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Prevalence of Aflatoxigenic Fungi in Cereal Grains and Their Related Chemical Metabolites

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Abstract

Aflatoxins (AFs) are chemical compounds with high toxic impacts produced by *Aspergillus* section *Flavi*. Toxigenic fungi contaminate foods and feed, thus presenting biological and chemical contamination resulting in health issues. Therefore, this study aims to evaluate the prevalence of toxigenic fungi in several cereal grains collected from different Egyptian governorates, as well as to evaluate the ability of toxigenic fungi to produce AFs using coconut agar medium (CAM), thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC). Results revealed that *Aspergillus* spp. were the predominant fungi in barley, white corn, yellow corn, and rice samples, followed by *Penicillium* spp. Data also showed that barley, white corn, yellow corn, and rice obtained from Alexandria governorate and wheat obtained from Beheira governorate were highly contaminated. On studying the ability of *A. parasiticus*, and *A. flavus* to produce AFs on CAM, 63.15% of the fungal isolates gave different blue and green fluorescence densities under a UV lamp at the reverse sides of the CAM plates. The fungal isolates showing fluorescence were further determined using TLC, whereas twenty-two fungal isolates were tested positive for the AFs production. The HPLC analyses revealed that twenty-one out of twenty-two fungal isolates produced the four types of AFs. Detectable aflatoxin B_1 (AFB_1) concentrations ranged between 0.02 and 875.03 ng/g; 0.07 and 13.55 ng/g for aflatoxin B_2 (AFB_2); 0.06 and 2.85 ng/g for aflatoxin G_1 (AFG_1); and 0.71 and 89.29 ng/g for aflatoxin G_2 (AFG_2). These results indicated the importance of a control system to regulate fungal and mycotoxin production in grains before their consumption or manufacturing to limit AFs contamination.

Keywords: cereals; Aspergillus spp.; aflatoxins; coconut agar medium; TLC; HPLC; chemical metabolites

1. Introduction

Cereal crops are considered the main source of human nutrition and are used as an additional source of nutrition in animals [1]. Cereals are susceptible to fungal proliferation, which could be accompanied by the production of various secondary metabolites during cultivation, harvest, storage, and processing [2, 3]. The growth of mycotoxigenic fungi on cereals increases due to the prevailing environmental conditions in the Middle East and Africa, such as high temperatures, high humidity, irregular rains, and frequent droughts [1]. Fungi are widely distributed and found wherever moisture is present and are major spoilage of foods and feedstuffs [4]. The distributions of different fungi in agricultural products have led to a reduction in quality and yield with significant economic losses [5, 6].

Toxigenic fungi that contaminate agricultural grains are divided into two groups; the first group are those that invade seed crops and are described as "field fungi" (*Alternaria, Cladosporium, Fusarium*, spp.), the second group gain access to seeds during plant development, and are described as "storage fungi" (*Aspergillus; Penicillium* spp.), and increase during storage [7]. *Aspergillus* and *Penicillium* species frequently grow on essential commodities such as maize, groundnut, tree nuts, spices, and cottonseed under storage conditions. Meanwhile, *Fusarium* species often infect growing crops such as wheat, barley, and corn and propagate in the plant [8].

Mycotoxins are produced by filamentous fungi mainly belonging to *Aspergillus*, *Fusarium*, and *Penicillium* genera and are considered the most important due to their high toxicity to humans and animals [9, 10]. Members of the genus *Aspergillus* are

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known to produce AFs, whereas the most economically important aflatoxin-producers belong to the *Aspergillus* section *Flavi*: *A. flavus*, *A. parasiticus*, and *A. nomius* [11-13]. Aflatoxins B_1 (AFB_1) is considered the most potent among the known AFs, associated with carcinogenicity and toxicity in humans and animals, and has been classified as a Group 1 human carcinogen by the International Agency for Research on Cancer [14].

As most mycotoxins are chemically and thermally stable during food processing, many international agencies are trying to achieve universal standardization of regulatory limits for mycotoxins in food, whereas about 13 mycotoxins are of concern [15]. These regulatory enforcements result in the rejection of contaminated food/feed followed by loss of markets and expense to the exporter [16].

Since the chemical hazard of mycotoxins remains a worldwide concern, thus it is essential to identify and characterize the current situation of mycotoxin-producing species to design management strategies to prevent crop contamination by mycotoxins. Therefore, this study aims to evaluate the prevalence of toxigenic fungi in several cereal grains collected from different Egyptian governorates and evaluate the ability of toxigenic fungi to produce AFs using CAM, TLC, and HPLC.

2. Materials and Methods

2.1. Chemicals and reagents

Aflatoxins B_1, B_2, G_1, and G_2 standards were obtained from Sigma Aldrich (St Loius, MO, USA). All chemicals and reagents used in this study were of analytical grade and were obtained from Merck, Darmstadt, Germany.

2.2. Sampling

Ninety samples of the different cereal grains (barley, white corn, yellow corn, rice, and wheat) were purchased unpacked from various retailers in six Egyptian governorates (Alexandria, Beheira, Cairo, Giza, Monufia, and Qalyubia). Cereal grain samples were transferred to the laboratory and stored under refrigeration conditions (4°C) in polythene bags until analysis [17].

2.3. Isolation of fungi

The cereal grains were surface sterilized using 1 % aqueous sodium hypochlorite solution for 1 min.; rinsing in sterile deionized water (3 times). The grains were blotted dry between sterile Whatman No. 1 filter papers before plating at a rate of 10 grains per plate on Potato Dextrose Agar (PDA, Conda, Spain) amended with chloramphenicol (0.1 g/L). Plates were incubated at 25°C for 5 days [18].

2.4. Morphological identification

Fungal pure cultures were inoculated into Petri plates and incubated for a total of seven days to be identified using morphological, macroscopic, and microscopic criteria using the keys of Pitt and Hocking [19], Lesliey and Summerell [20], and Silva et al. [21].

2.5. Evaluating the ability of toxigenic fungal species to produce aflatoxins

To differentiate between aflatoxin-producing and non-producing isolates, CAM was employed to screen the potential of fungal isolates to produce AFs. The medium was made by homogenizing shredded coconut (100 g) with hot distilled water (300 mL) for 5 min. The homogenate was filtered through cheesecloth, and the pH of the filtrate was adjusted to pH 7 with 2N NaOH. Agar (20 g/L) was added, and the liquid was heated to boiling before being chilled to around 50°C [22]. The liquid was then autoclaved, cooled to roughly 40 to 45°C, and put into sterile Petri dishes while being agitated. After that, the coconut medium plates were surface cantered with the appropriate fungal isolates and incubated at 25°C for 7 days. The plates were subsequently examined in the dark under UV light (365 nm) for fluorescence screening of Afs [22].

