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Occurance of Aflatoxins (AFs) in Corn and Peanut in Damietta Governorate, Egypt

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Abstract

Eighty random samples of corn and peanut that were purchased from different regions in Damietta Governorate (July 2010 to July 2011) were analyzed for aflatoxin (AFs) by HPLC and screened for the presence of fungi. Total count of fungi in corn ranged from 24 to 62 colony/g, the corn samples were found to be contaminated with six species of fungi belong to four genera; *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria*. The *Aspergillus* group was the most prevalent fungi in all examined samples, within these species, *A. niger*, *A. flavus*, *A. fumigatus*, *A. ochraceus* and *A. terreus*, in addition to *Fusarium equiseti* species. On the other hand, peanut samples were contaminated with eleven species belong to five genera, *A. flavus*, *A. parasiticus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. terreus*, *Penicillium chrysogenum*, *Penicillium nigricans*, *Alternaria alternate* , *Mucor sp.*, and *Chaetomium sp*. The total fungal count ranged from 48 to 73 colony/g peanut sample. AFs were detected in corn and peanut and its total concentrations ranged from 2.030 to 6.003±1.6 µg/kg and 4.23 to 5.69 µg/kg, respectively. This study concluded that the AFs level in the corn and peanut in the collected samples were below permissible levels.

Keywords: Aflatoxins, HPLC, corn, peanut, Fungi.

Introduction

The mycotoxin contamination of cereal commodities is a much older problem than its detection and characterization. Molded grains often caused different animal and human health problems long before the causal agents were known. The first mycotoxin detected was the aflatoxin B_1 (AFB_1) in the 1960s and since then hundreds of toxins have been described (D'Mello and Macdonald, 1997; Bartok *et al*., 2006). Aflatoxins (AFs) are typically found as secondary metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* and to a lesser extent *A. nominus* and other fungi (Migahed *et al.,* 2003). Epidemiological studies have shown that prolonged exposure to AFB_1 can cause liver cancer, especially in persons with hepatitis B antigens (Stubblefield *et al*., 1983). Consequently, the World Health Organization (WHO) classifies AFB_1 and its metabolites as a human carcinogens

(Anklam *et al*., 2002). AFs production is the consequence of a combination of fungal species, substrate and environment. The factors affecting AFs production can be divided into three categories: physical, nutritional and biological factors. Physical factors include temperature, pH, relative humidity of the atmosphere, storage or incubation time **(Ritter *et al*., 2011),** water activity, moisture content, light, aeration and level of atmospheric gases. AFs production in the substrate can happen in the field and in storage conditions between 20 and 40 ºC with a 10-20% of moisture and 70-90% of relative humidity in the air (Pitt and Hocking, 1997). The diseases caused by the ingestion of AFs are loosely termed 'aflatoxicoses' and can be a result of acute, sub-acute or chronic ingestion. AFs are known to be hepatocarcinogenic, mutagenic, teratogenic and immunosuppressive in both animals and human (Hussein and Brasel, 2001 and Papp *et al*., 2002). Fungal infection of seeds before and after harvest remains a major problem of food safety in most parts of Africa. Problems associated with this infection include loss of germination, mustiness, mouldy smell (Frisvad, 1995). Peanut is important substrates for the growth of different members of *Aspergillus* section *Flavi:* Invasion of peanut occurs as a result of drought stress and related factors (Cole *et al*., 1982). Fungal contamination of corn can occur in the field or in store, with the extent of contamination largely determining the rate of deterioration of stored grains (Chatterjee *et al*., 1990). The *Aspergilli* and *Fusaria* are the most commonly isolated contaminants of corn worldwide, with the most important species being *Aspergillus flavus, A. parasiticus, Fusarium verticillioides* and *Fusarium proliferatum* (Munimbazi and Bullerman, 1996; Ali *et al*., 1998). Therefore, the aim of this study was to analyze the composition and diversity of the fungal flora of corn and peanut and to investigate the presence of AFs in different samples of corn and peanut at Damietta governorate.

