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Enzyme Linked Immunosorbent Assay for Fumonisin B1 Detection in Local Corn Seeds from Baghdad-Iraq

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Abstract

Fungi produce a series of toxic compounds on corn, especially Fumonisin B1 (FB1) toxin produced by *Fusarium* spp. and promoting cancer activity in humans and animals. This study aimed to the isolation and identification of fungi associated with local corn seeds and the detection for the presence of FB1 by using ELISA technique. Thirty samples of corn ears were collected from silos and markets in Baghdad city during the period from November 2018 to March 2019. The present study found that *Fusarium* was the dominant isolate among fungi in terms of the relative density 57.07%, followed by *Aspergillus* 31.17%, *Rhizopus* 3.36%, Alternaria 2.88%, *Mucor* 2.16%, *Penicillium* 1.92%, *Trichothecium* 0.96%, and *Helminthosporium* 0.48%. FB1 was detected in all samples of the silos and markets with a concentration range of 13.69 - 175.54 µg/kg. There were no significant differences in FB1concentration among samples collected from the silos and markets. Also, no relationship was found between the number of infected seeds by *Fusarium* spp. and FB1concentrations.

Keyword: FB1, Fusarium, ELISA, Corn

فحص الأدمصاص المناعي بالأنظيم للتحري عن السم فيومونيسين ب 1 في حبوب الذرة المحلية، بغداد–العراق

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الخلاصة

تنتج الفطريات سلسلة من المركبات السامة على حبوب الذرة خصوصا السم فيومونيسين ب1 الذي ينتج من قبل *Fusarium* وهو سم محفز للسرطان عند الانسان والحيوان. هدفت الدراسة إلى عزل وتشخيص الفطريات المصاحبة لحبوب الذرة المحلية والتحري عن وجود السم فيومونيسين ب 1 باستخدام تقنية الاليزا. تم جمع ثلاثين عينة من عرانيص الذرة المحلية من مخازن الحبوب والأسواق المحلية في محافظة بغداد خلال الفترة تشرين الثاني 2018 الى اذار 2019. وأظهرت النتائج أن *Fusarium* كان من العزلات السائدة 57.07% تليه الفطريات *Aspergillus* 31.17%، *Rhizopus* 3.36%، *Alternaria* 2.88%، *Mucor* 2.16%، *Penicillium* 1.92%، *Trichothecium* 0.96 % و *Helminthosporium* 0.48 %. وقد تم الكشف عن وجود السم فيومونيسين ب1 في جميع عينات الذرة حيث كان تركيزه يتراوح بين 13.69 – 175.54 ميكروغرام / كغم. لا يوجد فرق معنوي في تراكيز السم فيومونيسين ب1 بين المخازن

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والأسواق وكذلك لا توجد علاقة بين عدد البذور المصابة بالفطر *Fusarium* ومستوى تراكيز السم فيومونيسين ب1.

Introduction

Different filamentous fungi are able to produce toxic compounds with small molecular weight, called mycotoxin [1]. *Fusarium*, *Aspergillus*, and *Penicillium* are the main toxigenic genera that are able to invade grains in the field and during storage period under suitable conditions of temperature and moisture [2]. Corn (*Zea mays* L.) is a worldwide staple food crop which can be invaded by certain fungi, especially *Fusarium* species that produce diverse toxins such as Fumonisin B1 [3]. FB1 is present mainly in corn and in other grains, such as oat, rice, and wheat [4]. Previous studies found that the consumption of FB1 has been related to human and animal diseases, with hepatotoxic, neurotoxic, teratogenic, nephrotoxic, immunosuppressive, carcinogenic, and pulmonary effects [5, 7]. Enzyme Linked Immuno-Sorbent Assay (ELISA) technique is the most popular method used for the detection of mycotoxin in food and feed [8]. There are several advantages to ELISA over other techniques; it is simple, low-cost, sensitive, and specific. It requires a small sample size and can analyze a large number of samples at the same time [9]. Therefore, this study aimed to the isolation and identification of fungi along with the direct detection of the presence of FB1 in corn ears by using ELISA.

Materials and Methods

1. Samples collection

Thirty samples of corn ears were collected from local markets and silos of Baghdad province (fifteen sample from silos and fifteen from markets), during the period from November 2018 to March 2019.