2.6. Extraction of Aflatoxins

To simulate the mycotoxin-producing ability of fungal isolates, grain corn agar (GCA) containing grain corn as a natural substrate was used as a semi-synthetic growth medium [23]. Fine-grain corn powder (30 g) and agar (15 g) were added to 1L of distilled water [24]. The medium was autoclaved at 121°C for 15 min, poured into Petri dishes, and left to solidify. Solidified GCA plates were refrigerated at 4°C before fungal inoculation.

The aflatoxigenic potentials of *A. flavus* and *A. parasiticus* (AFB_1, AFB_2, AFG_1, and AFG_2) were determined, whereas spore suspension (10 µl) of each fungal isolate was inoculated centrally on GCA plates and incubated in the dark at 30°C for 10 days. The extraction of AFs has been carried out following the method of Mohale et al. [25] with some modifications. In brief, 1000 µL of methanol was poured into Eppendorf tubes containing five mycelial plugs and vortexed. The tubes were then incubated at room temperature for 30 minutes before centrifugation (Spectrophuge 16 M, Labnet, Edison NJ, California, USA) at 10,000 X g for 5 min. The resultant extract was filtered into vials using nylon syringe filters.

2.7. Aflatoxin evaluation using TLC and HPLC

The extracts were then evaporated to dryness for TLC analysis and re-dissolved in chloroform (200µl).

Extracts were spotted on thin-layer chromatography silica gel 60 F254 plates (Merck, Darmstadt, Germany) along with AFs standard solution. A mixture of toluene: ethyl acetate: 90% formic acid (5: 4: 1, v/v/v) was used as mobile phase, and the bands were visualized under UV light (365 nm) [26]. Samples showing the ability to produce AFs were analyzed using HPLC.

The HPLC analysis was carried out using an Agilent 1260 series (Agilent Technologies, Centerville Rd, Wilmington, DE 19808, USA). The fluorescence detector was monitored at 365 nm excitation at 435 nm emission [27]. The separation was carried out using a C18 column (4.6mm x 250mm i.d., 5 µm).

2.8. Statistical analysis

Results were expressed as means ± standard deviation. With the SPSS 26 (IBM, USA) program, an analysis of variance was performed, and the differences between values were assessed for significance using ANOVA. The results were considered significant at $P \leq 0.05$. Fisher's Protected Least Significant Difference was also used to determine the difference between different means [28].

3. Results

3.1. Morphological identification of fungal isolates

Aspergillus genera had the highest occurrence among other fungal genera. *Aspergillus* section *Flavi* also had the highest frequency among other *Aspergillus* species, whereas two species were identified, namely *A. flavus* and *A. parasiticus*. On the other hand, *Fusarium*, *Penicillium*, *Rhizopus*, and *Alternaria* were identified by genus and not by species level.

Macroscopic observation revealed that *A. flavus* had a greenish colony that spread radially from the point of inoculation. As the colony progressively grew, it became slightly raised as mycelia piled, and the center became floccose and rough. Microscopic morphology revealed that conidial heads were typically radiate. Later splitting to form loose columns, biseriate but having some heads with phialides borne directly on the vesicle. Conidiophores were hyaline and coarsely roughened, often more noticeable near the vesicle. Conidia were globose to subglobose, pale green, and conspicuously echinulate.

Macroscopic observation of *A. parasiticus* revealed that it formed a rudiment of a white, aerial mycelium after 24 h. After 7-day growth, the *A. parasiticus* isolates formed an abundant, dark green aerial, dense mycelium of cottony appearance. The *A. parasiticus* colonies formed were flat and velvety, ivy green with woolly centres. The central zone of the colonies was green olive, and the borders were yellowish. Microscopic morphology revealed that conidia had rough, thick walls and were spherical. Conidia were distinctly roughened, globose to subglobose, and are borne on stalks, which are commonly covered in small spines

Macroscopic observation of *A. niger* colonies revealed that it is initially white, which quickly becomes black with conidial production. Microscopic morphology showed large, globose, dark brown conidial heads. Conidiophores were smooth-walled, hyaline, or turning dark towards the vesicle. Conidial heads were biseriate with the phialides born on brown, often septate metulae. Conidia were globose to subglobose, dark brown to black, and rough-walled.

Macroscopic observation of *A. ochraceus* was described as non-dense, flaky sporulated colonies ranging in colour between yellow and amber. The mycelium is white, with the reverse ranging from yellow to pale orange or grey gold. The sclerotia (when present) is opaque, ranging in colour from pink to purple, and that is the main distinguishable feature for the species microscopic morphology showed conidial heads radiating; conidiophores biseriate; stipes hyaline to brown, rough walled, vesicles globose, sometimes elongated; conidia globose to subglobose, finely roughened.

3.2. Frequency distribution of fungi in grains collected from different governorates

Table (1) showed the frequency distribution of fungi in barley samples obtained from some Egyptian governorates. Data revealed that *Aspergillus* spp. was the predominant fungi in barley samples, followed by *Penicillium* and *Alternaria* spp. In Alexandria governorate, barley samples were highly contaminated by *Fusarium* spp. (65.01%), followed by *Penicillium* (19.26%), and *Aspergillus* spp. (18.75%). Whereas in Beheira governorate, barley samples were contaminated by *Rhizopus*, *Fusarium*, *Aspergillus*, and *Penicillium* spp. with a percentage of contamination recording 50.37%, 34.98%, 16.96%, and 15.56%, respectively.

Meanwhile, in Cairo governorate, barley samples were highly contaminated by *Alternaria* spp. (27.97%), followed by *Penicillium* (19.26%) and *Aspergillus* spp. (18.75%). In Giza governorate, *Alternaria* spp. were also detected at a high contamination level, recording 32.05%, followed by *Rhizopus* spp. (24.81%), and *Penicillium* spp. (19.26%) respectively. Barley samples from Monufia governorate showed a lower contamination percentage, whereas *Penicillium*, *Alternaria*, and

Aspergillus spp. were detected at 13.26%, 12.00%, and 11.59%, respectively. For barley samples from Qalyubia governorate, data revealed that *Rhizopus* was the predominant fungi, followed by *Aspergillus* and *Penicillium* spp. at contamination levels of 24.81%, 15.18%, and 13.26% respectively. Generally, it could be concluded that barley samples from all governorates were contaminated by *Aspergillus*, *Penicillium*, and *Alternaria* spp. On the other hand, *Fusarium* spp. was not detected in Cairo, Giza, Monufia, and Qalyubia governorates samples.

