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Original Research Article

Detection Aflatoxin on the Imported Maize (*Zea mays* L) Using HPLC in the Areas of Baghdad

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Abstract: This study was conducted in the laboratory of fungi of the Department of biology of the collage of Science in Mustansiriyah University to investigate the contamination of grain (maize) displayed in the markets of the city of Baghdad mycotoxins type aflatoxin, was isolated the fungi associated with contamination of maize and the number of 10 isolates where the record *Aspergillus flavus* isolates the most isolates occur 7 *Aspergillus niger* isolates followed by 2 isolates and one isolated *Penicillium* sp. ، *Aspergillus flavus* isolates done Pathogenicity test to identify the isolates of the study. Four isolates were identified (Afla1, Afla2, Afla3, Afla4). The results of the aflatoxin detection in four different samples of positive yellow maize samples showed that all samples tested were contaminated with Aflatoxin, sampled from different regions, Adhamiya, Al-shaab and Palestine Street, had the highest pollution rate of 2149µg/g for Afla 1, while Afla 2 recorded the lowest pollution rate of 114µg/g.

Keywords: Maize, mycotoxins, *Aspergillus flavus,* Aflatoxin.

INTRODUCTION

Maize (*Zea mays* L) considered as one of the most significant grain crops in our world. It gives staple food to numerous populaces. In emerging nations maize is a significant kind of revenue to ranchers among whom many are asset poor. All around the world, maize is known as sovereign of oats since it has the most elevated hereditary yield potential among the grain. It is developed on almost 150 hectares in around 160 Countries having more extensive variety of soil, environment, biodiversity and the board rehearses that contributes 36 % in the worldwide grain creation.

The USA has the most noteworthy efficiency which is twofold than the worldwide normal (Ranum *et al.*, 2014). The infection of maize with fungus (molds) and mycotoxins represent a great Trouble for using in animal and human feeding (Krnjaja *et al.*, 2013) Contamination of grain in the field with fungi leads to the production of mycotoxins during cultivation, post-harvest, in storage, down to transportation and then processing. *Aspergillus flavus* ،*Fusarium graminearum*, *F. proliferatum* and *F. verticillioides* are important species of fungus can contaminate maize grains by produced aflatoxins, trichothecenes, fumonisins and zearalenone (Sun *et al.*, 2017).

Mycotoxins are serious by their presence in agricultural and food products; even at a low level concentricity threaten health of human and animal, which affects their immune response. Since the discovery of mycotoxin, the researchs focus on determination and detection tracks as well the induced poison towards animals and humans (Santos *et al.*, 2019). Afla toxins produced by most common food and feed contaminating species like *Aspergillus flavus* and *A. parasiticus*. There are 14 known aflatoxins, most of them are secondary metabolite formed endogenously in host managed by one major toxin, i.e., aflatoxin and derivatives G1, G2, B1 and B2, *A. flavus* produces B1 and B2 while *A. parasiticus* produces all of these four major toxins (Rawal *et al.*, 2011).

Aflatoxins are carcinogens that are produced by certain fungus (*Aspergillus flavus* and *A. parasiticus*) which isolated from the soil, rot vegetation, straw, and grains. Which are mainly found in poorly stored commodities such as cotton seed, corn, peanuts, millet, wheat, sunflower seeds, sorghum, sesame seeds, tree nuts, rice, and a variety of spices. Children are specially affected by aflatoxin exposure, which leading to stunting, delayed growth, liver cancer, and liver damage. Adults have a top tolerate to exposure, but are also remain in danger (Yu, 2020). The US Food and Medication Administration (FDA) activity levels for aflatoxin present in food or feed is 20 to 300 ppb. The filamentous fungi *Aspergillus flavus* is the essential causal specialist of food and feed defilement with the seriously harmful contagious metabolites, (Kumar, 2017). Aflatoxin B1 is the most common types of aflatoxin, is a poisonous parasitic metabolite known to be cancer-causing and teratogenic for the animals and humans. It is the main mycotoxin delegated a gathering 1a human cancer-causing agent by the Global Organization for Exploration on Malignant growth. Intense wellbeing impacts of aflatoxin openness from utilization of exceptionally sullied food incorporate liver cirrhosis and demise. Persistent utilization of sub lethal fixations is related with liver disease, development disability, and immune concealment (Probst *et al.,* 2011). Chronic exposure can effect in repressed immune response, malnutrition, hepatic lesions, centrilobular necrosis and fatty infiltration of the liver, and even hepatomas (Lewis *et al.*, 2005).

