2009) who recorded that the ability of aflatoxin production at (20, 25, 30°C) was high, but at 35°C the ability was decreased, and at 40°C the production of aflatoxin was very low.

Many results proved that there was relationship between relative humidity and aflatoxin level in wheat; the environmental can be affected by type and quality of toxins (Basilico, *et al*. 1995). Halt (1994), showed aflatoxin in wheat samples was in the range of (20 ng/kg) (standard of WTO), also Escobar and Reguerio, (2002) who studied aflatoxin contamination; it was found in wheat, but less than other analysis such as peanuts and corn. Polly, *et al*. (1991) explained that the amounts of mycotoxins in England wheat, with changing values, are little. Diener and Davis (1967) and Lander, *et al*. (1967) reported that relative humidity and temperature have ability to limit aflatoxin production in peanuts. Toteja, *et al*. (2006) found that the incidence ratio of AFB1 on parboiled rice were collected from 11 stations in India was (38.5%).Suárez-Bonnet, *et al.* (2013) found that the average total of aflatoxins in Spanish rice was (37.3 µg/kg), the range was from (1.6 to 1383 µg/kg).

Siruguri, *et al.* (2012) indicated that the stored rice samples did not pose any health concern with respect to aflatoxin contamination as per the criteria laid down by the Food Safety and Standards Authority of India. So, these results affirm that AFB1range was higher than AFB1 limits (1-20 ng/g) set by world wide range in majority of analysis sampling (FAO, 2004). We can deduce the inefficiency of the most of crop samples to consumption by human due to the concentration of AFB1 which exceeds the allowable limit.

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