Table (2) showed the frequency distribution of fungi in white corn samples obtained from some Egyptian governorates. *Aspergillus* spp. was the predominant fungi, followed by *Penicillium* spp. with a total fungal count recording 50.33 and 24.00, respectively. In Alexandria governorate, *Fusarium* spp. was the dominant fungi, followed by *Aspergillus*, and *Penicillium* spp. recording a contamination percentage of 48.58%, 18.53%, and 16.66%, respectively. Meanwhile, white corn samples were highly contaminated by *Rhizopus* spp., with a percentage of contamination recording 50.37% in the Beheira governorate. On the other hand, *Fusarium*, *Aspergillus*, and *Penicillium* spp. also contaminated white corn samples, with a percentage of contamination recording 28.53%, 19.86%, and 18.04%, respectively.

Results indicated that white corn samples obtained from Cairo governorate were highly contaminated by *Alternaria* (35.33%), followed by *Aspergillus* spp. (14.56%). *Alternaria* spp. contaminated white corn samples that were obtained from Giza (41.16%), followed by *Penicillium* (25.00%) and *Rhizopus* spp. (24.81%) respectively. Samples were also contaminated by *Aspergillus* but to a lower extent.

On the other hand, white corn samples obtained from Monufia governorate were also contaminated by fungal species but at a lower contamination level than other governorates. Both *Aspergillus* and *Penicillium* spp. were detected at contamination levels of 15.23% and 12.50%, respectively. For white corn samples from Qalyubia, results recorded a high contamination percentage for *Alternaria* (23.49%) and *Rhizopus* (24.81%) spp. Generally, it could be noticed that *Alternaria* spp. were not detected in white corn samples obtained from Alexandria, Beheira, and Monufia governorates. *Rhizopus* spp. were also not detected in white corn samples obtained from Alexandria, Cairo, and Monufia governorates. Results also revealed that white corn samples obtained from all governorates were contaminated by *Aspergillus*, *Fusarium*, and *Penicillium* spp.

Data in Table (3) showed the frequency distribution of fungi in yellow corn samples obtained from different Egyptian governorates, where *Aspergillus* spp. were the predominant fungi, followed by *Penicillium* and *Fusarium* spp. with a total fungal count recording 42.33, 19.67, and 8.66 respectively. *Fusarium* spp. highly contaminated yellow corn samples obtained from Alexandria governorate at a contamination level of 50.00%, followed by *Penicillium* (18.65%) and *Aspergillus* spp. (16.53%) respectively. Results also indicated that both *Alternaria* and *Rhizopus* spp. were not detected in samples obtained from Alexandria governorate. For samples obtained from Cairo and Giza governorates, *Fusarium* spp. was not detected.

On the other hand, *Alternaria* spp. was detected at a high contamination level, recording 75.18% and 24.81%, respectively. *Aspergillus* spp. were detected in yellow corn samples obtained from Cairo and Giza governorates, registering 14.17% and 22.04%, respectively, whereas *Penicillium* spp. were detected at contamination levels of 13.57% and 18.65%, respectively. In Monufia governorate, yellow corn samples were highly contaminated by *Rhizopus* spp. (40.11%), followed by *Aspergillus* (16.53%), and *Penicillium* spp. (11.84%) respectively. On the other hand, in Qalyubia governorate, yellow corn samples were also highly contaminated by *Rhizopus* (40.11%), followed by *Penicillium* (16.92%), *Fusarium* (15.35%), and *Aspergillus* spp. (12.59%) respectively. In general, it could be noticed that *Alternaria* spp. was not detected in samples obtained from Alexandria, Beheira, Monufia, and Qalyubia governorates.

Rhizopus spp. was not detected in samples obtained from Alexandria, Beheira, and Giza governorates. Meanwhile, *Penicillium* and *Aspergillus* spp. were detected in all samples obtained from different governorates.

The frequency distribution of fungi in rice samples obtained from different Egyptian governorates is shown in Table (4). *Aspergillus* spp. was the predominant fungi, followed by *Penicillium* and *Fusarium* spp. with a total fungal count recording 57.33, 32.67, and 16.66, respectively. *Fusarium* spp. highly contaminated rice samples obtained from Alexandria governorate (56.00%), followed by *Aspergillus* (24.99%) and *Rhizopus* spp. (19.87%) respectively. Results also showed that *Alternaria* spp.

was not detected in rice samples obtained from Alexandria governorate. The following fungal genera contaminated rice samples from Beheira governorate by the following fungi in descending order; *Fusarium* (25.99%), *Aspergillus* (22.10%), *Rhizopus* (19.87%), and *Penicillium* spp. (15.30%). Unlike the previous two governorates, rice samples from Cairo governorates were highly contaminated by *Alternaria* spp. (39.93%). *Rhizopus* spp. was also detected at a contamination level of 19.87%, followed by both *Penicillium* (14.29%) and *Aspergillus* spp. (12.21%). *Alternaria* spp. obtained from Giza governorate recorded a higher contamination level of 39.93%, followed by *Penicillium* (20.41%) and *Aspergillus* spp. (15.69%) respectively. On the other hand, samples for Monufia governorate were contaminated by *Rhizopus* at a contamination level of 40.36%, followed by *Penicillium* (18.36%) and *Aspergillus* spp. (12.78%) respectively. In Qalyubia governorate, rice samples were not contaminated by *Rhizopus* spp.

The frequency distribution of fungi in wheat samples obtained from different Egyptian governorates is demonstrated in Table (5). *Aspergillus* spp. was the dominant fungi, followed by *Penicillium* and *Fusarium* spp. with a total fungal count recording 59.67, 25.67, and 7.67, respectively. In Alexandria governorate, wheat samples were highly contaminated by *Fusarium* (52.15%), followed by *Aspergillus* spp. (21.78%). Results also revealed that both *Penicillium* and *Rhizopus* spp. were also detected at contamination levels of 14.29% and 16.50%, respectively. On the other hand, wheat samples obtained from Beheira governorate were contaminated by the following fungi; *Fusarium*, *Penicillium*, and *Rhizopus* spp. at contamination levels of 47.78%, 19.47%, and 16.50% respectively. In Cairo governorate, wheat samples were contaminated by *Rhizopus*, *Alternaria*, *Aspergillus*, and *Penicillium* spp. at contamination levels of 33.50%, 25.03%, 20.11%, and 11.68% respectively.

In Giza governorate, *Alternaria* was detected at a high contamination level of 55.02%, followed by *Rhizopus* and *Penicillium* spp. at 33.50% and 22.08%, whereas *Fusarium* spp. was not detected. In Monufia governorate, only *Aspergillus* and *Penicillium* spp. were detected in wheat samples, whereas *Fusarium*, *Alternaria*, and *Rhizopus* spp. were not detected. Fungal count in wheat samples obtained from Qalyubia governorate was considered lower than those found in other governorates; however, *Alternaria*, *Penicillium*, and *Aspergillus* spp. were only detected at contamination levels of 19.94%, 11.68%, and 11.17%, respectively.

