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RESEARCH ARTICLE

MYCOTOXIN AND TOXIGENIC FUNGI OCCURRENCE IN EGYPTIAN MAIZE

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Abstract

Many types of fungi such as *Fusarium*, *Aspergillus*, and *Penicillium* have been detected on Egyptian maize (Shamya) at field and earlier storage. Ten governorates located between latitudes 22 southwards to 32 northwards, led to a variety climate of temperature, wind and rainfall during the same season, this diversity impact on type and number of toxigenic fungi that grown on maize that followed by mycotoxins type and amount influences.. Analysis of maize showed that, the lowest and the highest number of fungal colonies were detected on Giza and Behira samples (79 and 98 colonies on 2012 year samples; 112 and 151 colonies on 2013 year samples per ten plates count, respectively). *Aspergillus* as a common fungus ranged from 29.9% - 61.3 %(year 2012) and from 55.9% – 84.1 %(year 2013) of total colonies count on maize. In 2013 year samples; total aflatoxins ranged from 14µg/kg to 34.9µg/kg (Giza and Asyut samples, respectively). Aflatoxins B_1 in all studied samples represented the highest amount of the four aflatoxins types (B_1, B_2, G_1, and G_2). Fumonisin B_1 ranged from 170µg/kg to 1915µg/kg (Giza and Dakahlia samples, respectively). The highest amount of ochratoxin A was 14.9µg/kg (Alexandria samples), while no ochratoxin A had detected in samples belonging to Giza, Sharkia, and Sohag. The highest amount of zearalenone was 3.5µg/kg (Gharbia samples), none zearalenone had detected in samples of Cairo and Dakahlia.

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Introduction

Many cereals and other crops are susceptible to fungal attack either in the field or during storage. These fungi may produce mycotoxins as secondary metabolites. There can be wide year to year fluctuations in the levels of mycotoxins in foods, depending on many factors, such as adverse conditions favoring fungal invasion and growth (Magan and Monica, 2004). Tropical conditions such as high temperatures and moisture, monsoons, unseasonal rains during harvest and flash floods led to fungal proliferation and production of mycotoxins (Bhat and Vasanthi, 2003). Poor harvesting practices, improper storage, marketing and processing can also contribute to fungal growth and increase the risk of mycotoxin production. The major mycotoxin-producing fungal genera are *Aspergillus*, *Fusarium* and *Penicillium*. Hundreds of different mycotoxins have been identified. However, according to their occurrence and toxicity, it is usually admitted that about 30 of them are of real significance for human and animal health (Bennett and Klich, 2003).

These toxic substances are known to be either carcinogenic (aflatoxin B_1, ochratoxin and fumonisin B_1); oestrogenic (zearalenone), neurotoxic (fumonisin B_1) nephrotoxic (ochratoxin) demartotoxic (trichothecenes); immunosuppressive (aflatoxin B_1, ochratoxin A, T-2 toxin) (Bankole and Adebanjo, 2003; Jestoi *et al.*, 2004; Coronel *et al.*, 2010). Aflatoxins are produced mainly by three fungal species belonging to *Aspergillus* genus: *Aspergillus flavus, A. parasiticus* and *A. nomius* (Varga *et al.*, 2011). This family of compounds is made of different molecules including aflatoxin B_1 (AFB_1), B_2, G_1 and G_2. AFB_1 is the most prevalent and toxic member of the

family and a correlation has been made between chronic exposure to aflatoxin B_1 and primary hepatic cancer (Abdel-Wahab *et al*., 2008). The AFB_1 is also thought to be responsible for growth retardation in children in Africa (Khlangwiset *et al*., 2011).

The food-borne mycotoxins likely to be of greatest significance in Africa and other tropical developing countries are the fumonisins and aflatoxins (WHO, 2006). While aflatoxins occur mostly in maize and groundnuts, the prevalence of fumonisins is 100% or close to it in all surveillance data that have been reported on maize from different parts of Africa (Bankole *et al*., 2006). Limited surveys have also established presence of ochratoxin A, trichothecenes and zearalenone in the continent (Muthomi *et al*., 2002; Bankole *et al*., 2006).

Udoh *et al.* (2000) reported that 33% of maize samples from different agro-ecological zones of Nigeria were contaminated with aflatoxins. Hell *et al.* (2000) found that the percentage of maize samples with more than 5μg/kg aflatoxin levels was between 9.9% and 32.2% in the different agro ecological zones of Benin before storage, these percentages increased to 15.0% and 32.2% after 6 months of storage. All the maize samples collected from silos and warehouses in Ghana contained aflatoxins at levels ranging from 20 to 355μg/kg.

Both temperature and humidity influence fungi which infect damaged crops with aflatoxin producers favored by warm conditions. Climate also influences the extent to which crops become wounded by mammals, birds and insects. Diverse insects carry aflatoxin producing fungi and specific insect/crop combinations have been repeatedly linked to aflatoxin contamination. These include corn borers on maize, pink bollworm on cotton, lesser corn stalk borer on peanut and the navel orange worm on pistachio. For insects, survival between seasons, dispersal across regions, and rates of population increases are all influenced by climate (Cotty and Jaime-Garcia, 2007).