The point of the current review is to explore the presence of aflatoxin in imported maize accessible in Baghdad utilizing HPLC.

METHODS

Collection of Samples

Five samples of yellow corn samples collected randomly from local areas of Palestine Street, Al-Adhamiya and Al-Shaab, and with 1 kg / sample, the samples were placed in polyethylene bags and prepared for laboratory testing.

Figure 1: Imported Maize (*Zea mays* L)

Isolation and Identification

The sample first surface cleaned by washing under sprinter fixture water to eliminate soil like the sand .cut contaminated parts and take tests with aspects (0.5 cm) disinfected surface with 1% sodium hypochlorite answer for 1 moment and afterward wash with refined water multiple times leave until drying, (0.5 cm) appropriated on Petri dishes containing Potato Dextrose Agar (PDP) enhanced with Chloramphenicol 250 mg/L. Three pieces were placed in the dish with three duplicates and brooded at 27 °C±2.for 7 days. a short piece of mycelium from every growth state was moved aseptically unto new plate containing the medium utilized. The parasites were cleaned by rehashed sub-refined. Furthermore, rehashed a few times until unadulterated growths were disengaged. Utilizing sterile vaccinating needle, minute piece of each seclude was taken and blend at the focal point of a clean minuscule slides containing drop of Lactophenol cotton blue stain, encompassed with cover slips and saw under the magnifying instrument. Recognizable proof was made concerning standard reading material, according to (Barnett and Hunter, 1998, Thiyam and Sharma, 2013).

Pathogenicity Test

The same method used by researchers (Khan *et al.,* 1999) to test the pathogenesis with some change in method used to measure the severity of injury, which is as follows:

The yellow corn was chosen flawless and of a similar size, clean the surface with 1% ethanol answer briefly and afterward wash with sanitized water multiple times. The corn was showered with a spore suspension of the tried parasite centralization of 10 6 g/ml. The control was immersed in sterile water. The corn was placed in a sterile bag to avoid friction and contamination. At 25 ° C for seven days, the area of the affected part was recalculated; the resulting fungus was isolated from each spot. Based on the results of the Pathogenicity test, four isolates were selected for the remaining tests.

Detection of Aflatoxin in Imported Maize Samples:

Follow the same method as described by researcher, 2015 (Ismael) and include the following steps:

1- The components of the CZapek's medium were Preparation as described by McGinnis, 1980.
2- Take a disk from the fungus culture in week old placed in the tubes of the liquid medium.
3- Put in incubate for 5 to 7 days at 25 ° C.
4- The mixture was filtered using sterile medical gauze. Then transfer ten mL of filtrate to suppress the separation, and then add 20 mL chloroform.
5- The chloroform-containing organic layer was isolated through a filtration paper with Na2So4 (sodium hydroxide) in order to dispose of the remaining water and dried with Evaporater evaporator at 45 ° C.
6- Add 1 ml of acetonitrile to the extractor and then filtered by filters (0.45 µm) and collect in a special HPLC tube. 0.2 µl of filtration was injected into the HPLC device under U.V. The light was wavelength (267 nm).
7- The amount of aflatoxin was estimated by the following equation:

$$\boldsymbol{Afla\ Conc.} = \frac{Peak\ area\ of\ sample}{Peak\ area\ of\ standard} \times Standard\ concentratin$$

RESULTS AND DISCUSSION

Isolation and Diagnosis of Fungi Associated with Imported Maize:

The results showed isolating 10 isolates of fungi, divided into three fungal species belonging to (*Penicillium, Aspergillus*), of total (5) samples of imported maize, tested. *Aspergillus flavus* figure (1) was the most common species found (7) isolates in all five samples followed by *Aspergillus niger* (2) isolates and then *Penicillium* sp. (1) Isolates. These results are consistent with several studies on isolating and diagnosing the fungus associated with imported maize in terms of the number of isolates to the sample size (Tao *et al.,* 2020 & Chulze, 2010). Also agree with the study (Niaz and Dawar, 2009) and (Bhattacharya and Raha, 2002) who isolated the storage fungus accompanying maize.