Figure 1(a-e) showed the total fungal count in different governorates for barley, white corn, yellow corn, rice, and wheat. Data revealed that grain samples (barley and white corn) obtained from Alexandria governorate was highly contaminated, followed by Giza governorate (Figure 1a, b). Results indicated that yellow corn and rice obtained from Alexandria were highly contaminated, followed by Beheira and Giza governorates, respectively (Figure 1c, d). Meanwhile, wheat samples obtained from Giza governorates were highly contaminated, followed by Alexandria governorate. Data in Figure 2 (a-e) presented the total fungal count for each fungal genus for barley, white corn, yellow corn, rice, and wheat. Results revealed that *Aspergillus* was the dominant genus in all grains studied, followed by *Penicillium.*

3.3. Production of aflatoxins by aflatoxigenic fungal species

Data in Figure (3) demonstrated the ability of identified *A. flavus* and *A. parasiticus* isolated from cereal grains to produce AFs using CAM showing visible fluorescence at 365 nm. One hundred and twenty out of one hundred and ninety toxigenic isolates (63.15%) gave different blue and green fluorescence under a UV lamp at the reverse sides of the CAM plates.

The fungal isolates showing fluorescence were further determined using thin-layer chromatography (Table 6). Results demonstrated that out of one hundred and twenty, twenty-two fungal isolates (18.33%) tested positive for the production of AFs, and were selected for further quantification using HPLC.

The HPLC analyses revealed that twenty-one out of twenty-two fungal isolates (95.45%) produced AFs. Data showed that all fungal isolates produced AFB_1 and AFG_2, whereas 16 fungal isolates only produced AFB_2, and 17 fungal isolates produced AFG_1.

It was also observed that all aflatoxin-producing isolates produced AFB_1 in the range of 0.02 to 875.03 ng/g agar plugs. The highest concentration of AFB_1 was produced by the isolate WcC6, isolated from white corn. All aflatoxin-producing isolates produced AFG_2 in the range of 0.71 to 89.29 ng/g agar plugs. The highest concentration of AFG_2 was produced by the isolate YcB58, isolated from yellow corn. Results indicated that fungal isolates produced AFG_2 at higher concentrations than other AFs.

Table (1): The detection of fungal species from Barley samples obtained from different Egyptian governorates

Governorates	*Aspergillus*		*Fusarium*		*Penicillium*		*Alternaria*		*Rhizopus*	
	Total Fungal count	%*	Total Fungal count	%*	Total Fungal count	%*	Total Fungal count	%*	Total Fungal count	%*
Alexandria	7.00±2.65 [a]	18.75	4.33±1.15 [c]	65.01	5.33±2.52 [a]	19.26	1.00±1.00 [a]	12.00	0.00±0.00 [a]	0.00
Beheira	6.33±1.53 [a]	16.96	2.33±1.53 [a]	34.98	4.33±1.53 [a]	15.56	1.00±1.00 [a]	12.00	0.67±0.58 [a]	50.37
Cairo	7.00±2.00 [a]	18.75	0.00±0.00 [b]	0.00	5.33±1.53 [a]	19.26	2.33±0.58 [a, c]	27.97	0.00±0.00 [a]	0.00
Giza	7.00±1.00 [a]	18.75	0.00±0.00 [b]	0.00	5.33±1.53 [a]	19.26	2.67±0.58 [b]	32.05	0.33±0.58 [a]	24.81
Monufia	4.33±1.53 [a]	11.59	0.00±0.00 [b]	0.00	3.67±1.53 [a]	13.26	1.00±1.00 [a]	12.00	0.00±0.00 [a]	0.00
Qalyubia	5.67±1.53 [a]	15.18	0.00±0.00 [b]	0.00	3.67±1.15 [a]	13.26	0.33±0.58 [a, d]	3.96	0.33±0.58 [a]	24.81

Results are mean ± SD (n=3); Numbers within each column superscript with the different letters are considered significant $P \leq 0.05$.
Numbers within each raw superscript with the different letters are considered significant $P \leq 0.05$.
*Percentage of contamination for each fungal species in each government.

Table (2): The detection of fungal species from white corn samples obtained from different Egyptian governorates

Governorates	*Aspergillus*		*Fusarium*		*Penicillium*		*Alternaria*		*Rhizopus*	
	Total Fungal count	%*	Total Fungal count	%*	Total Fungal count	%*	Total Fungal count	%*	Total Fungal count	%*
Alexandria	9.33±0.58 [a]	18.53	5.67±1.15 [c]	48.58	4.00±1.00 [a]	16.66	0.00±0.00 [a]	0.00	0.00±0.00 [a]	0.00
Beheira	10.00±1.00 [a]	19.86	3.33±5.78 [a]	28.53	4.33±0.58 [a]	18.04	0.00±0.00 [a]	0.00	0.67±0.58 [a]	50.37
Cairo	7.33±0.58 [a]	14.56	0.33±0.58 [b]	2.82	3.00±1.00 [a]	12.50	2.00±1.00 [a, c]	35.33	0.00±0.00 [a]	0.00
Giza	9.67±0.58 [a]	19.21	0.00±0.00 [b]	0.00	6.00±1.00 [a]	25.00	2.33±0.58 [b]	41.16	0.33±0.58 [a]	24.81
Monufia	7.67±0.58 [a]	15.23	0.67±0.58 [b]	5.74	3.00±1.00 [a]	12.50	0.00±0.00 [a]	0.00	0.00±0.00 [a]	0.00
Qalyubia	6.33±1.53 [a]	12.57	1.67±0.58 [b]	14.31	3.67±0.58 [a]	15.29	1.33±0.58 [a, d]	23.49	0.33±0.58 [a]	24.81

Results are mean ± SD (n=3); Numbers within each column superscript with the different letters are considered significant $P \leq 0.05$.
Numbers within each raw superscript with the different letters are considered significant $P \leq 0.05$.
*Percentage of contamination for each fungal species in each government.