Maize has become a staple for many millions in warm regions throughout Africa, Asia, and the Americas. This crop is particularly vulnerable to influences of climate as exemplified by recent experiences with lethal aflatoxicoses in Kenya (Lewis *et al*., 2005). The quantity of aflatoxin producing fungi associated with crops and soils varies with climate. Aflatoxin producing fungi are native to warm arid, semi-arid, and tropical regions with changes in climate resulting in large fluctuations in the quantity of aflatoxin producers (Bock *et al*., 2004).

Climate influences not only the quantity but also the types of aflatoxin producers present. This result in aflatoxin-producing fungi differing geographically (Cotty, 1997). Furthermore, the average aflatoxin producing potential of fungal communities varies with geography with some regions having communities with greater aflatoxin-producing potentials and, as a result, crops grown in those regions are more vulnerable to contamination (Cotty, 1997; Cotty and Jaime-Garcia, 2007).

White maize (Shamya) is considered as one of the main crops in recent years in Egypt, importance of white maize is due to its usage in bread production by mixing the wheat flour with white maize flour, mixing has been done to solve problem of wheat shortage in Egypt. The aim of this study was to isolate and identify toxigenic fungi and to determine mycotoxins in white maize (Shamya) in Egypt to know the situation of maize that used in one of the most important Egyptian staple food (Bread), to study effects of climate changes during two years (2012 and 2013), also to study geographical position impacts of different Egyptian governorates on types and numbers of toxigenic fungi that could be found on maize (Shamya).

Materials and Methods

Basic apparatus; chemicals and solvents:

Conical flask – measuring cylinders - vials, borosilicate – glass funnel – Buchner funnel – screw cap lined with foil or teflon.

Wrist Action Shaker, Model EL680, Eberbach Co. – Rotary evaporator System Cole-Parmer; Diagonal, 115 VAC – A high-speed blender (15000 rpm) with a 1 L glass jar and cover (General Electric), a model JFSD – 100 grinding/subsampling mill for maize seed – EW-28615-00 – UV light Chamber - Micropipette (5 µl - 100 µl adjustable) . Cleanup cartridges were presented from Romer labs. Inc. (USA). HPLC grade H_2O was prepared with a ZD20 four bowl Milli-Q water system (Millipore), chromatography column, 25 mm (i.d) X 300 mm length.

HPLC grade solvents were obtained from Sigma (Germany). Other chemicals used through the study were of analytical reagent grade and obtained from Merck (Germany).

Media for isolation:

Czapek dox agar with 60µg/ml chloramphenicol (CDAC), and potato dextrose agar amended with 60µg/ml chloramphenicol (PDAC) were used for isolation of fungi from maize seeds.

Collection of samples:

A total of 50 samples were collected from 10 governorates (5 samples from each one according to Grain Inspection, Packers & Stockyards Administration (GIPSA) - USAD). Samples (1Kg each) were randomly collected from local storages just on arrive time and stored in refrigerator at 4 °C. Samples were collected during two winter seasons 2011 – 2012 (year 2012), and 2012 – 2013(year 2013).

Isolation and purification of fungi:

Potato Dextrose Agar (PDA) supplemented with 60µg/ml chloramphenicol (PDAC) was used for isolation of fungi. Ten plates of PDAC for each sample were embeded by 10 seeds on each plate. Before embedding of the seeds, samples were hold at (- 20°C) for 72 hours to kill mites and insects that might interfere with analysis, then sieve was used to get rid of it. (Amra, 1984).

Approximately, 100 grams of each sample were transferred into sterile 500 ml beaker containing 300 ml distilled water with 5% NaOCl (sterilizing solution). Maize seeds were emerged in this solution for 3 minutes and after that, it was washed twice with distilled water. Flamed forceps was used to transfer and embed 10 seeds into the solidified agar of PDAC media; each seed had approximately a diameter of 1 cm free zone around it. Plates were incubated for 5 days at 25 °C. Each fungal growth was picked up and diluted with sterile distilled water. One milliliter from the dilution was plated into PDA medium using pour plate technique. Incubation was carried out for 5 days at 25 °C.

Plates were inspected to fungal growth, total count of colonies were recorded. Stereo microscope was used to isolate single spores. by using fungal loop, loop load was transferred to small graduated eppendorf 1ml contains autoclaved water with tween 80, agitating with vortex mixer for 2 minutes then spread on plate media (Czpeak Dox Agar media – PDA – for *Aspergillus sp*. or *Penicillum sp*. – Agar water for *Fusarium sp*.), incubated for 12 / 24h in UV incubation. Plates were inspected after incubation under microscope for single spore with small hyphen, cut a diamond shape around it with sharp knife loop then transferred it onto small size PDA or CDA plate, re-incubated for 4 – 5 days to fungal grew, re-cultured on small size plates of Czapek-Doks Agar media to isolate single spores of *Aspergillus* or *Penicillum;* for *Fusarium* agar water media had been used. According to Wang *et.al*, (1996) with some modification, four days fungal grew on maize seed PDAC media plates were used to isolate conidiophores of each fungi type under stereo-microscope. Growing colonies were selected and purified. (Castellari *et al*., 2010) The culture obtained were grown on PDA slant and maintained at 4 °C.