2. Fungal Isolation and Identification

Isolation of fungi from grains was performed on 50 seeds from each corn sample. The surfaces of seeds were sterilized by 2% sodium hypochlorite solution for one minute and washed by sterilized D.W. three times. The grains were left for drying by sterilized filter papers, then 10 grains were seeded on potato dextrose agar (PDA: an enrichment medium for the isolation of all fungi) and malachite green agar 2.5 (MGA 2.5: a selective medium for the isolation of *Fusarium* spp.) media containing chloramphenicol (50 mg/L). The plates were incubated for seven days at 25°C. All fungal isolates were identified to species level by using Scotch tape preparation and slide culture technique, depending on macroscopic and microscopic appearance [10]. The values of relative density (RD), isolation frequency (IF), and incidence (IN) of infected seeds per sample were calculated according to Jedidi *et al.* [11] and Tsedaley *et al.*[12].

$$RD\% = \frac{No.of\ isolated\ fungi\ species}{Total\ No.of\ fungi} \times 100 \quad (1($$

$$IF\% = \frac{No.of\ samples\ occurrence\ of\ fungi\ species}{Total\ No.of\ samples} \times 100 \quad (2)$$

$$IN\% = \frac{No.\ infected\ seeds}{Total\ No.\ of\ seeds} \times 100 \quad (3)$$

3. Detection of FB1 by ELISA

Preparation of samples was performed according to the manufacturer's instructions, as in the following; 5 gm of grinded maize was added to 25 ml of 70 % methanol in a container and the mixture was shaked for 20 minutes. The sample was centrifuged at 4,000 rpm/10 minutes. One ml of the supernatant was diluted with 1 ml of D.W., then 50 µl of the diluted supernatant per well was used in the experiment. Detection of FB1 by competitive ELISA technique was performed using BiooScientific ELISA kit. The plate was measured optically after adding stop buffer using plate reader with 450 nm wavelength

Results and Discussion

Thirty samples were analyzed for the presence of Fumonisin B1 and mycoflora in corn ears. Figure 1 shows isolation frequency and relative density values of fungi isolated from all samples. The relative density results showed that *Fusarium* was the dominant isolate among the other fungi (57.07%), followed by *Aspergillus* (31.17%), *Rhizopus* (3.36%), *Alternaria* (2.88%), *Mucor* (2.16%), *Penicillium* (1.92%), *Trichothecium* (0.96%) and *Helminthosporium* (0.48%). Whereas, isolation frequency results demonstrated that *Aspergillus* was the dominant isolate (59.38%), followed by *Fusarium* (43.75%). In silo samples, the relative density of *Fusarium* spp. (66.17%) was higher than that recorded in the samples from local markets (41.06%), as shown in Figure 2. The present research identified two main fungus genera (*Fusarium* and *Aspergillus*) that are associated with silo and markets' corn seeds. This result agrees with those of previous studies [2, 12] which reported that *Fusarium* and *Aspergillus* are the main genera encountered on corn seeds in tropical areas. Corn seeds could be infected by these two genera during field or storage periods [12]. Also, when corn become contaminated with *Fusarium* spp. during pre and post-harvest, this leads to an increase in FB1 content; therefore, some studies considered that *Fusarium* spp. is a field and storage organism [13-15].