Table (3): The detection of fungal species from yellow corn samples obtained from different Egyptian governorates

Governorates	*Aspergillus*		*Fusarium*		*Penicillium*		*Alternaria*		*Rhizopus*	
	Total Fungal count	%*	Total Fungal count	%*	Total Fungal count	%*	Total Fungal count	%*	Total Fungal count	%*
Alexandria	7.00±1.00 [a]	16.53	4.33±0.58 [b, d]	50.00	3.67±0.58 [a]	18.65	0.00±0.00 [a]	0.00	0.00±0.00 [a]	0.00
Beheira	7.67±1.53 [c]	18.11	2.33±0.58 [a]	26.90	4.00±1.00 [a]	20.33	0.00±0.00 [a]	0.00	0.00±0.00 [a]	0.00
Cairo	6.00±1.00 [a]	14.17	0.00±0.00 [b, c]	0.00	2.67±0.58 [a]	13.57	1.00±1.00 [b]	75.18	0.33±0.58 [a]	19.76
Giza	9.33±0.58 [c]	22.04	0.00±0.00 [b]	0.00	3.67±0.58 [a]	18.65	0.33±0.58 [a, b]	24.81	0.00±0.00 [a]	0.00
Monufia	7.00±1.00 [a]	16.53	0.67±0.58 [b]	7.73	2.33±1.15 [b]	11.84	0.00±0.00 [a]	0.00	0.67±0.58 [a]	40.11
Qalyubia	5.33±1.15 [a, b]	12.59	1.33±1.53 [a, e]	15.35	3.33±1.15 [a]	16.92	0.00±0.00 [a]	0.00	0.67±0.58 [a]	40.11

Results are mean ± SD (n=3); Numbers within each column superscript with the different letters are considered significant $P \leq 0.05$.
Numbers within each raw superscript with the different letters are considered significant $P \leq 0.05$.
*Percentage of contamination for each fungal species in each government.

Table (4): The detection of fungal species from rice samples obtained from different Egyptian governorates

Governorates	*Aspergillus*		*Fusarium*		*Penicillium*		*Alternaria*		*Rhizopus*	
	Total Fungal count	%*	Total Fungal count	%*	Total Fungal count	%*	Total Fungal count	%*	Total Fungal count	%*
Alexandria	14.33±1.53 [a, c]	24.99	9.33±1.53 [d]	56.00	5.00±1.00 [a, b]	15.30	0.00±0.00 [a]	0.00	0.33±0.58 [a]	19.87
Beheira	12.67±1.15 [a]	22.10	4.33±0.58 [a]	25.99	5.00±1.00 [a]	15.30	0.00±0.00 [a]	0.00	0.33±0.58 [a]	19.87
Cairo	7.00±1.00 [a, c]	12.21	0.00±0.00 [b, c]	0.00	4.67±0.58 [a, b]	14.29	1.33±0.58 [b]	39.93	0.33±0.58 [a]	19.87
Giza	9.00±1.00 [a]	15.69	1.33±0.58 [b]	7.98	6.67±0.58 [b]	20.41	1.33±0.58 [a, b]	39.93	0.00±0.00 [a]	0.00
Monufia	7.33±0.58 [a, c]	12.78	1.00±1.00 [b]	6.00	6.00±1.00 [a, b]	18.36	0.00±0.00 [a]	0.00	0.67±0.58 [a]	40.36
Qalyubia	7.00±1.00 [b]	12.21	0.67±1.15 [a]	4.02	5.33±0.58 [a, b]	16.31	0.67±0.58 [a]	20.12	0.00±0.00 [a]	0.00

Results are mean ± SD (n=3); Numbers within each column superscript with the different letters are considered significant $P \leq 0.05$.
Numbers within each raw superscript with the different letters are considered significant $P \leq 0.05$.
*Percentage of contamination for each fungal species in each government.

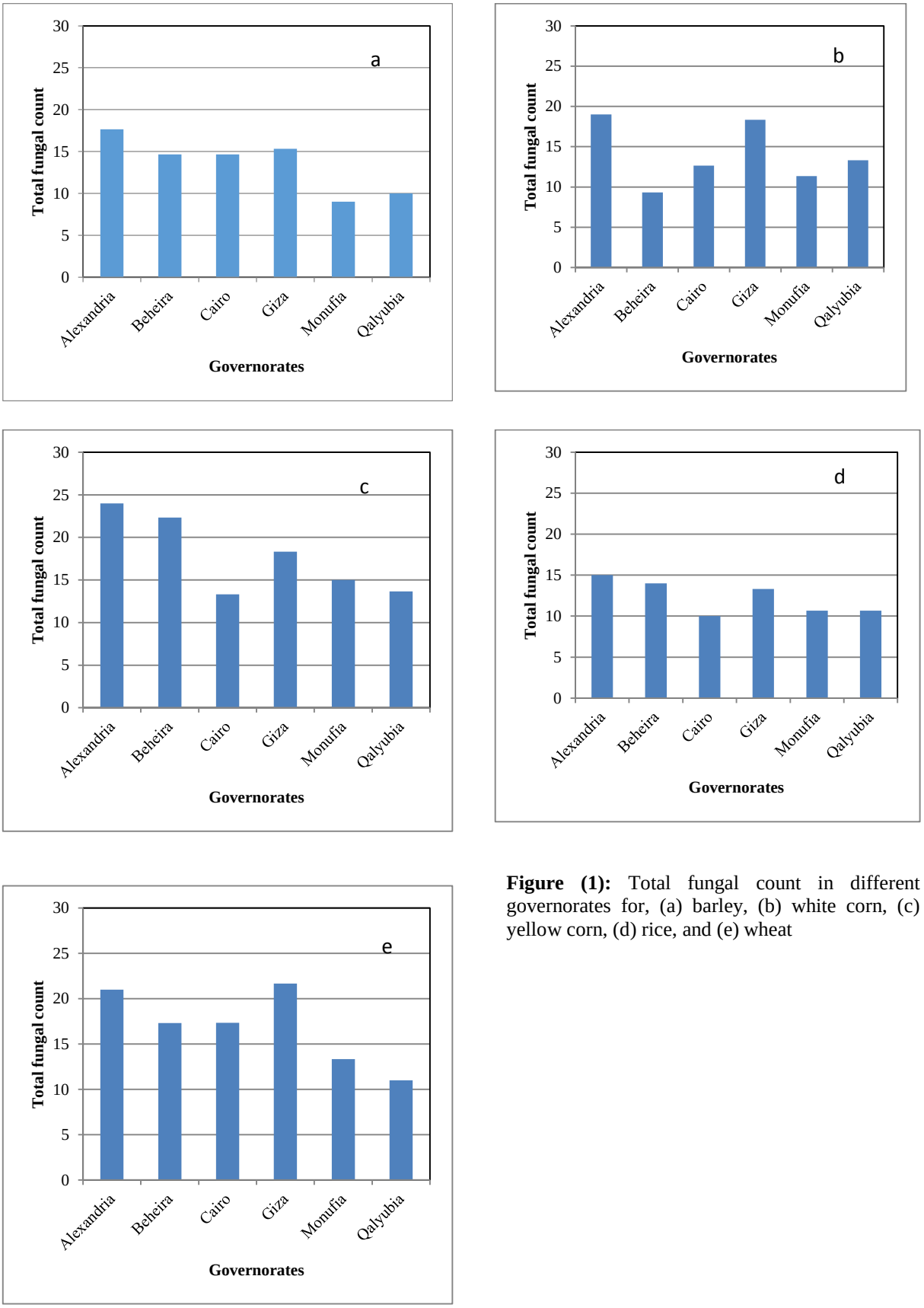


Figure (1): Total fungal count in different governorates for, (a) barley, (b) white corn, (c) yellow corn, (d) rice, and (e) wheat