MATERIALS AND METHODS

Sampling

A total of eighty samples (forty of each product) of local corn and peanut were collected from five regions in Damietta Governorate (EL Zarka, Faraskour, Kafr Saad, Damietta City and New Damietta City). Eight samples were collected from two locations (A, B) for each region. All of the samples were randomly purchased from local markets and were put in sealed polythene bags and transported to the laboratory where they were stored at -4°C until analysis. The monitoring program was carried out for one year from July 2010 to June 2011.

Methods

Isolation and Identification of Fungi associated with peanut and corn samples

Fungi were isolated and cultured according to the methods of Lichtwardth *et al.* (1958); Mislivec (1977) and Ichinoe *et al.* (1983) peanut and corn samples were thoroughly mixed to obtain homogeneity and five disinfected grains of peanut and corn each were randomly selected from samples of each location, surfaced sterilized using 5% sodium hypochlorite solution and washed aseptically with ten successive 100 ml volume of sterilized distilled water before plating, directly plated in petri dishes on Czapek's Dox agar medium, and incubated at 25°C for 4 days. The fungal colonies that developed from the infected grains were isolated, purified and maintained on slants Potato Dextrose Agar (PDA) medium. Subculturing was repeated successively until pure fungi were obtained and were identified according to the generic or species level according to (Gilman, 1957; Barnett and Hunter, 1972).

Extraction of aflatoxins by BF method

Aflatoxins (AFs) were extracted from peanut and corn according to method of Association of Official Analytical Chemists (AOAC, 2000). In brief, fifty grams of blended corn and peanut powder were added to 250 ml of methanol/water mixture (55/45 by volume) with 200 ml hexane and 4 grams sodium chloride and blending for 1 min. at high speed into a blender. Then all content was centrifuged for 5 min at 2000 rpm, after separation takes place within 30 min. Pipette 25 ml of lower aqueous methanol phase in a separating funnel, add 25 ml chloroform, stopper and shake 30–60 seconds. Drain bottom chloroform layer through anhydrous sodium sulphate into a 250 ml beaker, repeat extraction, collect in a beaker, and evaporate combined chloroform extract to between 2 ml and just dryness under nitrogen stream. Dried residue was dissolved in 200-µl benzene/acetonitrile mixture (98/2 by volum), spot on TLC or HPTLC along with known aflatoxin standards.

Determination of AFs by HPLC

50 µl hexane and 50 µl of trifluoracetic acid were added to dry residue of each sample and standard and vortexed for 30 sec. Kept for 10 min at 40°C in water bath, then evaporated to dryness under nitrogen. 450 µl water/ acetonitril mixture (9/1, v/v); was added to each vial to dissolve residue and vortexed and used for HPLC analysis**.** All samples were measured at National Research Centre, Cairo, Egypt.

HPLC conditions: The mobile phase consists of acetonitrile/water/methanol (1:6:3, by volume). The separation was performed at ambient temperature at a flow rate of 1.0 ml/min. The injected volume was 20 µl for either the standard solutions or the sample extracts. The fluorescence detector was operated at excitation wavelength of 365 nm and an emission wavelength of 450 nm. AFs concentration in samples was determined from the standard curve, using peak area for quantity estimation **(AOAC, 2000).**

RESULTS and DISCUSSION

Fungal species in corn and peanut samples

The results indicated that corn samples were contaminated with six species belonging to two genera, *Aspergillus* and *Fusarium* were the most prevalent fungi in all examined samples. Where, *A. niger* constituted 39% of the total fungal count of the isolates, followed by *A. flavus* (28%). *A. fumigatus* and *A. ochraceus* (7%) for each. While *A. terreus* represented the lowest occurrence of (6%). *Fusarium equiseti* was 12% of the total fungal count. In addition, it was obvious that the corn samples collected from Damietta City recorded the higher total fungal count (TFC = 62.0 colony/g corn sample), followed by Faraskour (56.0 colony/g), El-Zarka and Kafr Saad regions (52.0 and 47 colony/g, respectively). New Damietta City represented the lowest one (24.0 colony/g), **(Fig. 1).**

