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Prevalence extent of aflatoxigenic fungi and their toxins level in corn and corn-based products

Ahmed Lotfy E. Mohmoud, Ayat H. Abdel-aziz, Elhagag A. Hassan

Botany and Microbiology Department, Faculty of Science, University of Assiut, Egypt.

*Corresponding Author:ahlotfy10@aun.edu.eg

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ABSTRACT

In the current study, the physicochemical analysis of 48 different corn and corn-based products samples collected from Assiut Governorate revealed that among the corn sources, white corn had the highest concentrations of total carbohydrates, protein, sodium , magnesium, chloride, phosphate and sulphate. As for, corn-based products, flamenco recorded the highest content of moisture, lipids, proteins, nitrate, phosphate and sulphate. Mycological analysis of these samples resulted 37 fungal species in addition to one varity belonging to nine fungal genera. Aspergillus flavus and A. parasiticus were consistently the most common species in all samples. It was concluded that the physicochemical properties of collected samples displayed the main role governing the biodiversity and abundance of isolated fungi. Using TLC technique, results indicuted that from 71 Aspergillus isolates tested for their potential ability to produce aflatoxins B1 ,B2 ,G1 and G2. Thirty-four fungal isolates could produce these toxins with different abilities. The study revealed that 50% of the tested isolates related to each of A. flavus and A. parasiticus in addition to 20% of those releated to A. flavus var. columnaris could produced aflatoxins with different abilities. It was noticed that all collected corn and corn-based products samples were differently contaminated with aflatoxins. The HPLC technique was used for confirmation and quantification of aflatoxins levels in the extracts of the most aflatoxins-producing potential fungal isolates of A. flavus (AFC3) and A. parasiticus (APP56). In addition, aflatoxins level was estimated in the two samples from which the two previous fungal isolates were recovered. A. flavus (AFC3) produced aflatoxins B1 and G2 with a total concentration (34.832 ng/g),while A. parasiticus isolate (APP56) produced B1 , B2 and G2 with a total concentration (21.82 ng/g). As for cornflakes sample from which AFC3 was isolated, it contained B1 (9.206 ng/g) and G2 (1.531 ng/g), whereas popcorn sample from which APP56 was isolated contained B1 (2.942 ng/g) and G2 (17.433 ng/g). Presence of aflatoxigenic aspergilli in corn and corn-based products gives the probability of aflatoxins formation in these samples. In addition,the natural occurrence of aflatoxins in cornflakes and popcorn with a level which exceeds a lawable limit of these toxins in many countries all over the world represents hazard and threatens the public health.

INTRODUCTION

Food security is one of the most pressing issues around the world, indicating a failure to meet the population's food needs due to a lack of funding [1]. Egypt has long been considered one of the most exposed countries in the world when it comes to food security [2]. Corn (*Zea mays* L.) is one of the most popular important economic cereal crops for human consumption and animal feed all over the world. Along with its use as a feed crop, a food staple and animal feed it is an important source for human health that is used for the treatment of blood pressure, cholesterol, urinary disorders, and other medical uses, it also provides income to farm households and traders [3]. It contains more proteins and antioxidants than many other cereal grains [4]. Consequently, in recent years corn and corn-based products gain the main attention for the economic and scientific interests in both developing and industrial countries. As, corn cultivation lands in developing countries represent around half of all cultivated land, where corn flour is consumed as a food staple for poor people and corn stalks are used to feed farm animals during the dry season. On the other hand, in countries with an industrialized economy, corn is primarily utilized as fodder for livestock and as a raw material for manufacturing industrial products e.g., as feed, silage, breakfast food and processing (breakfast cereals, corn chips, grits and flour), industrial starch and popcorn [5]. Egypt is one of the largest importers of corn due to increased consumer demand and low corn production. It is critical to the state's strategy to restore and develop farmlands, which can be used extensively for corn cultivation [6]. This would result in a rise in corn production and a decline in corn imports from other countries[7-8].According to the Food and Agricultural Organization (FAO), mycotoxins infect between 25% and 50% of crops around the world. According to reports, a variety of fungal species found in corn belongs to the genera *Fusarium*, *Aspergillus*, and *Penicillium*, all of which have been linked to the production of mycotoxins that cause mycotoxicosis in humans and animals. Fungi and their mycotoxins in corn grains have been documented by several researchers [9-14].