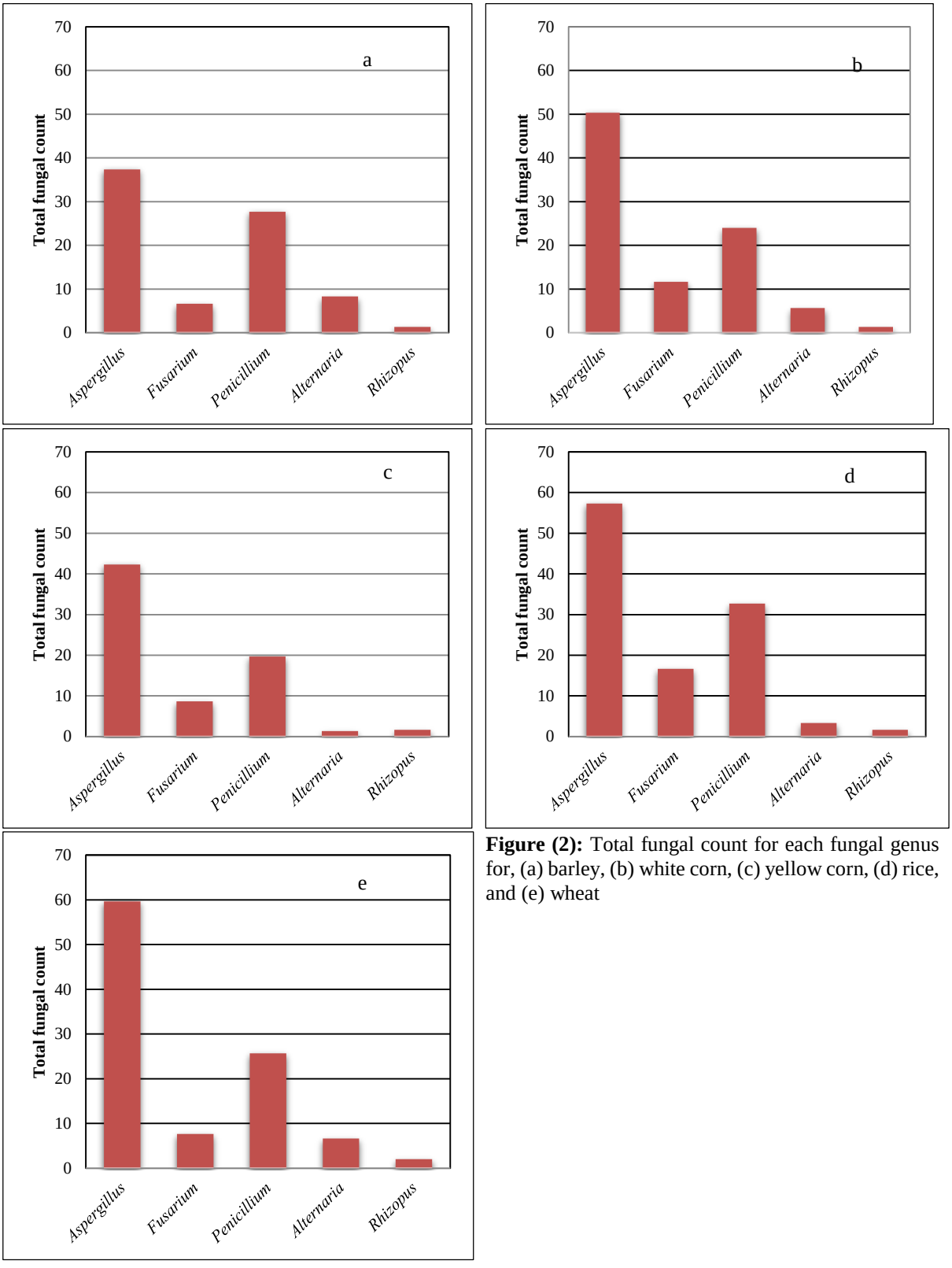


Figure (2): Total fungal count for each fungal genus for, (a) barley, (b) white corn, (c) yellow corn, (d) rice, and (e) wheat

4.Discussion

The most often used method for fungal isolation and identification is morphological characterization. This study used Potato Dextrose Agar Medium, which allowed adequate fungal growth and sporulation, thus permitting good evaluation. For *A. flavus*, the morphological identification was in line with Okayo et al. [29], who reported that the isolates representative of *A. flavus* had a greenish colony that spread radially from the point of inoculation. Results were also consistent with Thathana et al. [30]. The morphological description of *A. parasiticus* was in line with that reported by Nikolić et al. [31]. Nyongesa et al. [32] reported similar observations who stated that the *A. parasiticus* colonies on PDA were green with white mycelia and roughened with age. Morphological identification of *A.niger* was in agreement with Varga et al. [33], who revealed that the colonies originally appeared as white-yellow in colour and then conidia head turned to dark brown-black. Finally, the *A. ochraceus* colony had a characteristic yellow colour, whereas the macroscopic appearance conidiophores were powdery in mass, and the characteristics were chalky yellow to pale yellow-brown [34].

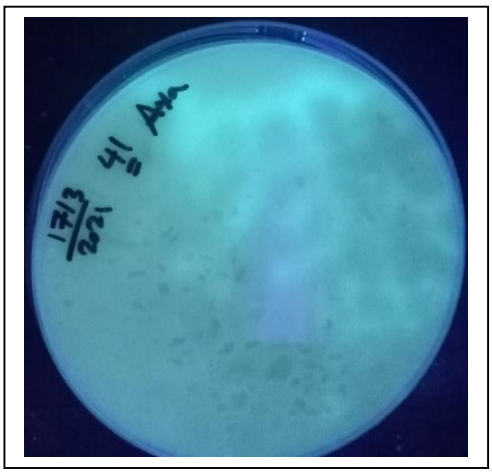
Several fungal species were isolated as the natural contaminant of cereals (barley, white corn, yellow corn, wheat, and rice). The isolated genera included *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria*, and *Rhizopus*. Results showed that *Aspergillus* spp. was the most isolated genera from various cereal grains from different governorates, followed by *Penicillium* and *Fusarium* spp.