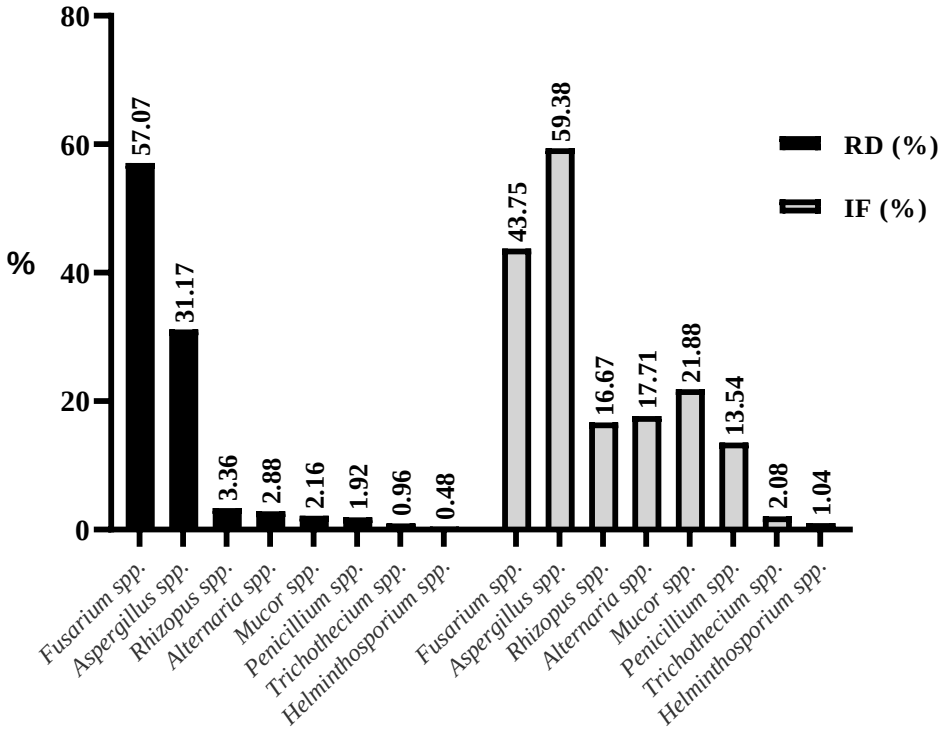

Figure 1-Isolation frequency and relative density of fungi isolated from corn samples of silo and markets in Baghdad city.

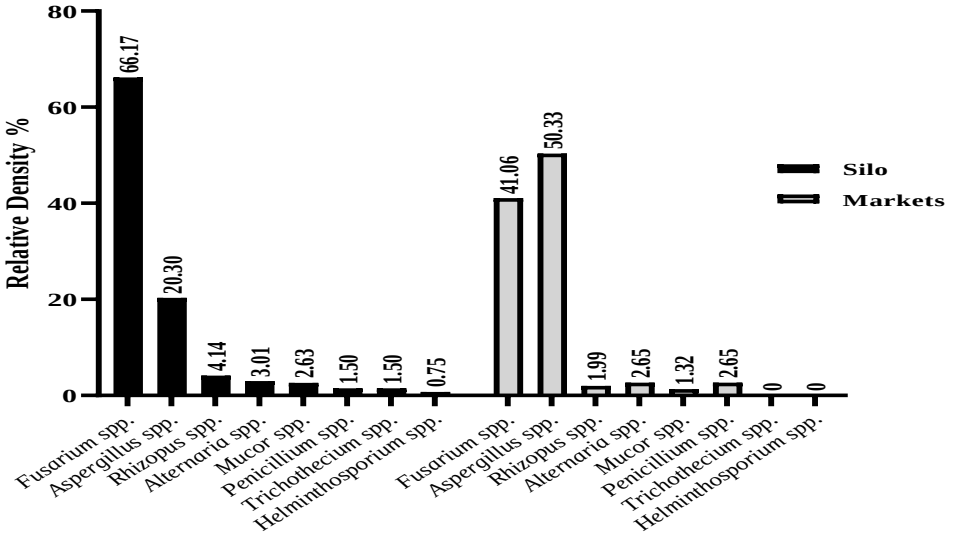

Figure 2-Relative density of fungi genera isolated from corn samples of silo and markets in Baghdad city.

The results revealed that FB1 concentration was 175.54 µg/kg and 172.88 µg/kg in silo and market samples, respectively, as shown in Table (1). There were non-significant differences in the mean of FB1 concentrations between silo and market samples. This finding agrees with that of a previous study which considered *Fusarium* spp. as a field fungus [16]. Also, the present study found no relationship between the number of infected seeds by *Fusarium* spp. and FB1 concentration, as shown in Figure 3. Fumonisins production by *Fusarium* spp. is dependent on *fum* gene cluster (*fum1* - *fum9*), and the responsible gene for FB1 biosynthesis is *fum1*[17]. In addition, FB1 production is *Fusarium* species-dependent and the main producers of FB1 are *F. verticillioides* and *F. proliferatum*.