The identification procedure should start with identification of the fungi to genus level morphologically, using the books by Domsch *et al*. (2007) and Samson *et al.* (2010). When the fungus is identified to genus level, different identification procedures may be used. *Penicillium* and *Aspergillus* identification are usually inoculated on the media Czapek yeast extract agar (CYA), malt extract agar (MEA), yeast extract sucrose agar (YES), while Fusarium isolates are identified on Specifikke nutrient-arme agar (SNA), potato dextrose agar (PDA). *Alternaria* species are identified via Dichloran Rose Bengal Yeast Extract Sucrose (DRYES) agar and potato carrot agar (PCA) (Simmons, 2007). *Penicillium* isolates can be identified to species level according to Samson *et al*. (2010). *Aspergillus* species can be identified to species level using Samson *et al*. (2010). *Fusarium* species can be identified according to Samson *et al.* (2010). The identifications can be validated using authenticated cultures of each species and by comparison to the descriptions in the taxonomic works. (Domsch *et al*., 2007; Samson *et al*., 2010)

The cultural characteristics of the selected fungal isolates on PDA media were recorded. The fungal isolates were also examined microscopically. The slide agar method (Benson, 2001) was used for preparation of isolates. Slides were examined microscopically under low and high power after staining with lacto phenol cotton blue stain.

Detection and determination of mycotoxins:

Aflatoxin, Fumonisin B1, Ochratoxin A, and Zearalenone were detected in maize samples according to the TLC methods described by (Stroka *et al*., 2000) for qualitative, and by HPLC for quantitative determination using multi-technique according to A.O.A.C. (2007).

Aflatoxins:

Preparation of standards

Aflatoxin standards were received as crystals: Calculated volume of benzene -acetonitrile (98+2) was added to the container of dry aflatoxins B_1, B_2, G_1, and G_2, to give a concentration of 8 - 10µg / ml. Solution was vigorously agitated for 1 minute on vortex shaker and it was transferred without rinsing to convenient sized glass vials.

Extraction

Fifty grams of powdered representative sample was mixed in a 500 ml conical flask with 25ml water, 25 gm diatomaceous earth (celite) and 250 ml chloroform. The flask was securely stoppered with masking tape and shaken on a wrist action shaker for 30 minutes to extract the toxin. Filter through fluted filter paper. When filtration was slow, mixture was transferred to a Buchner funnel pre-coated with about 5 mm layer of diatomaceous earth and filtered using light vacuum. First 50 ml of filtrate were collected.

Cleanup using Immuno – affinity columns

The extract was diluted to less than 5% acetonitrile using phosphate_buffered saline. Then 18 mL of the resulting solution was loaded into the Afla Star™ column from Romer Labs®. The column was rinsed using 20 ml of water and eluted using acetonitrile. Acetonitrile was evaporated to dryness.

Preliminary TLC

To uncap vial containing cleaned up residue, 200µl benzene – acetonitrile (98+ 2) were added and resealed with polythene stopper. Shaking vigorously was applied to dissolve the toxin. Polythene stopper was punctured to accommodate needle of 10µl syringe. In subdued incandescent light and as rapidly as possible 10µl were spotted on imaginary line 4cm from bottom of TLC plate. Vials were kept for quantitative analysis. On same plate 10µl of aflatoxin standards were spotted. Fifty ml acetone - chloroform (1+9) was placed in trough of unlined developing tank. Plate was immediately inserted and the tank was sealed.

Plate was developed for 40 minutes or until aflatoxins reach R_f 0.4 – 0.7. After that, the plate was removed from tank; solvents were evaporated at room temperature and viewed under long wave UV lamp in a viewing chamber. Pattern of the four florescent spots (B_1, B_2, G_1, and G_2) were observed.

Derivatization using Trifluoroacetic Acid (TFA)

Two hundred micro-liter of the eluent were transferred to a 5mL reaction vial and 880µL of a derivatization agent (70:20:10 water: TFA: acetic acid) were introduced. The mixture was shaken by hand, and reacted at 65 ºC for 9 minutes then cooled to room temperature prior to HPLC analysis.

Fumonisin:

Preparation of standards

Stock solution of fumonisin B_1 at concentration of 250µg/ml in acetonitrile – water solution (1:1, v/v) was prepared. The standard work solution containing fumonisin B_1 at concentration 50µg/ml, solution was stored at 4ºC.

Extraction

Maize samples were ground to yield material of such size that almost retained between 250 to 500µm mesh screens. To 500ml plastic blender container, 50 grams of grinding material were transferred, 100 ml of methanol – water solution (3:1, v/v), blended the contents for 5 minutes. Blended mixture was centrifuged at 500xg then filtered supernatant through fluted filter paper. The pH of filtrate was adjusted to 5.8 (from 5.8 to 6.5 by using drops of NaoH 1M if it was required).