The presence of microfungi could be attributed to the infection of corn at the pre-harvest stage (in the field) and/or at the post-harvest stage (during storage) [15]. Aflatoxins are fungal secondary metabolites generated by *Aspergillus flavus* and *A. parasiticus*. Aflatoxin is a broad term that refers to four different forms of mycotoxins, namely B_1, B_2, G_1, and G_2, that are a major health concern in food and feed. Aflatoxin B_1 is one of these forms that are typically present in high concentrations in contaminated food and feed and potentially responsible for about 23% of liver cancer cases all over the world. Humans may consume mycotoxins mostly directly from foods or through animal-based food containing mycotoxins as residues. Depending on the mycotoxin type, the ingestion can cause deterioration of liver or kidney function. in addition, they act as neurotoxins and interfere with cellular protein synthesis causing effects ranging from human body immunodeficiency to organ necrosis [16-17]. The presence of aflatoxins in cereals and

cereal-based food is also restricted by the European Commission, which has set maximum limits of aflatoxins in cereals varying from 4 to 10 $ng.g^{-1}$[18].The current study was designed to throw the light on aflatoxigenic aspergilli's prevalence and natural occurrence of aflatoxins in corn and corn-based products.

MATERIALS AND METHODS

Collection of corn and corn-based products samples:

Six types of corn and corn-based products including white, yellow corn, popcorn, corn flakes, Doritos and Flaminco were collected during 2018-2019 from different areas (Assiut, Manfalout, El kosia and Dairout) in Assiut Governorate. A total of 48 samples were collected (8 samples from each type). two hundred and fifty gram of each sample were collected in clean sterilized plastic bags from every site. The samples were transferred immediately to the laboratory, homogenized, then were kept in the refrigerator at 4°C prior to further experiments.

Physicochemical analyses of corn and corn-based products samples:

The moisture content and total content (ash) were measured according to AOAC,[19]. The total lipids were determined calorimetrically using kits of **DIAMOND Diagnostics**, Egypt. The total proteins were determined **by** CBB G-250 dye-binding method as described by Bradford[20]. The anthrone-sulphuric acid method was used for the determination of carbohydrates[21-22]. The soluble sodium (Na^{+}), potassium (k^{+}), calcium (Ca^{+2}), magnesium (Mg^{+2}), chloride (Cl^{-}), phosphate (PO_4^{-3}), nitrate (NO_3^{-}), sulphate (SO_4^{-2}) and bicarbonate (HCO_3^{-}) were determined according to APHA, [23]. The physicochemical analysis for each sample was treated individually in triplicates.

Isolation and identification of fungi:

Toxigenic fungi were isolated from corn and corn-based products using serial dilution and direct techniques on a selective medium "Dicloran rose-bengal chloramphenicol (DRBC)"composed of (g/L): Peptone, 5.0; Glucose, 10.0; KH_2PO_4, 1.0; $MgSO_4.7H_2O$, 0.5; Dichloran, 0.002; Rose-bengal, 0.025 the pH after sterilization was 5.6±0.2 [23]. The three plate replicates inoculated with each collected sample were incubated at 28±1 º C for 7 days. The grown cultures were transferred on fresh potato dextrose agar (PDA) plates for purification and identification. Each pure culture was identified based on cultural and microscopic characteristics using identification keys [25-32]. These recovered identified fungal cultures were kept on a PDA slant and maintained at 4 º C for further studies.

Correlation of physicochemical properties and isolated fungi

The correlation of the determined physicochemical properties with the occurrence of fungal communities was determined using Canonical correspondence analysis (CCA) using PAST (PAleonotological STatistics) Software version 3, USA.

Aflatoxins-producing potential of the isolated identified fungi:

A total of 71 fungal isolates belonging to *A. flavus* and *A. paraciticus* obtained during this study were screened for their aflatoxin-producing potential.Before cultivation in the liquid medium, the selected strains were cultivated on Potato dextrose agar (PDA) solid medium according to *Filtenborg and Frisvad* [33]. To obtain sufficient inoculum for cultivation on a liquid medium, seven-day-old culture of each fungus grown at 25 ± 1 °C were used for inoculum preparation. A disc (1cm) taken by cork-porer from the edges of fungal culture was used as inoculum [34]. Liquid yeast extract sucrose medium (YES) was used for fungal growth and toxin production. It contained (g/L): yeast extract ,1.0; peptone ,10.0; sucrose, 30.0; K_2HPO_4 ,1.0; $MgSO_4.7H_2O$,0.5; $FeSO_4.H_2O$,0.01; KCl, 0.5; $NaNO_3$, 2.0 the pH after sterilization was 6.5. The medium was autoclaved for 15 min at 121 °C. In each100 ml flask, 25 ml of YES medium was taken, inoculated and cultivated under static conditions at 28 °C for 14 days.The experiment was carried out in triplicates.