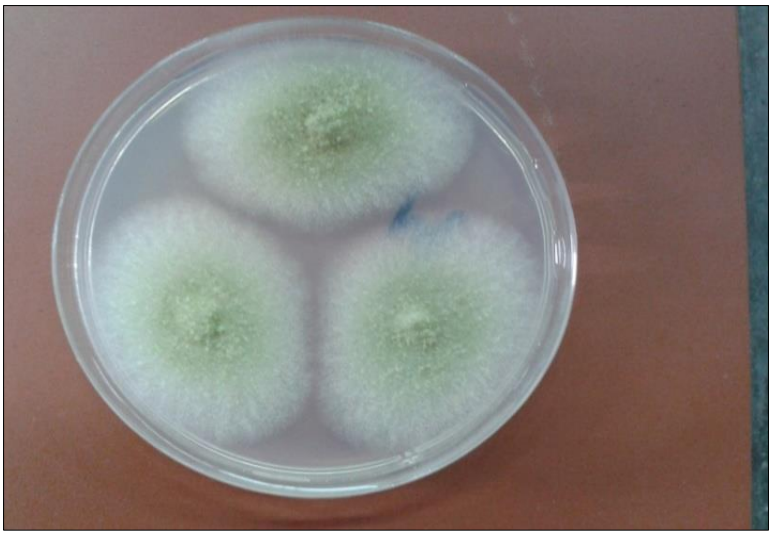

Figure 2: *Aspergillus flavus* on PDA

Pathogenicity Test

The results of the Pathogenicity test showed that all 7 isolates of *Aspergillus flavus* had high Pathogenicity compared with control grains. It was observed the damage *A. flavus* cover grain figure (2) The infection of full yellow maize within three days of the incubation period is due to the content of the corn contains components rich in carbohydrates, carbohydrates, vitamins, sugars and amino acids, which are a whole fertile environment for the growth of fungus The difference in fungal isolates tested in their pathogenic capacity may be due to the amount of toxic substances

produced by these isolates as well as their quality. This study is consistent with another study in the same field (Koenning *et al.*, 2007).

Figure 3: Pathogenicity test

The results of this test were selected four isolates of high susceptibility to conduct a test to detect the presence of Aflatoxin in isolates (Afla1, Afla2, Afla3, Afla4).

Detection of Aflatoxin in Imported Maize Samples

Aflatoxin was found in all four isolates of *Aspergillus flavus* that were taken from imported maize and tested by HPLC. The concentration of aflatoxin in each isolate varied from one to the others. Isolation (Afla 1) recorded the highest concentration of aflatoxin (2149) µg / mL followed by isolation (Afla 4) where it recorded (233) µg/mL. (Afla 3) with a concentration of (215) µg / mL, while the lowest concentration (114) µg / ml for isolation (Afla 2).

Table 1: Aflatoxin levels using the HPLC method

Sample	Afla 1	Afla 2	Afla 3	Afla 4
Concentration of aflatoxin µg / mL	2149	114	215	233

The apparent results show the difference in concentrations between samples.

1- This difference is similar to several researches in this field, which is due to the different sample areas and conditions of sorting, storage and treatment. (Younis and Malik, 2003.) (Herzallah, 2009) (Keith and Susan, 2010).
2- The different levels of aflatoxin concentrations are due to the presence of mycotoxin-producing fungi before harvesting and during storage; there may be an increase in level (Chulze, 2010). (Neme and Mohammed, 2017).

CONCLUSION

In recent times, the elimination of the dangers of contamination of mycotoxins has become one of the most important challenges facing the competent authorities for its wide spread and high toxicity. Some developing countries find it difficult to implement special strategies to reduce the risk of these toxins because of their lack of resources, technology and infrastructure for food control and best practices for storage and thus increase the number of people at risk of fungal poisoning through contaminated food and especially grains. To prevent contamination of food products with fungi, the following points should be considered:

1- Activating the laws of agricultural quarantine at the border crossing points to prevent the entry of contaminated foodstuffs, grains and vegetables.
2- Serious attention to provide stores with high specifications of quality by determining the humidity and control with good ventilation and lighting.

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