Cleanup:

The SPE cartridge was fitted to SPE manifold; cartridges were conditioned by washing with 5 ml methanol, followed by 5 ml methanol – water solution (3:1, v/v), 10 ml of filtered extracted was applied to cartridge, maintaining flow rate at 1 ml/minute. Cartridge was washed with 5 ml methanol – water solution (3:1, v/v), followed by 5 ml methanol. Before cartridge dryness, fumonisin B_1 was eluted with10 ml acetic acid – methanol solution

(1:99, v/v), flow rate was adjustable at 1 ml/ min, all eluted solutions were collected in a glass vials then contents of vial were dried under steam nitrogen at 60ºC.

Derivatization:

Re-dissolve residue from cleaned up vial in 200µl methanol, 25µl was transferred to small test tube, then 255µl of O-phthaldialdihide (OPA) reagent was added, tube contents were mixed well, within 1 minute 10µl had taken by micro-syringe and injected into HPLC system.

Ochratoxin A:

Preparation of standards

Stock solution of ochratoxin A at concentration of 50µg/ml in acetic acid – benzene solution (1:99, v/v) was prepared. The standard work solution containing ochratoxin A at concentration 10µl/ml, solution was stored at 4ºC.

Extraction

Sample of fifty grams was weighed for analysis, transferred to a blender jar, 25ml of (0.1 M) phosphoric acid and 250ml chloroform were added, blended for 5 minutes at medium speed; 10 grams of celite were added near the end of blended operation. Throw glass fiber paper covered with 10 grams of celite on Buchner funnel elute were filtered, from this filtrate about 80 ml were collected. Five ml of filtrate solution were transferred to separating funnel 10 ml of 3% sodium bicarbonate were added, and then centrifuged (2min /2000rpm – 753 xg) in case of emulsion formed. Upper phase was collected for column extraction.

Cleanup:

A C18 column was placed on manifold ports with 50ml flasks inside manifold to collecting condition and wash solvents. Each column was washed twice by 2 ml methanol, 2 ml water, 2 ml of 3% sodium bicarbonate. Five ml bicarbonate extract was pipetted to C18 column followed by 2ml 0.1M phosphoric acid then, 2ml of water were added. Washes solutions were discarded, ochratoxin A was eluted with 8ml of solution (95ethyl acetate : 5 methanol : 0.5 acetic acid) into vial containing 2 ml water, mixed well then, upper phase was raised twice with 1 ml ethyl acetate and added to ochratoxin A , evaporated to dryness.

Zearalenone:

Preparation of standards

Stock solution of zearalenone at concentration of 50µg/ml in acetic acid – benzene solution (1:99, v/v) was prepared. The standard work solution containing ochratoxin A at concentration 10µl/ml was stored at 4ºC.

Extraction

Fifty grams of sample were weighed for analysis, transferred to glass stoppered extraction flask, 25 g of celite and 20 ml water were added, and flask was rotated to accomplish some mixing. A volume of 250ml of chloroform were added, a wrist action shaker used for 15 minutes, filtered throw fluted paper then, filtrate of 50ml was collected. Filtrated solution was transferred to separating funnel then; 10ml of NaCl were added and mixed well. The lower layer was discarded; 50ml chloroform were added then shacked well for 1 minute. Citric acid solution of 50 ml was added to separator, mixed and zearalenone was extracted with 50 ml CH_2Cl_2, shacked for 1 minute, after separated two phases, lower phase was drained through $NaSO_4$ over wool ball filter, re-extracted with 50 ml CH_2Cl_2, and lower phase was drained through $NaSO_4$ over wool ball filter again. $NaSO_4$ over wool ball filter was washed with 15 ml of CH_2Cl_2, and then CH_2Cl_2 was evaporated to dryness under nitrogen.

Instrumental condition

HPLC mobile phase:

1. Methanol : acetonitrile (V/V 50:50) with 0.1% formic acid for aflatoxins.(Stroka *et al*.,2000).
2. Methanol: sodium dihydrogen phosphate (0.1 M) (V/V 80:20) adjusted to an apparent pH of 3.35 for fumonisins. (Sydenham *et al*., 1996).
3. Water: acetonitrile: acetic acid (V/V /V 51:48:1) were used for ochratoxin A. (Mantle *et al.*, 2000).
4. Water: acetonitrile (V/V 55: 45) were used for zearalenone (Kolf-Clauw *et al*., 2007).

Triple quadruple HPLC and Analytical Column RP-18 end-capped, 150x4.6mm, 5µm particle size. Mycotoxins were quantified by HPLC (Water, 474) with fluorescence detector (excitation λ_{exc}, 330 nm; emission

λ_{em}, 460 nm for aflatoxin and ochratoxin), (λ_{exc} 274 nm, λ_{em} 440 nm for zearalenone). Flow rate was 1.0 ml per minute and injection volume was 10µl. The detection limit of the analysis was 0.01µg Kg^{-1}. Quantification was achieved with a computing integrator (Millenium32 v.3.05 software, Milford, Massachusetts, U.S.A). Mycotoxin was quantified on the basis of the HPLC fluorimetric response compared with that of a range of standards.

Statistical analysis:

Analysis of variance was carried out using M Stat program V.6 software (Stat Soft Inc, USA), differences among the means were determined for significance at $P < 0.05$ using Fisher least significance difference test ($LSD_{0.05}$), (Gomez and Gomez, 1984).