In peanut samples, it was found that *A. fumigatus* was the predominant and constituted (32 %) of the total fungal count of the isolates, followed by *A. niger* (19 %) and *A. flavus* species (15%). Whereas *A. terreus*, *Alternaria alternate*, *A. parasiticus*, *Penicillium nigricans* and *Mucor* were 11%, 7%, 6%, 4% and 2%, respectively. *A. ochraceus*, *Chaetomium spp*. and *Penicillium chrysogenum* recorded 1.0 % for each one. In addition, the peanut samples collected from Kafr Saad recorded the higher total fungal count (TFC = 73.0 colony/g). Followed by Damietta City and Faraskour regions (66.0 and 62.0 colony/g), respectively. New Damietta City and El-Zarka regions recorded (Total count = 57.0 and 48.0 colony/g), respectively, **(Fig. 2).**

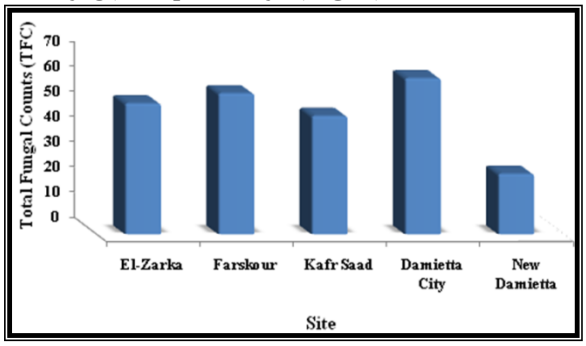

Fig. (1): TFC associated with corn samples collected from different regions in Damietta Governorate.

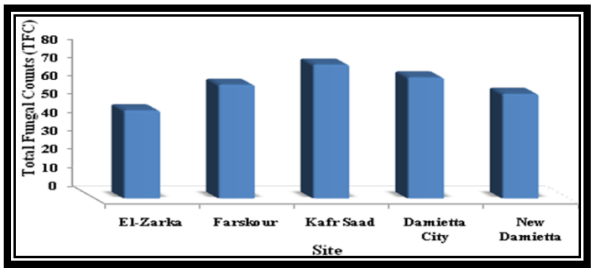

Fig. (2): TFC associated with peanut samples collected from different regions in Damietta Governorate.

The infection of corn by such these species may be attributed to poor post-harvest handling or associated with insect damage in the field and many contamination events (Cotty, 2001). Also may be attributed to wounding of the developing crop by birds, mammals, insects, mechanically (e.g. hail) or the stress of hot dry conditions results in significant infections (Odvody *et al.*, 1997; Guo *et al.*, 2003). Weather conditions such as warm and moist conditions may affect crops infections such as warm, moist conditions either in the field or during transportation and storage, or use. Under high humidity, initially dry seed develops water content conductive to contamination (Choudhary and Sinha, 1993; Cotty *et al*., 1994). Our results also agreed with the results of Ito *et al*. (2001) who mentioned that *Aspergillus* species are important colonizers of corn. Among the *Aspergillus* species, *A. flavus*, *A. parasiticus*, *A. nomius*, *A. pseudotamarii*, *A. bombycis* and *A. ochraceoroseus* are of concern due to their toxigenic potential for producing AFs. Also our results agreed with the results of Mostafa and Kazem (2011); they found that the incidences of *Fusarium* spp. were the highest (35.2%), followed

by *Aspergillus* spp. (2.9%), *Penicillium* spp. (1.1%), *Rhizopus* spp. (2.3%), *Mucor* spp. (1.4%), and *Alternaria* spp. (0.2%).With respect to the reasons of fungal presence in crops, De Nijs *et al*., (1996) explained that the storage conditions may affect the fungal growth, and they added that to avoid growth of *A. flavus* in grains during storage the moisture levels should be <14%. Perhaps, it is likely that most grains can become contaminated with AFs during storage and levels that were present in the grain pre-harvest may increase if the grain is not sufficiently dried and stored. Makun *et al.* (2010) had explained that fungi incidence is likely to be higher in badly stored grains than those in the field. They also added that the exposure of marketed samples to the ubiquitous fungi and their spores in the air and the aerobic condition of the atmosphere would encourage more fungal growth than the relatively anaerobic conditions of storage systems such as sack. This might explain the higher fungi occurrence in market samples than those from the stores.