Table 1- Concentration of Fumonisin B1 in corn samples collected from silo and markets

Location	Number	Mean (µg/kg)	Standard Error	Min.	Max.	ANOVA *p*
Silo	15	112.05	11.71	24.43	175.54	0.753 (NS)*
Markets	15	117.70	13.37	13.69	172.88	

*NS: Not significant ($p < 0.05$)

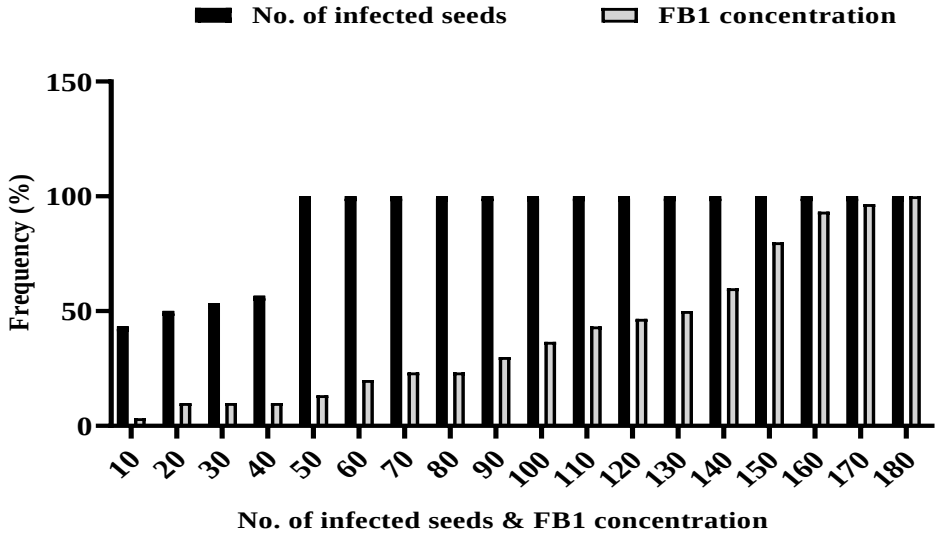

Figure 3-Relationship between number of infected seeds by *Fusarium* spp. and Fumonisin B1 concentration

The concentration of FB1 in the present study was low when compared with previous studies in Iraq [20]. This may be due to the use of new strategies in plant disease control. Also, this comparison showed that the production of FB1 in corn was higher than that in other plants (rice and wheat) in Iraq and other countries, as presented in Table 3. This indicates that corn and its products are the main contaminated foods by FB1 [4]. Corn became more susceptible to toxigenic fungi that produce FB1, due to many reasons such as harvesting methods, drying process, and methods of transport to other countries, which could cause mechanical damage to the corn. In addition, storage conditions, including high humidity, temperature, insects, rodents, and long period of storage, can damage the grains rapidly and elevate corn mycoflora during storage; therefore, stored and broken maize are more vulnerable than freshly harvested maize [21, 22]. Subsequently, ingestion of contaminated grains by FB1 can affect human and animal health, causing hepatotoxic and nephrotoxic effects [4, 5, 23].

Table 3-A comparison of Fumonisin B1 concentration between the present and previous studies in Iraq and other countries

Ref.	FB1 Concentration (µg/kg)	Plant	Detection method	Country
Present study	13.69 - 175.54	Corn	ELISA	Iraq
Al-Zobaidy[24]	0	Rice	ELISA	
Hamad & Akif[20]	467.19 - 570.0	Corn	HPLC	
Hammood & AL-Nazzal [25]	0.103 - 2.240	wheat	ELISA	
Shephard et al [26]	10–3980	Corn	HPLC	Iran
Alkadri et al [27]	5–6	wheat	HPLC	Syria
El-Sayed et al [28]	10 -780	Corn	HPLC	Egypt
Omurtag [29]	0.30–0.32	Corn	HPLC	Turkey
Altıparmak & Tunali [13]	0.11 - 8.48	Corn	HPLC	
Katta et al [30]	0.1 - 3.5	Corn	HPLC	Washington
Liu et al [31]	0.2 - 3.0	Corn	HPLC	Taiwan
Munawar et al [32]	> 0.25	Corn	ELISA	Indonesian

Conclusions

The present study found no significant differences in FB1concentrations between corn samples collected from silos and markets. Also, there is no relationship between number of infected seeds by *Fusarium* spp. and FB1concentration.

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