Results and discussions

Maize samples were collected from ten governorates of Arab Republic of Egypt, these governorates included (Alexandria, Asyut, Behira, Cairo, Dakahlia, Gharbia, Giza, Kafr El sheykh, Sharkia, and Sohag), Data presented in Table (1) show the common fungi that were isolated from Egyptian maize samples of the studied governorates in year 2012 (2011-2012).

Plate count and type of fungi were done twice; first at the pre- refrigeration time of samples and secondly at the end of the storage period (3 months), the count of fungi and also the genera were almost the same. Types of fungi were different from place to place; many factors could play a key role in this respect. Climate condition is one of the most important factors that have a great effect. In this respect Giza was the highest governorate in temperature averages. On the other hand Alexandria was the lowest governorate. Kafr El Sheykh and Alexandria were higher in wind speeds, also in humidity levels. Behira had high levels of dew point, but for rain fall levels, Alexandria was the first. All these variables have an effect on fungi types and kinds of secondary metabolites or toxins (Egyptian Metrological Authority).

Type and number of mycotoxin producing fungi on samples of Giza governorate (Middle site Governorate) differed from those appeared on maize samples collected from Asyut (Southern governorate) and Alexandria (Northern governorate). Dominant type of fungi in Asyut samples was *Aspergillus*, but in case of Giza samples, *Fusarium* was the dominant (Table 1, Figure 1). All types of fungi (*Asspergillus, Alternaria, Fusarium, Penicillium,* and *Rhizopus*) were found in maize samples of Alexandria governorate (Table 1 and Figure 1) as well as samples of Behira and Dakahlia.

Aspergillus was the most dominant fungus in all studied samples except samples collected from Giza. Average percentage of fungi that grew on maize samples of year 2012 were; *Aspergillus* (41%), *Alternaria* (4.3%), *Fusarium* (26.6%), *Penicillium* (26.3%), and *Rhizopus* (5.5%).

Sum of *Aspergillus* group (*Aspergillus flavus* + *Aspergillus niger*) were more than 50% of the total fungi on maize samples of most governorates (Table 2). Behira and Kafr El Sheykh were the highest governorates for total fungi colonies count on the plate agar media samples of maize seeds (151 and 144 colony, respectively), whereas Giza was found to be the lowest governorate in total fungi colonies count(112 colony). Results appeared in Table (2) indicate that in the Northern governorates high relative humidity values (Data not given here) had specific effect on total fungi colonies count. *Alternaria* and *Rhizopus* fungi appeared in four and six governorates respectively; those governorates are in Northern and Middle area of Egyptian country, Upper Egypt governorates had no *Alternaria* or *Rhizopus* fungi.

Aspergillus sp. was the most prevalent presence fungus, in generally, the average percentages of fungi that grew on maize grain of year 2013 were; *Aspergillus* (65.1%), *Alternaria* (0.99%), *Fusarium* (18.6%), *Penicillium* (17.1%), and *Rhizopus* (1.08%).

Data presented in Tables (1) and (2) show that there were variations in types and percentage of fungi on maize collected from various governorates in years 2012 and 2013. The lowest percentage of *Aspergillus* was noted in samples of Giza and Dakahlia in the first year (29.9% and 32.5%, respectively), in the second year, Alexandria was the lowest governorate (55.9%).

Samples collected from Asyut and Sohag, South governorates, had the highest percentage of *Aspergillus* in the first year (2012) where Asyut and Sohag samples were the highest in *Aspergillus* percentages (60% and 61.3%,

respectively). This result was not so different from the second year (82.4% and 84.1%, respectively). On the other hand; those governorates had the lowest percentages of *Fusarium* (12.9% and 7.5% in the first year; and zero and 2.4% in the second year, respectively).

As for *Penicillium* percentages of fungi presented on the seeds, it ranged from 22.4% to 30.1% in the first year of the ten governorates with average 26.3%. This ratio had changed in the second year to be in a range from 13.5% to 22.3% with average 17.1%.

Total fungal count for all governorates was higher in the second year, the percentage of increment ranged between 36.96% in Sohag governorate, 47.4% in Giza, 54.1% in Asyut and Behira, 58.4% in Sharkia, 64.19% in Gharbia, 65% in Dakahlia, 70.2% in Alexandria, 72.5% in Cairo, finally it had been reached 82.3% in Kafr El Sheykh. According to the meteorological data from Egyptian Metrological Authority (EMA) website which displayed daily information about weather parameters (rainfall, wind speed, RH, and dew point), relative humidity and wind speed increased in the second year, so; may such parameters had affected the total fungal count of maize samples.

Table (1): Total colonies, numbers of colonies and percentage of each isolated fungal genus from maize collected from ten Egyptian governorates at year 2012.