Also, the obtained results are in accordance with the results of El-Magraby and El-Maraghy (1988) who reported that 43 species of fungi, belonging to 16 genera were isolated from groundnut seed samples; the most dominant genera were *Aspergillus*, *Penicillium* and *Fusarium*: *A. fumigatus* (62% of samples), *A. flavus* (57%), *A. niger* (55%), *Penicillium chrysogenum* (52%) and *Fusarium oxysporum* (55%); they were the most frequently isolated species. Additionally, our present results agreed with Nakai *et al*. (2008) who found that a predominance of *Fusarium spp*. (67.7% in hulls and 25.8% in kernels) and *Aspergillus spp*. (10.3% in hulls and 21.8% in kernels). These results agreed also with those of Euloge *et al. (*2012), they found that the fungal isolates from peanut cake products samples include *A. flavus, A. parasiticus*, *Fusarium* spp., and *Penicillium* spp. The contamination of peanuts with fungi, especially aflatoxigenic species, may be due to the occurrence of rain while peanut pods are drying in the field after uprooting (Horn and Dorner, 1999). Diener *et al*. (1982) and Sanders *et al*. (1984) stated that infection of peanut by *A. flavus* can start in different growth stages of the plant and in different organs. Flowers and pegs can be contaminated when temperatures are high during the morning (27-30°C), but the infection occurs more often through the pegs after their penetration into the soil and during fruit and seed development. Pitt and Hocking (2006) mentioned that water activity (a_w) of the substrate also plays an important role in fungal growth and is defined as the ratio between the vapor pressure of a material (p) and the vapor pressure of pure water (p^o) under the same conditions ($a_w = p/p^o$). Moss (1991) stated that factors such as soil moisture content, damage caused by insects, mineral deficiency and stress play an important role in fungal contamination. However, high concentrations of AFs are related to the growth of *A. flavus* and *A. parasiticus* after harvest when storage conditions are propitious. Davison *et al*. (1982) reported that one important factor that contributes to the contamination of stored peanuts is the high moisture content of peanut grains during post-harvest drying and the inability to maintain adequate moisture during storage. Many investigators have correlated the presence of fungi and weevils in stored grains and suggested that these insects serve as mechanical vectors of viable fungal structures from a contaminated to a non-contaminated environment (Ferreira-Castro *et al*., 2012).

Detecion of Aflatoxins (AFs)

I. In corn

The percentage of incidence of contaminated corn samples as 3 out of 8 (37.5%) of the corn samples collected from El Zarka, 4 out of 8 (50%) of the samples collected from Faraskour region, also 4 out of 8 (50%) of the samples collected from Kafr Saad region. While, 2 out of 8 (25%) of the corn samples collected from Damietta and only one sample of corn collected from New Damietta (12.5%) were contaminated with AFs (Fig. 3). The total AFs levels in corn samples were 6.003±1.60, 2.497±0.38, 3.510±0.91, 4.060±0.69 and 2.03 µg/kg for El Zarka, Faraskour, Kafr Saad, Damietta City and New Damietta City, respectively (Fig.4 and Fig.5a,b). Concentration of AFs in corn samples showed a significant difference between different types of AFs where AFB_1 recorded the highest level through all sites with mean concentration of 1.95±1.08, followed by AFG_1, AF B_2 and AF G_2 with mean concentrations of 0.944±0.77, 0.420±0.31 and 0.272±0.27 µg/kg, respectively, in the same order. According to (Egyptian Regulations, 1990 and FAO, 2004), the maximum limit of AFs in corn and peanut products cannot exceeds 20 and 10 µg/kg, respectively. AFs concentrations in our samples were lower than the permissible limits set by international organizations. These results agreed with Yazdanpanah *et al*. (2001) who