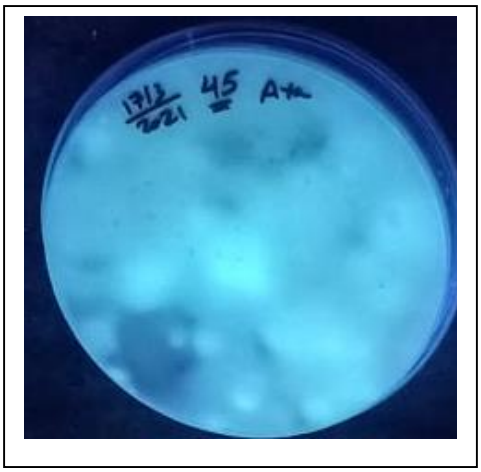
Similar observations were stated for the contamination of barley. In Italy, barley was contaminated by the following genera of *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium* [35]. In Poland, *Fusarium* was among the most frequently identified microscopic fungi genera in barley samples [36]. In Egypt, similar results were reported by Badr and Amra [37], who revealed that barley obtained from different governments was contaminated with *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria*, and *Rhizopus*. Regarding corn contamination, a study in 2016 revealed the contamination of maize by the mycotoxigenic species of the genera *Aspergillus* and *Penicillium* [38]. Similar observations were reported by Aristil et al. [39]. Munkvold et al. [40] and García-Díaz et al. [41] stated that *Aspergillus*, *Fusarium*, and *Penicillium* genera had been associated with pre-and post-harvest corn.

In earlier studies in Malaysia, the proliferation of *Fusarium* spp. was particularly emphasized, whereas several *Fusarium* spp. contaminating corn ears, bracts, and kernels were detected [42, 43]. In Egypt, Sahab et al. [44] revealed that *Aspergillus* was the most predominant fungi (50.9 and 60.3 %), followed by *Alternaria* (14.5 and 15.6 %) in maize samples collected from Cairo and Giza governorates, respectively. Lately, Hussain et al. [18] isolated *Aspergillus*, *Penicillium*, and *Fusarium* from white and yellow corn samples collected from Egypt. Regarding the contamination of rice in Brazil, Nicolli et al. [45] isolated *Fusarium* spp. In Kenya, *Aspergillus* spp. was the main genera contaminating rice [46]. Meanwhile, in Saudi Arabia, rice was contaminated by mycotoxigenic species of the genera *Aspergillus*, *Fusarium*, and *Penicillium* [47]. On studying 150 samples of freshly harvested wheat grains collected in Brazil, a predominance of *Alternaria*, *Fusarium*, and *Epicoccum* were observed [48]. In Italy, the main component of the wheat durum fungal community was *Alternaria*, whereas *Fusarium* was the second most prevalent genus of the fungal community [49].

Numerous fungi of the genus *Aspergillus* were the most abundant in the tropical regions of the world [50] and were involved in the food spoilage linked to the production of AFs. The fluorescence of positive colonies, when exposed to UV at 365 nm, was used to screen for aflatoxin formation on CAM. Similar results were reported by Thathana et al. [30] who found that 23% of fungal isolates showed fluorescence when exposed to UV light. Recently, Khan et al. [51] reported that 45% of the *A. flavus* isolates using UV fluorescence screening exhibited aflatoxin production. Coconut agar medium was effective for *A. flavus* and *A. parasiticus* growth and aflatoxin production since the surface of CAM is also highly absorbent to UV light, making it a suitable background for spotting zones of fluorescent AFs surrounding fungal colonies [52]. The TLC was employed in this investigation to validate aflatoxin formation by the isolates and identify the AFs

2





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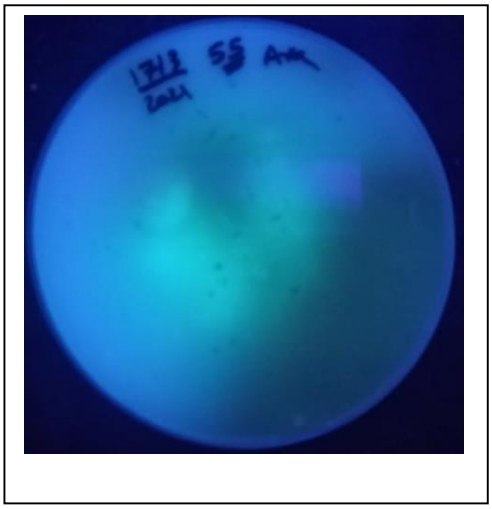

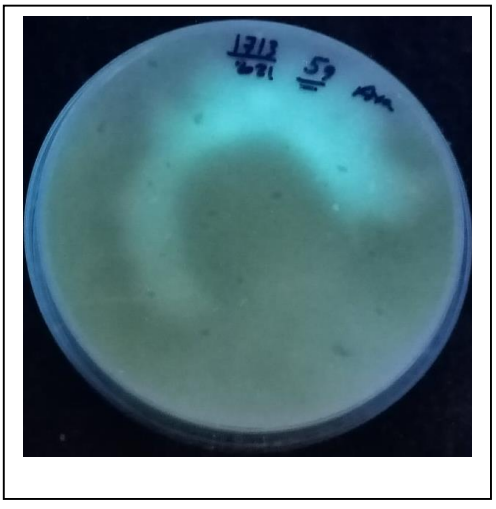

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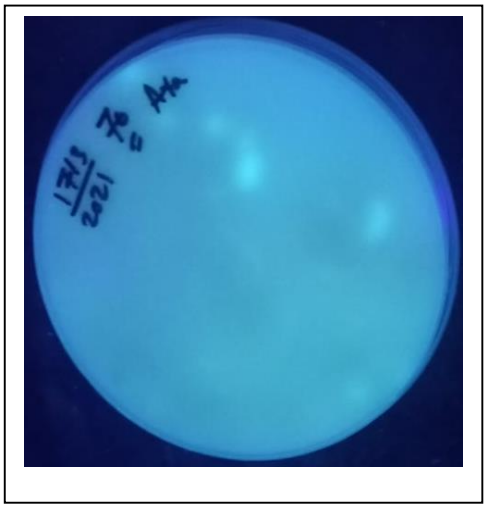

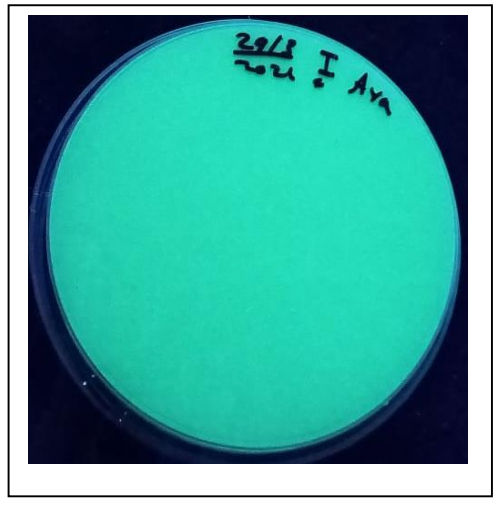

Figure (3): Coconut agar plates of *Aspergillus flavus* and *Aspergillus parasiticus* showing visible fluorescence under 365 nm, and indicating the possible production of aflatoxins. Plates 1and 2 are *Aspergillus flavus* isolated from WcQ41, WcA45. Plates 3, 4 and 5 are *Aspergillus parasiticus* isolated from YcB56, YcB59, and YcQ70. Plate 6 nonproducing aflatoxin isolated from WcMI.