Governorates	Total fungi colonies	Types of isolated fungi									
		Aspergillus		*Alternaria*		*Fusarium*		*Penicillium*		*Rhizopus*	
		No.	%	No.	%	No.	%	No.	%	No.	%
Alexandria	**84**	**32^{d} ±1.41**	**38.1**	**2 ± 0.7**	**2.4**	**27^{a} ± 1**	**32.1**	**20^{b} ±0.9**	**23.8**	**3 ±0.89**	**3.6**
Asyut	**85**	**51^{a} ±1.14**	**60**	**0.0**	**0.0**	**11^{f} ±0.2**	**12.9**	**$23^{c,d}$ ±1.6**	**27.1**	**0.0**	**0.0**
Behira	**98**	**35^{d} ± 4**	**35.7**	**4 ± 1**	**4.1**	**29^{b} ±1.41**	**29.6**	**22^{a} ±1.14**	**22.4**	**8 ±0.74**	**8.2**
Cairo	**80**	**$30^{d,c}$ ±2**	**37.5**	**0.0**	**0.0**	**25^{c} ±0.94**	**31.3**	**21^{b} ±1.58**	**26.3**	**4 ±0.57**	**5**
Dakahlia	**80**	**$26^{b,c}$ ±3.4**	**32.5**	**5 ±2**	**6.3**	**20^{c} ±1.62**	**25**	**21^{d} ±1.7**	**26.3**	**8±0.91**	**10**
Gharbia	**81**	**$33^{d,c}$ ± 1.7**	**40.7**	**0.0**	**0.0**	**25^{c} ±1.14**	**30.7**	**20^{d} ±1.37**	**24.7**	**3 ±0.43**	**3.7**
Giza	**76**	**23^{e} ±1.8**	**29.9**	**0.0**	**0.0**	**27^{e} ±1.3**	**35.1**	**22^{b} ±1.14**	**28.6**	**4±0.61**	**5.2**
Kafr El-sheykh	**79**	**28^{b} ±1.4**	**35.4**	**0.0**	**0.0**	**25^{d} ±0.7**	**31.6**	**23^{d} ±2.3**	**29.1**	**3±0.47**	**3.8**
Sharkia	**89**	**$35^{b,c}$ ± 1.8**	**39.3**	**0.0**	**0.0**	**27^{c} ±1.4**	**30.3**	**$22^{b,c}$ ±1.1**	**24.7**	**5 ±0.71**	**5.6**
Sohag	**92**	**57^{a} ± 1.14**	**61.3**	**0.0**	**0.0**	**7^{f} ±0.37**	**7.5**	**28^{e} ±0.79**	**30.1**	**0.0**	**0.0**
egaravA	**84.6**	**35**	**41**	**3.6**	**4.3**	**22.3**	**26.6**	**22.2**	**26.3**	**4.8**	**5.6**
L.S.D		**6.28**		**0.58**		**2.85**		**3.76**		**1.07**	

- Means with different superscript letters are significantly different ($P \leq 0.05$)
- Data are expressing as means ± SD.

Table (2): Total colonies, numbers of colonies and percentage of each isolated genus from maize collected from ten Egyptian governorates in 2013.

Governorates	Total fungi colonies	Types of isolated fungi									
		Aspergillus		*Alternaria*		*Fusarium*		*Penicillium*		*Rhizopus*	
		No.	%	No.	%	No.	%	No.	%	No.	%
Alexandria	143	$80^{e} \pm 1.84$	55.9	1±0.22	0.7	$37^{a} \pm 0.81$	25.9	$23^{c,d} \pm 0.67$	16.1	2 ± 0.2	1.4
Asyut	131	$108^{a} \pm 1$	82.4	0.0	0.0	0.0±0.24	0.0	$23^{c,d} \pm 0.86$	17.6	0.0	0.0
Behira	151	$85^{d,c} \pm 1.4$	56.3	0.0	0.0	$35^{a} \pm 0.86$	23.18	31 ±0.94	20.5	0.0	0.0
Cairo	138	$83^{d,e} \pm 1.72$	60.1	0.0	0.0	$26^{c,d} \pm 0.66$	18.8	28 ±0.83	20.3	1 ± 0.37	0.72
Dakahlia	132	$83^{d,e} \pm 2.42$	62.9	1±0.25	0.75	$29^{b} \pm 0.66$	21.96	$17^{e} \pm 0.87$	12.9	2 ± 0.4	1.51
Gharbia	133	$85^{d,c} \pm 1.42$	63.9	0.0	0.0	$25^{d} \pm 1.34$	18.8	$22^{d} \pm 0.58$	16.5	1 ± 0.32	0.75
Giza	112	64 ± 1.86	57.1	2±0.21	1.79	21 ±0.89	18.75	25 ±1	22.3	0.0	0.0
Kafer El-sheykh	144	$94^{b} \pm 1.59$	65.3	0.0	0.0	$25^{d} \pm 1.14$	17.4	$23^{c,d} \pm 0.54$	15.6	2 ± 0.45	1.4
Sharkia	141	$89^{b,c} \pm 1.08$	63.1	1± 0.32	0.71	$28^{b,c} \pm 0.66$	19.9	$22^{d} \pm 0.8$	15.6	1 ± 0.32	0.71
Sohag	126	$106^{a} \pm 1.1$	84.1	0.0	0.0	3 ±0.44	2.4	$17^{e} \pm 0.94$	13.5	0.0	0.0
Average	134.1	87.7	65.1	1.3	0.99	25.4	18.6	23.1	17.1	1.5	1.08
L.S.D		5.11		1.4		2.37		2.35		1.57	

- Means with different superscript letters are significantly different ($P \leq 0.05$)
- Data are expressing as means ± SD.