Extraction of aflatoxins from fungi:

The liquid culture containing fungal growth of each isolate was homogenized in 25 ml chloroform for 5 min in high-speed blender. The organic phase was separated from the aqueous phase by a separation funnel, filtered through Whatman filter paper No.1, dehydrated over anhydrous sodium sulphate, and evaporated to near dryness on a rotary evaporator. The residues of each extract were redissolved in 2 ml of chloroform kept in small vials until the detection process

Extraction of aflatoxins from the natural sources

Twenty-five grams of each milled maize sample were weighted separately and put in 250 ml Erlenmeyer flasks. 100 ml methanol-water (5.7: 1) were added to each flasks, stoppered and protected with a foil. The flasks were shaken vigorously for 30 min. The produced mixture was filtered through a Whatman No. 1 filter paper and the residue was discarded [35].

Clean-up of mycotoxins extracts.

Twenty milliliters of the obtained filtrate was transferred into a 125 ml capacity separating funnel, and 20 ml of 10% sodium chloride solution was added and mixed well. Then, 12.5 ml of n-hexane was added, and the mixture was shaken for 1 min. The

separating funnel was allowed for 10 min. to separate the aqueous phase from the organic phase.The lower phase (aqueous) was drained into a second 125 ml separating funnel, wh

Liquid-liquid partition

To the aqueous phase in the separating funnel, 12.5 ml of chloroform was added and shaken for 1 min. Phases were allowed to separate and the lower phase (organic) was allowed to pass through a bedrock of sodium sulphate into a 250 ml Erlenmeyer flask. The above steps were repeated for all samples. After the chloroform extraction, the extracts were concentrated by evaporating to dryness on a rotary evaporator. The residues of each extract were redissolved in 2ml chloroform and kept in a small vial [36].

Detection of aflatoxins

A. Thin layer chromatography (TLC) technique

Aluminum sheets (20 x 20 cm) precoated with silica gel G 60 without Fluorescence indicator, No 1.05553, Layer thickness 0.2 mm (Merck, 64271 Darmstadt, Germany)were used as plates. 50 µl of each sample extract was spotted on TLC plate. The spotting was done using a micropipette. The TLC plates were developed in toluene-ethyl acetate-acetic acid (8:1:1, v/v) as a mobile phase. The plate was dried at room temperature until the excess solvent disappeared. Aflatoxins were detected under long-wave UV light (365 nm). Standard solution of aflatoxins (Sigma, Chemical Co. St. Louis, MO, USA) was prepared. Aflatoxins-producing potential of the tested fungal isolates was determined according to intensity of fluorescence compared with Standard solutions.

B. High-performance liquid chromatography (HPLC) technique

Aflatoxins were also quantitatively estimated by the high-performance liquid chromatography (HPLC) according to Sibanda *et al.*,[37]. The mobile phase was water: acetonitrile: methanol (55: 30:15 v/v/v). Aflatoxins were detected using a fluorescence detector with an excitation wavelength of 295 nm and an emission wavelength of 330 nm [38]. The HPLC analysis of the crude extract was carried out using high performance liquid chromatography instrument (Agilent Technologies 1200 Series, G1321A FLD with column Zorbax Eclipse Plus C18) stands at the Analytical Chemistry Unit, Assuit University.

RESULTS

Physicochemical analysis of collected samples:

Data in table (1) showed the physicochemical analysis for 48 samples of different corn and corn-based products sources (8 samples from each source) collected from Assiut Governorate. The obtained results were expressed as a range based on the lowest and highest value for each parameter. Among the corn sources, white corn showed the highest occurrence of total carbohydrates, protein, sodium, magnesium, chloride, phosphate and

sulphate content representing, 32.46, 159, 52, 9,8,1.38 and 9.2 mg/g respectively. On the other hand, popcorn showed lowest occurrence of total ash, carbohydrates, proteins, sodium, potassium, and magnesium content recording, 16.6, 30.84, 64, 51,15.3, 7mg/g Similarly ,for corn-based products, flamenco recorded the highest content of moisture (22 mg/g), lipids (43.6 mg/g), proteins (153 mg/g), nitrate (1.44 mg/g), phosphate (0.51 mg/g) and sulphate (20.6 mg/g). Adversely, Doritos contains the lowest occurrence of total carbohydrates, potassium, chloride, and bicarbonate compared to other sources of corn-based products. Seifi [39] reported that, moisture content of corn was in the range of 4.73-22%. In another study by Giménez [40] in which the moisture content of corn was 11.10% and corn nutrients include protein,lipids, ash (8.07, 0.58, 0.32 g/100g), respectively. Moreover corn nutrients and phytochemicals include protein ,total lipid,carbohydrate (9.42, 4.74, 74.26g/100g)Similarly.and minerals (calcium 7mg/100g,magnesium 127mg/100g, potassium 287mg/100g,sodium 35mg/100g) [41]. Prasanthi [42] stated that,popcorn and cornflakes contained moisture (7.62,5.83 (g %)), protein (7.16,7.45(g %)), fat 8.46,4.58(g %)), ash(1.92,0.70(g %)) and carbahydrates (50.80,77.21(g %)).