determined AFs in nine corn samples and they found that AFB_1 and AFB_2 were detected in 8 (88.8%) and 6 (66.6%) samples at a mean level of 15.83 and 2.99 ppb (median 1.72 and 1 ppb), respectively. The results were in agreement with those of Abdullah *et al*. (2011) and with Lutfullah and Hussain (2012) who reported that the percentage of contamination for AFs in corn samples (40%) and they added that the highest contamination levels of AFs were found in one corn sample (13.0 µg/kg). On the other hand, the results were in disagreement with Sanchis *et al*. (1995), Da Silva *et al.* (2000) and Bokhari (2010) who detected AFs ranged from 1 to 24.8 ppb. The high total aflatoxin concentrations may be attributed to the high agricultural activities all the year. A variety of moulds routinely infect the world's cereal crops. Under certain field or storage conditions, some moulds can produce toxic metabolites "mycotoxins". Aflatoxins (AFs) are a group of highly carcinogenic mycotoxins produced primarily by the fungus *Aspergillus flavus* (Kumar *et al*., 2008). Sanchis and Magan (2004) explained that corn and some other cereals are excellent media for fungi growth and mycotoxin contamination which attack either the plant in field or in cereal grains during the storage (pre- or post-harvested contaminations). Also they added that some of these pathogenic fungi produce mycotoxins which are considered secondary metabolites and it can cause diseases for both human and animals. The main fungal species, which infect maize are *A. flavus*, *A. parasiticus* and *A. nomius*. These fungal species grow well in the range of 19-35°C and produce maximum aflatoxins at 28°C. Ali *et al*. (2005) attributed such these contaminations to climate of the region; they stated that the climate in Egypt, with its uniform high temperature and high relative humidity, is conducive for growth of AFs producing fungi on stored grains. They also added that the incidence of AFs in foods and feeds is relatively high in the tropical and sub-tropical regions due to the warm and humid weather conditions that provide ideal conditions for the growth of the aflatoxigenic moulds.

II. In peanut.

Peanuts are important substrates for fungal growth and subsequent AFs production by different members of *Aspergillus* section *Flavi*: *A. flavus* Link, *A. parasiticus* Speare, *A. nomius*, *A. pseudotamarii* and *A. bombycis*. Recently, additional new AFs producing species have been isolated from peanuts in Argentina (*Aspergillus arachidicola* spp. and *Aspergillus*. *minisclerotigenes* spp. (Pildain *et al*., 2008). The incidence % of AFs in peanut samples shows that 4 out of 8 (50%) of the peanut samples collected from El Zarka, 4 out of 8 (50%) of the peanut samples collected from Faraskour, 5 out of 8 (62.5%) of the samples collected from Kafr Saad region 6 out of 8 (75%) of the peanut samples collected from Damietta City and four samples of the peanut collected from New Damietta region were contaminated with AFs (Fig. 6). The significantly difference between the four types of toxin concentrations, where AFB_1 recorded the highest level through all sites with mean concentration of 2.49±0.24, followed by AFG_1, AFG_2 and AFB_2 with mean concentrations of 1.265±0.20, 0.766±0.13 and 0.595±0.11 µg/kg, in the same order. It was found that the mean levels of total AFs recorded for the samples collected from different areas were 4.23±1.7, 5.68±1.40, 5.06±1.98, 5.23±1.50, and 5.4±2.10 µg/kg for EL Zarka, Faraskour, Kafr Saad, Damietta City and New Damietta City, respectively, (Fig. 7 and Fig. 8). The results obtained showed that all AFs concentrations in peanut samples are lower than the Egyptian and international permissible limits accorging to (Egyptian Regulations, 1990 and FAO, 2004). The results are in agreement with Iqbal *et al*. (2013) who found that peanut samples contaminated with AFs with mean concentration in raw peanut shell of (6.4 µg/kg) and raw peanut without shell (9.6 µg/kg) lower than permissible limits. On the other hand, the results are different from those of Nakai *et al*. (2008); Kamika and Takoy (2011); Michele *et al*. (2012); Euloge *et al.* (2012) and Ezekiel *et al*. (2013) as they found AFs in peanut with concentrations exceeded the maximum limit of 5 µg/kg prescribed by WHO and EU, and 10 µg/kg prescribed by FAO and Egyptian regulations. Although AFs contamination of peanuts occurs during post-harvest curing and storage, the most significant contamination usually occurs prior to harvest during periods of late season drought stress as peanuts are maturing. The losses caused by fungal infection are mainly due to the rejection of food with visible fungal growth and /or to its probable content of mycotoxins (Olaru *et al.*, 2008).