Table (5): The detection of fungal species from wheat samples obtained from different Egyptian governorates

Governorates	*Aspergillus*		*Fusarium*		*Penicillium*		*Alternaria*		*Rhizopus*	
	Total Fungal count	%*	Total Fungal count	%*	Total Fungal count	%*	Total Fungal count	%*	Total Fungal count	%*
Alexandria	13.00±1.73 [a]	21.78	4.00±1.00 [c]	52.15	3.67±0.58 [a]	14.29	0.00±0.00 [a]	0.00	0.33±0.58 [a]	16.50
Beheira	8.33±1.15 [a]	13.96	3.67±0.58 [a]	47.84	5.00±1.00 [a]	19.47	0.00±0.00 [a]	0.00	0.33±0.58 [a]	16.50
Cairo	12.00±1.00 [a]	20.11	0.00±0.00 [b]	0.00	3.00±1.00 [a]	11.68	1.67±1.15 [a, c]	25.03	0.67±0.58 [a]	33.50
Giza	11.67±0.57 [a]	19.55	0.00±0.00 [b]	0.00	5.67±1.53 [a]	22.08	3.67±0.58 [b]	55.02	0.67±0.58 [a]	33.50
Monufia	8.00±1.00 [a]	13.40	0.00±0.00 [b]	0.00	5.33±0.58 [a]	20.76	0.00±0.00 [a]	0.00	0.00±0.00 [a]	0.00
Qalyubia	6.67±1.53 [a]	11.17	0.00±0.00 [b]	0.00	3.00±1.00 [a]	11.68	1.33±0.58 [a ,d]	19.94	0.00±0.00 [a]	0.00

Results are mean ± SD (n=3); Numbers within each column superscript with the different letters are considered significant $P \leq 0.05$.
Numbers within each raw superscript with the different letters are considered significant $P \leq 0.05$.
*Percentage of contamination for each fungal species in each government.

Table (6): Ability of some *Aspergillus* species to produce aflatoxins using TLC and HPLC

Isolate No.	TLC	Aflatoxins concentration ng/g agar plugs				
		AFB_1	AFB_2	AFG_1	AFG_2	Total AFs
WQ1*	+	0.70±0.16	0.17±0.07	1.00±0.06	1.85±0.35	3.73
WcC6*	+	875.03±169.02	13.55±2.86	ND	4.24±2.79	892.82
WQ7*	+	0.52±0.04	0.19±0.03	0.86±0.02	5.26±0.08	6.83
WQ12*	+	0.31±0.13	0.14±0.04	2.26±0.36	5.06±1.38	7.76
WcA33**	+	0.14±0.16	0.07±0.08	0.20±0.22	0.77±0.85	1.18
WcA35*	+	0.09±0.10	ND	0.06±0.06	3.09±3.43	3.24
WcA39*	+	0.18±0.04	ND	0.41±0.01	0.71±0.45	1.30
WcQ40**	+	1.43±0.07	0.16±0.04	0.43±0.48	9.85±0.83	11.87
WcQ41**	+	0.19±0.03	0.14±0.06	0.06±0.09	3.17±0.38	3.56
WcA44*	+	0.02±0.02	ND	ND	4.25±4.72	4.27
WcA45*	+	1.04±0.02	0.18±0.01	0.80±0.09	9.32±0.43	11.34
WcA46*	+	0.09±0.10	0.13±0.15	0.23±0.04	3.39±3.11	3.84
YcB56**	+	0.60±0.05	0.18±0.03	2.26±0.06	4.52±0.05	7.56
YcB57**	+	0.63±0.07	0.38±0.03	2.85±0.08	6.91±0.31	10.77
YcB58**	+	0.91±0.92	ND	ND	89.29±89.65	90.20
YcB59**	+	0.87±0.16	0.30±0.03	2.65±0.40	6.31±0.30	10.13
YcQ70**	+	0.56±0.10	0.49±0.28	1.57±0.11	2.25±2.17	4.87
BG86*	+	1.58±0.08	0.25±0.01	ND	15.66±1.97	17.49
RA110*	+	0.42±0.10	0.09±0.03	0.10±0.11	1.06±0.09	1.67
RA112*	+	0.38±0.09	0.10±0.02	0.06±0.10	1.03±0.18	1.57
RA198**	+	0.10±0.11	ND	0.12±0.13	3.83±4.25	4.05

Results are mean ± SD (n=2); ND: Not detected
Cereal code: W: Wheat; B: Barley; Wc: White corn; Yc: Yellow corn; R: Rice
Government code: A: Alexandria; B: Beheira; C: Cairo; G: Giza; Q: Qalyubia
Aspergillus flavus*; * Aspergillus parasiticus*

production since most of them fluorescent strongly in long-wave UV light [53]. Strongly

Aflatoxigenic *Aspergillus* species gave reliable results regarding aflatoxin production on CAM as confirmed by TLC and HPLC. Sohrabi and Taghizadeh [54] reported similar results. A positive association between the TLC and HPLC methods for aflatoxin production in fungus species has also been confirmed Akinola et al. [55]. Many researchers have investigated fungal contamination and mycotoxin production in Egyptian crops and commodities. El-Shanshoury et al. [56] reported that *A. flavus* isolated from cereals and peanuts could produce AFB_1, AFB_2, and AFG_1. Abbas et al. [57] isolated *Aspergillus* species from different samples and identified them as *A. flavus*, whereas most of them were AFB_1 and AFB_2 producers. Recently, El-Sayed et al. [58] revealed that three of five *A. flavus* strains produced AFB_1 and AFB_2. These results were considered different than those reported in our study, whereas both *A. flavus* and *A. parasiticus* isolates produced the four types of AFs. Takahashi et al. [59] reported similar results, which indicated that strains of *A. flavus* could produce B and G aflatoxins.

5.Conclusion

Our results showed that several fungal species were isolated from cereal grains, whereas *Aspergillus* and *Penicillium* were the predominant genera. Corn grain agar medium allowed the production of AFs by *A. flavus* and *A. parasiticus*. The UV fluorescence screening of CAM and TLC for detecting and identifying AFs provided a quick and reliable means to distinguish between the aflatoxigenic and non-aflatoxigenic strains. Further studies are needed to minimize fungal contamination of cereal grains.

6. Conflicts of interest

There are no conflicts to declare

7. References

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