Table 1: The physicochemical analysis of the collected samples.

Sources / Parameters	Corn sources			Corn-based sources		
	Popcorn*	**White corn***	**Yellow corn***	**Cornflakes***	**Doritos***	**Flamonco***
Moisture (%)	2-29	5-9	1-17	1-17	1-12	1-22
Total ash (%)	7.4-16.6	9.6-18.6	11.1-25.4	9.4-19.2	7.4-16.2	6-13.2
Total carbohydrate **	4.38-30.84	5.41-32.46	5.45-31.83	8.71-25.85	4.68-35.26	8.49-30.38
Total lipids **	18.3-49.9	6.3-43.6	6.3-49.9	3.1-30.9	25.3-41.4	8.4-43.6
Total prot. **	44-64	45-159	49-108	50-102	52-98	45-153
Na^+ **	4-51	6-52	5-43	16-49	8-62	8-62
K^+ **	1.5-15.3	3.7-14.8	3.6-35.1	3.2-10.8	2.5-22.6	3.5-14.6
Ca^{2+} **	1-4	1-3	1-2	2-8	2-3	1-2
Mg^{2+} **	1-7	2-9	2-6	3-9	4-9	2-4
Cl^- **	6-7	5-8	5-7	5-7	4-7	5-7
HCO_3^- **	10-20	10-40	10-30	20-50	10-40	20-40
NO_3^- **	0.04-0.65	0.06-0.49	0.02-0.17	0. 31-1.22	0.08-0.53	0.15-1.44
PO_4^{-3} **	0.16- 0.49	0.11-1.38	0.14-0.11	0.11-0. 91	0.11-1.14	0. 32-0.51
SO_4^{-2} **	0.9-6.9	1.2-9.2	1.2-7.9	1.7-8.4	1.8-14.9	5.7-20.6

* The range based on results of testing of eight samples from each source per parameter.

** milligram per gram (mg/g)

Isolation and identification of fungi:

Thirty-seven fungal species, In addition to one species variety belonging to nine fungal genera were collected in the present investigation. Results indicuted that, cornflakes samples showed the highest occurrence of fungal taxa (7 genera + 16 species), followed by Doritos (6 genera +15 species), popcorn (6 genera + 9 species), yellow corn (5 genera +12 species), white corn (4 genera + 13 species) and flamonco (3 genera + 9 species) as shown in table 2. *Aspergillus flavus* and *A. paraciticus* were consistently the most common species in all samples. *A. niger* and *A. terreus* were recorded in moderate occurrence on popcorn and cornflakes. Also, *Penicillium deculxi* was moderate in its

occurrence on white and yellow corn. Moreover, yeasts have been isolated from Doritos at a moderate level. Other fungal flora like; *Alternaria* sp., *A. carbonarous*, *A. nidulans*, *Chaetomium* sp., *Cladosporium* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp. and *Rhizopus* sp. were varied between low and rare occurrence. Our results agree with those reported by (Abdel-Kader *et al.* [43] ; Assawah & Elarosi [44] and Moubasher *et al.* [45]). The greater numbers of species that contaminate food stuffs and produce aflatoxins were belonging to *Aspergillus*, *Penicillium* and *Fusarium* species. Similar data were reported by Udagawa, [46]; Bullerman, [47]; El-Magraby *et al*. [48]; Kpodo *et al*. [49]; CA[50]; Gonzáslez *et al*. [51]; Rustemeyer *et al*. [52].

Table 2: Isolation of fungal flora of corn and corn-based products from diverse cities in Assiut Governorate.