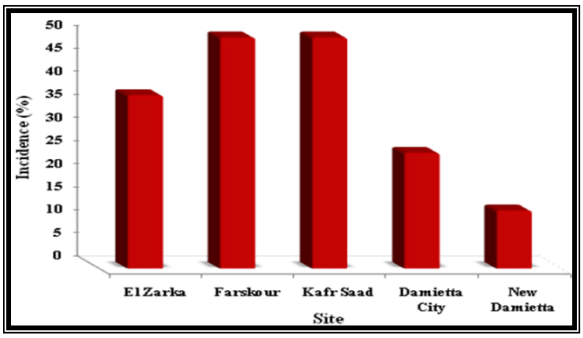

Fig. (3): The incidence percent of AFs in corn samples.

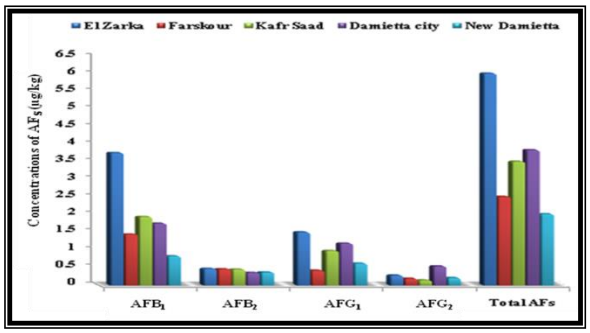

Fig. (4): Mean concentrations of AFs of corn samples.

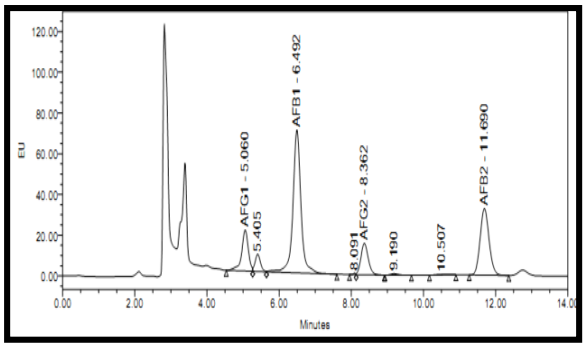

Fig. (5a): HPLC chromatogram standard of AFs.

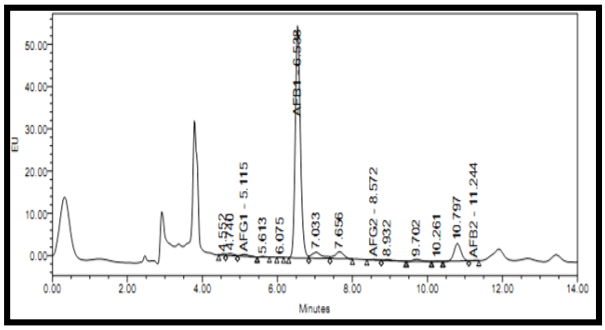

Fig. (5b): HPLC chromatogram of AFs in positive sample of corn from El-Zarka.

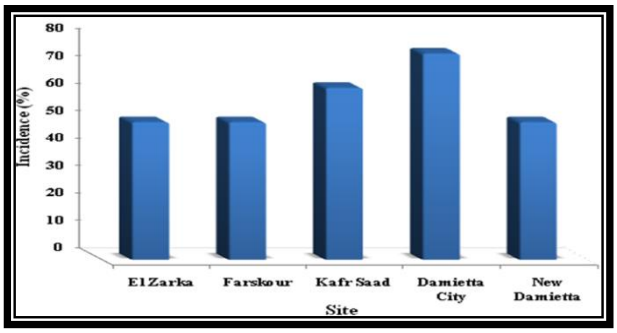

Fig. (6): The incidence percent of AFs in peanut samples.