Total aflatoxins were determined in contaminated samples with *Aspergillus* fungi using HPLC, results are presented in Table (3) . Total aflatoxins ranged from 14 – 34.9µg/Kg, being the highest in maize belongs to Asyut, and the lowest in Giza samples. Aflatoxin B_1 ranged from 9.4µg/Kg (Cairo samples) to 21.8µg/Kg (Asyut samples). Aflatoxin B_2 ranged from 1.7µg/Kg (Gharbia samples) to 8.1µg/Kg (Asyut samples). No Aflatoxin G_1 was detected in Giza and Sohag samples, while Alexandria samples had the highest level of the Aflatoxin G_1 (7.9µg/Kg). No aflatoxin G_2 could be detected in samples of Giza, Kafr El Sheykh, and Sharkia. The highest amount of aflatoxin G_2 (1.1µg/Kg) was detected in Alexandria samples. (Table 3)

The highest amount of total aflatoxins found in Asyut samples were compatible with presence of *Aspergillus* as common fungi in this respect, also that means most of those strains were aflatoxin producer as recorded in Table(3). According to meteorological data in Egypt that recorded by Egyptian Metrological Authority, RH values and rainfall measuring in Alexandria were the highest in governorates investigated here, in Asyut ; the dew point was the highest value compared to other governorates. This information could explain the reason of highly aflatoxins levels that measured in samples of Asyut and Alexandria. Total aflatoxins in Giza found to be the lowest levels allover governorates, the lowest levels of aflatoxin B_1 were recorded in Cairo region followed by Giza. Referring to meteorological parameters data, this area (Cairo and Giza) had a dry weather.

Other mycotoxins (ochratoxin A , fumonisin B1, and zearalenone) were determined in maize samples by using HPLC techniques for 2012 samples(Data are presented in Table 4). Fumonisin B_1 ranged from 59µg/ Kg

(Giza samples) to 1455µg/Kg (Dakahlia samples). The highest amount of ochratoxin A was found in Alexandria samples (9.88µg/Kg) followed by Kafr El Sheykh samples (8.21µg/kg), while no ochratoxin A was detected in Giza, Sharkia, and Sohag. The highest amount of zearalenone was recorded in Kafr El Sheykh samples (1.67µg/Kg), no zearalenone was detected in Cairo, Dakahlia, Giza, and Sohag samples (Table 4).

According to samples of 2013 Fumonisin B_1 ranged from 170µg/ Kg (Giza samples) to 1915µg/Kg (Dakahlia samples). The highest amount of ochratoxin A was found in Alexandria samples (14.9µg/Kg), while no ochratoxin A was detected in Giza, Sharkia, and Sohag. The highest amount of zearalenone was recorded in Gharbia samples (3.5µg/Kg), no zearalenone was detected in Cairo and Dakahlia samples (Table 4).

Tables (3): Aflatoxins levels in maize samples from various Egyptian regions in 2013

Aflatoxins Levels / Governorates	Total Aflatoxins µg/ Kg	Aflatoxin B_1 µg/ Kg	Aflatoxin B_2 µg/ Kg	Aflatoxin G_1 µg/ Kg	Aflatoxin G_2 µg/ Kg
Alexandria	29.1	15.9± 0.22	4.2±0.32	7.9± 0.37	1.1±0.0
Asyut	34.9	21.8±1.25	8.1± 0.37	4.2±0.45	0.8±0.2
Behira	22.3	13.6±1.25	4.9±0.32	3.2±0.86	0.6±0.25
Cairo	17.5	9.4±0.75	2.3± 0.6	5.1±0.25	0.7±0.2
Dakahlia	21.9	16.2±0.4	3.7±0.37	1.6±0.31	0.4±0.12
Gharbia	20.3	16.9±0.37	1.7±0.25	1.3±0.2	0.4±0.0
Giza	14	11.3±1.68	2.7± 0.68	ND	ND
Kafr El Sheykh	20	14.8±0.89	4.1±0.82	1.1±0.24	ND
Sharkia	24.7	18.4± 0.52	4.2± 0.2	2.1±0.25	ND
Sohag	22.2	14.5± 0.73	7.4± 0.45	ND	0.3±0.2
Average	22.69	15.28±0.81	4.33±0.44	3.31±0.29	0.61±0.01