Sources / Fungal isolate	Corn sources						Corn-based sources					
	Popcorn		White corn		Yellow corn		Cornflacs		Doritos		Flamenco	
	NCI	OR	NCI	OR	NCI	OR	NCI	OR	NCI	OR	NCI	OR
***Alternaria alternata*, (Fr.) Keissl.**	1	R	0	0	0	0	1	R	3	L	0	0
***Aspergillus awamori* Nakaz.**	2	L	3	L	1	R	2	L	1	R	5	M
***A. carbonarius* (Bainier) Thom**	0	0	0	0	0	0	1	R	1	R	2	L
***A. flavus* Link**	6	H	6	H	6	H	7	H	6	H	6	H
***A. flavus* var. *columnaris* Raper & Fennell**	0	0	4	M	0	0	2	L	4	M	3	L
***A. fumigatus* Fresen.**	2	L	2	L	2	L	2	L	2	L	1	R
***A. niger* Tiegh.**	5	M	6	H	8	H	5	M	3	L	6	H
***A. nidulans* (Eidam) G. Winter**	0	0	0	0	0	0	1	R	0	0	0	0
***A. oryzae* (Ahlb.) Cohn**	0	0	4	M	0	0	0	0	0	0	0	0
***A. parasiticus* Speare**	6	H	7	H	6	H	6	H	6	H	6	H
***A. tamarii* Kita**	1	R	2	L	0	0	0	0	0	0	0	0
***A. terreus* Thom**	4	M	6	H	2	L	4	M	4	M	1	R
***Chaetomium hispidum* Fr.**	1	R	0	0	0	0	0	0	0	0	0	0
***Cladosporium chlamydospora* Matsush.**	0	0	0	0	1	R	1	R	0	0	0	0
***Fusarium avenaceum* (Fr.) Sacc.**	0	0	1	R	0	0	0	0	0	0	0	0
***F. oxysporum* Schltdl.**	0	0	0	0	2	L	0	0	1	R	0	0
***F. phyllophilum* Nirenberg & O'Donnell**	0	0	0	0	0	0	0	0	1	R	0	0
***F. poae* (Peck) Wollenw.**	1	R	0	0	0	0	0	0	0	0	0	0
***F. proliferatum* (Matsush.) Nirenberg**	0	0	0	0	0	0	0	0	1	R	0	0
***F. pseudonygamai* O'Donnell & Nirenberg**	0	0	1	R	0	0	0	0	0	0	0	0
***F. solani*, (Mart.) Sacc.**	1	R	0	0	0	0	0	0	0	0	0	0
***F. sterilihyphosum* Britz, Marasas & M.J. Wingf.**	0	0	0	0	0	0	0	0	1	R	0	0
***F. verticillioides* (Sacc.) Nirenberg**	0	0	1	R	1	R	0	0	0	0	0	0
***Mucor abundans* Povah**	0	0	0	0	0	0	1	R	1	R	0	0
***Penicillium canadense* G. Sm.**	0	0	0	0	0	0	1	R	0	0	0	0
***P. chrysogenum* Thom**	0	0	1	R	0	0	0	0	2	L	0	0
***P. corylophilum* Dierckx**	0	0	0	0	0	0	1	R	0	0	0	0
***P. citrinum* Thom**	0	0	0	0	0	0	1	R	0	0	0	0
***P.deculxi* Delacr. Biourge**	6	H	4	M	5	M	6	H	4	M	3	L

Continue table (2)

Sources / Fungal isolate	Corn sources						Corn-based sources					
	Popcorn		White corn		Yellow corn		Cornflacs		Doritos		Flamenco	
	NCI		OR		NCI		OR		NCI		OR	
***P. rugulosum* Thom**	0	0	0	0	0	0	0	0	0	0	1	R
***P. steckii* K.W. Zaleski**	0	0	0	0	0	0	1	R	2	L	0	0
***Rhizopus arrhizus* A. Fisch.**	0	0	0	0	0	0	1	R	0	0	0	0
***Saccharomyces cerevisiae* (Desm.) Mey**	4	M	3	L	4	M	3	L	4	M	3	L

N.C.I. = number of cases of isolation out of 8 samples of each type; OR= Occurrence remarks; High occurrence(H) = 8-6 cases; Moderate occurrence (M) = 5-4 cases; Low occurrence (L) = 3-2 cases; Rare occurrence ® = 1 cases; zero = not detected.