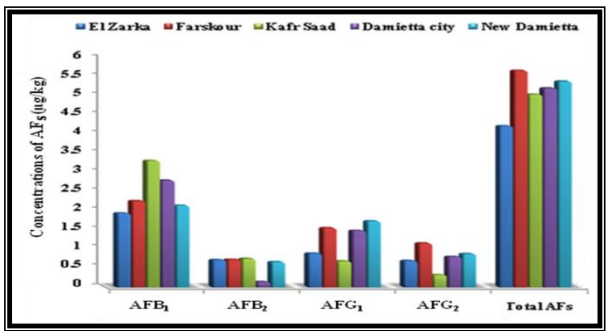

Fig. (7): Mean concentrations of AFs contamination in peanut samples.

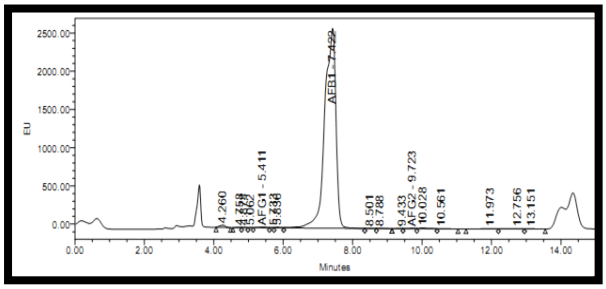

Fig. (8): HPLC chromatogram of AFs in positive sample of peanut from Faraskour region.

Conclusion

In conclusion, the concentrations of AFs in corn and peanut samples collected from Damietta Governorate were lower than international permissible limits. The authors strongly recommend that desired control of mycotoxins can be achieve by reducing fungal infection of crops by rapid drying and correct storage of the harvested crops using effective anti-mould preservatives. Properly designed, mycoflora and mycotoxin surveys and monitoring programmes can reduce the fungal and mycotoxins in our foods. In addition, good agricultural practices (GAP) can be applied to control fungal contamination of crops.

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الملخص العربي

عنوان البحث: تواجد الأفلاتوكسين في الذرة والفول السودانى في مناطق مختلفة بمحافظة دمياط، مصر

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تم تجميع عدد ٨٠ عينة من الذرة والفول السودانى عشوائيا من مناطق مختلفة بمحافظة دمياط وهى الزرقا، فارسكور، كفر سعد، دمياط القديمة و دمياط الجديدة خلال الفترة من (يوليو ٢٠١٠ إلى يوليو ٢٠١١) وقد تم تحليل العينات باستخدام جهاز الكروماتوجرافى السائل عالى الكفاءة، بالإضافة إلى فحص وجود الفطريات والافلاتوكسين بالعينات. وقد أظهرت النتائج أن عينات الذرة ملوثة بستة من الأجناس وأربعة من الأنواع حيث كان لفطر الأسبرجلس السيادة في الظهور في كل عينات الدراسة، حيث تراوح العد الفطري الكلى لعينات الذرة مابين ٢٤ و ٦٢ مستعمره/جم ذرة، بينما تبين أن عينات الفول السودانى ملوثة بإحدى عشر من ألاجناس و خمس أنواع وقد تراوح العد الفطري الكلى لها مابين ٤٨ و ٧٣ مستعمره/جم فول سودانى). كما أوضحت النتائج تواجد طبيعى لسموم الأفلاتوكسين في الذرة والفول السودانى، حيث تراوح التركيز الكلى لسموم الأفلاتوكسين فى الذرة مابين ٢,٠٣ و ٦,٠٠٣± ١,٦، وفى الفول السودانى مابين ٤,٢٣ و ٥,٦٩ جزء في البليون. ونستنج من الدراسة أن التركيز الكلى لسموم الأفلاتوكسين فى عينات الذرة والفول السودانى اقل من الحدود السموح بها مما لا تمثل خطرا على صحة الإنسان.