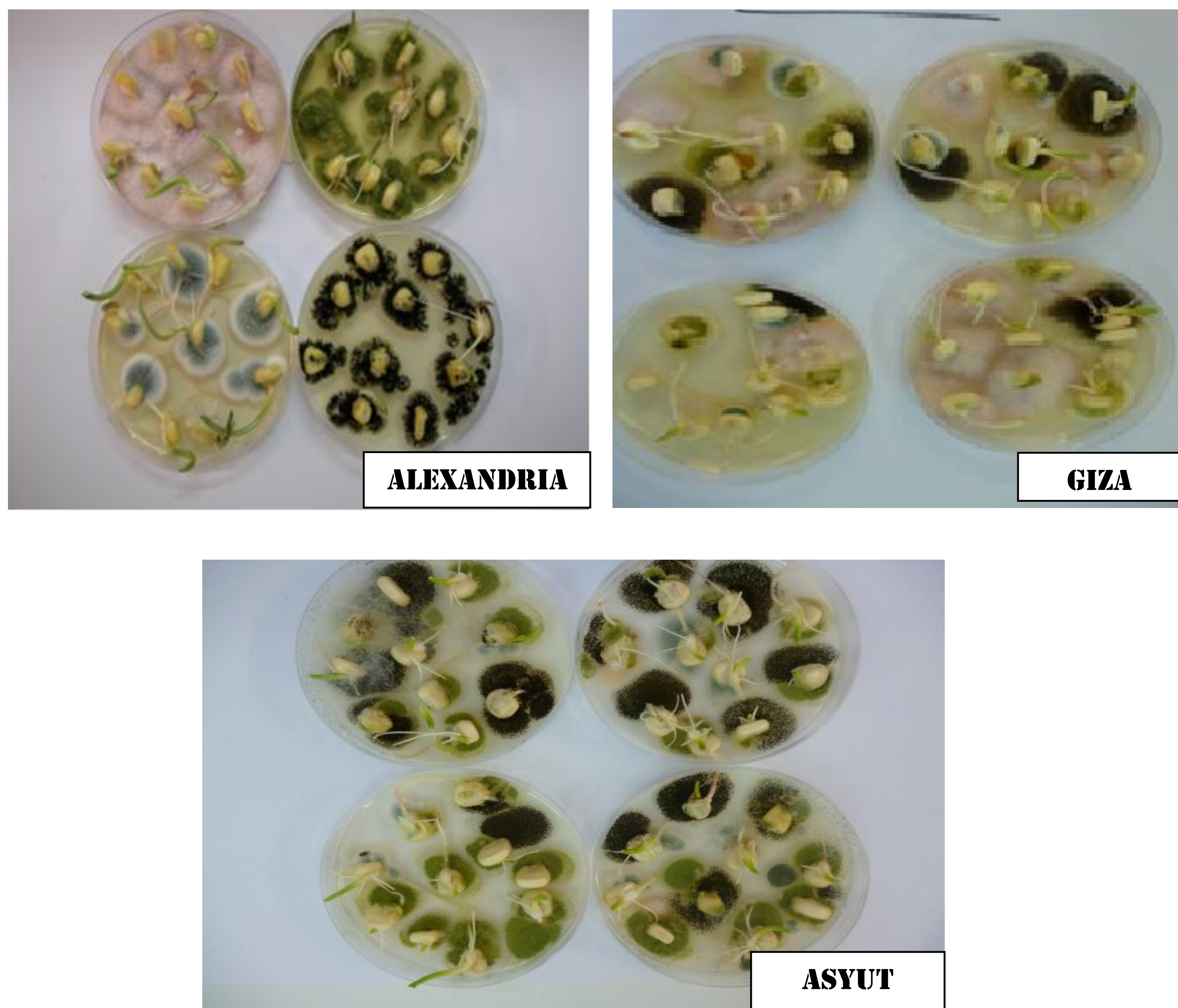
- Data are expressing as means ± SD.
- ND = not detected , LSD = 2.282

Tables (4): Mycotoxins levels in maize samples from various Egyptian regions in 2013

Governor-ates	Mean of positive samples µg/kg					
	Fumonisin B_1 (2012)	Fumonisin B_1 (2013)	Ochratoxin A (2012)	Ochratoxin A (2013)	Zearalenone (2012)	Zearalenone (2013)
Alexandria	876±1.79	1285±2.82	9.88±1.2	14.9±0.88	2.4±1.48	3±0.2
Asyut	161±2.4	273±2.14	5.7±0.67	7.15±0.64	1.03±0.66	2.1±0.41
Behira	729±3.36	1390±3.1	4.95±0.71	6.50±0.51	1.4±1.1	2.8±0.37
Cairo	1214±2.77	1545±2.4	2.21±0.54	2.75±0.37	ND	ND
Dakahlia	1455±2.22	1915±1.7	1.14±0.25	4.30±0.44	ND	ND
Gharbia	656±3.14	1125±1.4	1.14±0.41	1.23±0.58	1.2 ±0.48	3.5±0.2
Giza	59±1.98	170±0.86	ND	ND	ND	1.5±0.17
Kafr El Sheykh	789±2.64	1185±1.97	8.21±1.54	11.2±0.26	1. 67±0.37	1.3±0.25
Sharkia	343±2.88	752±2.1	ND	ND	1.44±0.55	3.2±0.41
Sohag	67±1.55	180±1.2	ND	ND	ND	0.8±0.2
Average	497±2.94	982±1.96	3.32±0.53	6.86±0.53	1.01±0.46	1.82±0.22

- Data are expressing as means ± SD.
- ND = not detected , LSD = 3.641

Figure (1): Fungal growth around maize embeded in PDAC medium representing three governorates at North, Middle, as well as Upper Egypt.



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