Correlation of physicochemical analysis and fungal occurrence

Canonical correspondence analysis (CCA) was assayed to determine the impacts of physicochemical properties on the fungal composition of the collected samples as shown in Fig. (1). From the analysis of physicochemical properties of the corn and corn-based samples collected from Assiut Governorate, the relation of fungal composition data and physiochemical properties showed that magnesium, calcium ions, and phosphate correlated with the occurrence of *A. fumigatus*, *Alternaria alternata* and *A. terreus*. Whereas the water, lipid, protein, carbohydrate concentrations, chloride, sodium, nitrate and sluphate ions exhibited an obvious relationship with *Aspergillus awamori*, *A. tamarii*, *A. flavus*, *A. flavus* var. *columnaris*, *A. carbonarius*, *A. niger*, *F. poae* and *P. canadense.* On the other hand, ash content, K^+ and HCO_3^- were interacted with the dominance of *F. pseudonygamai*, *F. phyllophilum*, *F. solani*, *F. oxysporum* and *P. steckii* Fig.(1). It is concluded physicochemical properties of collected samples displayed the main role governing the biodiversity and abundance of isolated fungi. Interestingly, it was reported that the growth, microbial abundance and diversity are regulated by various physical and chemical parameters such as pH, temperature, organic matter, P and K and salinity[53-54]. As well as, Muneer *et al.*, [55] stated that, variations in the physicochemical properties were the main factors controlling fungal community structure in different habitats as NO_3, Ca and Mg exhibited a significant correlation with fungal composition. Drott *et al.*, [56] reported that soil is a natural habitat for *Aspergillus flavus*, and consequently, their aflatoxin production is closely related to the soil chemical properties, pH value, nutrients and water contents [57].

Aflatoxins-producing potential of isolated fungi:

As shown in table 3 among Seventy-one isolates of *A. flavus*, *A. flavus* var. *columnaris* and *A. paraciticus* screened for their aflatoxins-producing potential, 34 isolates could produce different types of aflatoxins (B_1 , B_2 , G_1 and G_2).Results indicated that,16 isolates of *A. flavus* in addition to 17 of *A. paraciticus* isolates and one isolate of *A. flavus var. columnaris* produced aflatoxins with different abilities . From the positive aflatoxins- producing isolates, *A. flavus* (AFC3) isolated from cornflakes and *A. paraciticus* (APP56) isolated from popcorn were the most producers. Aflatoxins-producing potential of some tested isolates was shown in fig.(2).

In accordance with our results, Saleemi [58] reported that, 43 and 67% of *A. flavus* and *A. parasiticus*,respectively isolated from maize and maize-gluten meal from Pakistan were aflatoxigenic. In another study by Yassin [59] in which most *A. flavus* isolates 75% and 67% of *A. flavus* var. *columnaris* isolates produced aflatoxins in corn and popcorn samples, collected from markets throughout the Riyadh region of Saudi Arabia. Tran-Dinh [60] found that, 25% of the strains of *A. flavus* isolated from corn samples produced aflatoxins. The incidence of aflatoxigenic *A. flavus* recovered from sweet corn kernels and soil samples collected from different localities in Malaysia was (30%) [61].

Table 3: Aflatoxins-producing potential of the recovered fungi on YES medium after 14 days at 28 ºC using TLC.

Isolate code	Fungal isolate	Source	Aflatoxins-producing potential
AFC 1	*A. flavus*	Cornflakes	-
AFC 2		Cornflakes	+
AFC 3		Cornflakes	++++
AFC 4		Cornflakes	−
AFD 5		Doritos	+++
AFD 6		Doritos	++
AFD 7		Doritos	−
AFD 8		Doritos	−
AFD 8		Doritos	−
AFF 10		Flamonco	+++
AFF 11		Flamonco	−
AFP 12		Popcorn	+++
AFP 13		Popcorn	−
AFP 14		Popcorn	−
AFP 15		Popcorn	+++
AFW 16		White corn	+++
AFW 17		White corn	−
AFW 18		White corn	+
AFW 19		White corn	−
AFW 20		White corn	+
AFW 21		White corn	+
AFW 22		White corn	−
AFW 23		Yellow corn	++
AFY 24		Yellow corn	−
AFY 25		Yellow corn	−
AFY 26		Yellow corn	+
AFY 27		Yellow corn	+++

Table 3: Continue

Isolate code	Fungal isolate	Source	Aflatoxins-producing potential
AFY 28		Yellow corn	++
AFY 29		Yellow corn	–
AFY 30		Yellow corn	–
AFY 31		Yellow corn	–
AFY 32		Yellow corn	–
AFVD 33	*A. flavus* var. *columnaris*	Doritos	–
AFVF 34		Flamonco	–
AFVW 35		White corn	+++
AFVW 36		White corn	–
AFVY 37		Yellow corn	–
APC 38	*A. paraciticus*	Cornflakes	+++
APC 39		Cornflakes	++
APC 40		Cornflakes	–
APC 41		Cornflakes	+++
APC 42		Cornflakes	+++
APD 43		Doritos	–
APD 44		Doritos	+++
APD 45		Doritos	–
APF 46		Flamonco	++
APF 47		Flamonco	–
APF 48		Flamonco	++
APF 49		Flamonco	+
APF 50		Flamonco	–
APF 51		Flamonco	–
APP 52		Popcorn	++
APP 53		Popcorn	–
APP 54		Popcorn	++
APP 55		Popcorn	–
APP 56		Popcorn	++++
APP 57		Popcorn	–
APP 58		Popcorn	–
APP 59		Popcorn	–
APW 60		White corn	–
APW 61		White corn	+

Table 3: Continue

Isolate code	Fungal isolate	Source	Aflatoxins-producing potential
APW 62	*A. paraciticus*	White corn	−
APW 63		White corn	−
APW 64		White corn	++
APW 65		White corn	++
APY 66		Yellow corn	+++−
APY 67		Yellow corn	−
APY 68		Yellow corn	−
APY 69		Yellow corn	++
APY 70		Yellow corn	+
APY 71		Yellow corn	−

* Aflatoxins-producing potential : (-): non-producing isolate., (+): low producing, (++): moderate-producing, (+++): high-producing isolates, (++++):veryhigh-producing isolates.

Figure. 3 Thin layer chromatography plates showing aflatoxins-producing potential of some tested isolates at long wavelength 365 nm.

Natural occurrence of aflatoxins in corn and corn-based products samples:

All tested samples were naturally contaminated by aflatoxins with different levels. In accordance with our results, El-Sayed [62] reported that, presence of aflatoxin B_1 in yellow corn, popcorn and white corn which collected from districts in Egypt. Also, they detected the presence of aflatoxins, namely, aflatoxin B_1 and G_1 in cornflakes as corn-based products. In another study by Amin [63] in which corn-based products collected from Cairo and El-Qaliubia Governorates were tested for aflatoxin contamination,The authors found that aflatoxin B_1 prevalence was 40% and 28% of corn-based products obtained from Cairo and El-Qaliubia, respectively. In addition, aflatoxin B_2 was detected as 4% and 24% of corn-based products collected from Cairo and El-Qaliubia, respectively. Similary, aflatoxin B_1 was found as contaminates for yellow corn samples

collected from different Governorates in Egypt, namely, Giza, Beni-Suef, Qulobia and Kafr El-Sheikh with the frequency of detection 34.3%, 21.5%, 37.3% and 34.7%, respectively [64]. Fifteen per cent of sweet corn kernels and soil samples collected from different localities in Malaysia were contaminated with aflatoxins [59.]

Confirmation and quantification of aflatoxins by HPLC technique:

The HPLC technique was used for confirmation and quantification of aflatoxins produced by the most active aflatoxins-producing isolates namely *A. flavus* (AFC3) and *A. parasiticus* (APP56). Data presented in table (4) and figure (2) showed that, *A. flavus* no. (AFC3)which was isolated from cornflakes produced aflatoxin B_1 (21.239 $ng.g^{-1}$) and aflatoxin G_2 (13.493 $ng.g^{-1}$). As for *A. parasiticus* no.(APP56) isolated from popcorn produced aflatoxin B_1 (11.666 $ng.g^{-1}$), aflatoxin B_2 (4.462 $ng.g^{-1}$) and aflatoxin G_2 (5.692 $ng.g^{-1}$).

In a similar study, Almeida [64] stated that *A. flavus* isolates could produced aflatoxin B_1 30.750 $ng.g^{-1}$ and/or aflatoxin B_2 11 $ng.g^{-1}$ to 22 $ng.g^{-1}$ on freshly harvested corn hybrids. In other research, Keller [66] indicuted that Aflatoxins levels produced by toxigenic isolates of *A. flavus* and *A. parasiticus* isolated from corn silage ranged from 2 to 45 $ng.g^{-1}$ and from 2 to 100 $ng.g^{-1}$, respectively. In the same respect ,Yassin [67] recorded that *A. flavus* and *A. flavus var. columnaris* isolates associated with corn and popcorn samples could produced aflatoxins. The range of production was ranging from 1 to 8 $ng.g^{-1}$.

Table 4: Aflatoxins concentration of the most aflatoxins-producing potential fungal isolates AFC3, APP56 .

Fungal isolate	source	Aflatoxin concentration ($ng\ g^{-1}$)			Total aflatoxins concentration ($ng.g^{-1}$)
		B_1	B_2	G_2	
AFC3 isolate	**Cornflakes**	**21.239**	**ND**	**13.593**	**34.832**
APP56 isolate	**Popcorn**	**11.666**	**4.462**	**5.692**	**21.82**

*AFC3 isolate (*A.flavus*) ,APP56 isolate (*A.parasiticus*).
* ND= not detected

Rusults in table (5) and figure (4) showed that the two samples (cornflakes and popcorn) from which AFC3 and APP56 were isolated were contaminated whith aflatoxin B_1 and aflatoxin G_2. Cornflakes contained 9.206 $ng.g^{-1}$ and 1.531 $ng.g^{-1}$ of aflatoxin B_1

and aflatoxin G_2 ,respectively. As for popcorn sample aflatoxin B_1 and aflatoxin G_2 were recorded as 2.942 $ng.g^{-1}$ and 17.433 $ng.g^{-1}$, respectively. Aflatoxin contamination detected in two samples was found to be higher than the maximum tolerable limit currently proposed by the European Commission for aflatoxins [18].

In accordance with our results, Majeed [68] reported that corn and corn products samples had been found contaminated with aflatoxins. Average levels of aflatoxin B_1 and total aflatoxins in corn were 7.90 and 12.08 $ng.g^{-1}$, while that in corn products were 5.47 and 7.85 $ng.g^{-1}$,respectively. In another study, Firdous [69] found that 52% of corn-based products samples were contaminated with aflatoxin B_1 and B_2.The contamination level with aflatoxin B_1 ranged from 2.0 to 1405. $ng.g^{-1}$,while that aflatoxin B_2 recorded 1.0 to 55.2 μg.kg−1. In another study, kerneled corn sold samples were contaminated with Aflatoxin B_1 and aflatoxin G_2 . The contamination level with aflatoxin B_1 ranged from 5.03 $ng.g^{-1}$ to 465.31 $ng.g^{-1}$,while that aflatoxin G_2 recorded 1.59 $ng.g^{-1}$to 57.1 $ng.g^{-1}$[70].

Table 5: Aflatoxins concentration of the contaminated samples from which the the most aflatoxins-producing potential fungal isolates were isolated.

Samples	Aflatoxin concentration ($ng\ g^{-1}$)			Total aflatoxins concentration ($ng.g^{-1}$)
	B_1	B_2	G_2	
Cornflakes	**9.206**	**ND**	**1.531**	**10.737**
Popcorn	**2.942**	**ND**	**17.433**	**20.375**

* ND= not detected

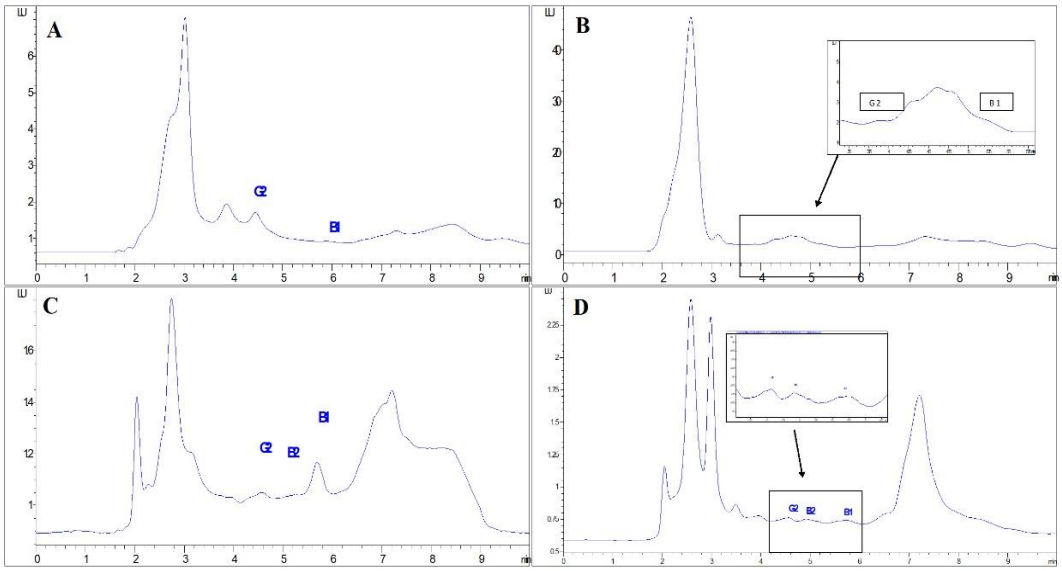

Figure 4. Quantification of aflatoxins by high-performance liquid chromatography for (A) *A.flavus*, (B) *A.paraciticus*, (C) cornflakes and (D) popcorn.

CONCLUSION

The current study revealed that, *A. flavus* and *A. paraciticus* are the most frequent contaminants of corn and corn-based products in Assiut Governorate. The potent toxigenic *Aspergillus* isolates recovered from corn and corn-based products indicated that rigorous quarantine and healthy storage conditions should be adopted with importing commodities to avoid contamination with toxigenic fungi and consequently prevent hazards to human and animal health.

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