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# Research Article
# Mycotoxigenic Fungi and Mycotoxins in Egyptian Barley under Climate Changes

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## Abstract

**Background:** Barley was planted in more than hundred countries worldwide in 2013. The world output in 1974 was around 148 million tons, ever after, there has been a trivial come down in the produced amount of barley worldwide. Mycotoxins are one of the most important groups of anti-nutritional substances found in feed. **Materials and Methods:** Thirty barley feeding samples and 12 of Human Consuming (HC) samples were collected from 6 regions, each region was represented by a withdrawal of five representative samples directly after harvesting from the major storage locations within governorates under study. **Results:** Total Fungal Count (TFC) ratios varied from 31-83% on 2014 to 54-92% on 2015 in feed sample. *Aspergillus* sp. was the dominant fungus in 2 years of study for feed and HC. In 2014, total aflatoxins ranged from 11.6-26.4 and 11.3-23.2 μg $kg^{-1}$ for feed and HC samples, respectively. The $AFB_1$ in feed samples was between 9.5-17.9 and 7.9-18.7 μg $kg^{-1}$ on 2014 and 2015, respectively. For ochratoxin A, levels ranged from 0.17-0.55 and 0.3-2.1 μg $kg^{-1}$ for feed samples on 2014 and 2015, respectively. Zearalenone maximum levels were 3.4 and 1.6 μg $kg^{-1}$ for 2014 and 2015, respectively. **Conclusion:** Climate changes were a great factor that had impacts on mycotoxigenic fungal growth, fungal count, toxin type and its amount, that impact had reflected results appeared in food safety and food security.

Key words: Aflatoxins, *Aspergillus*, ochratoxin A, zearalenone, climate effects, food safety

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Barley belongs to the grass family, it is also self-fertile, the diploid kind in chromosomes. The wild predecessors of housebroken barley are considerable in woodlands and grasslands during fructify Crescent area of Asia and North Africa and is abundant in confused homeland, waysides and groves. Outgoing that area, overland barley is least widespread and may often found at confused homeland. One of the most useful benefits of barley was its uses for depreciation the ratio of sugar in the blood, lowering blood pressure and cholesterol, while also uses for encouraging weight loss. Again barley is used for digestive malady inclusive stomach pain, diarrhea and inflammatory bowel conditions, investigated by Ammari *et al.*[1].

According to Behall *et al.*[2] and McIntosh *et al.*[3], fibers of barley may depress cholesterol and blood pressure in human suffering of high cholesterol. Barley might also decrease sugar ratio and insulin levels in blood[4]. Barley appears to linger stomach discharge time. Such could support hold sugar ratio stable in blood and inspire the feeling of being full, that may assist to cog appetite. Several mankind are used barley for rising power and prospect. Other benefits include cancer prohibition and remediation of a lung troubles named bronchitis. Barley is utilized in curing boils of skin. For food, barley is applied as a provenance of carbohydrates, vitamins, proteins and fatty acids. In industrialization, barley is utilized as a cereals food, also as a natural sweetener, finally it may use as an ingredient in alcoholic beverages making.

Mycotoxins are secondary metabolites of fungi by low molecular and organic characters and they are toxic to most organisms. These substances are produced by fungal mycelium but they can also be included in spores[5]. Mycotoxin considered as fungal metabolites that may contaminate food and feed chains that were a purpose of toxic effects in higher creatures that consumed impure commodities and impact on barley competitive production on either domestic or export markets. Barley is one of the most common sheep feed commodities. It is also predisposed to mycotoxins occurrence particularly aflatoxins, deoxinivalenol and zearalenol causing harmfulness, especially in animal reproduction. The exceeding critical levels along with low housing conditions in animal hygiene, mycotoxicoses signs can occur to farm animals. Mycotoxins are a public health worry for Egyptian populations. Grains, crops, oregano and animal products are the master provenance of Egyptian mycotoxins[6]. The dose and their duration action are the decisive factors for the overall toxic effects. Higher toxic effects are caused by synergistic effects of multiple mycotoxins in the feed[7]. The changes of the occurrence of mycotoxins in stored commodities can occur during stored feed.

Toxigenic mold species are at most performed by *Penicillium*, *Aspergillus* and *Fusarium*, however, *Alternaria* among the others is important because it also considered food/feed contaminant and plant pathogens. Numerous *Aspergillus* and *Penicillium* species are recognized as mycotoxins producer. Other toxigenic fungi considerably grown on cereals are *Fusarium*, *Alternaria*, *Trichoderma*, *Chaetomium*, *Acremonium* and *Paecilomyces*[8]. Mycotoxins are minor metabolites generated by fungi that might besmear food or feeds even raw materials used in manufacture them. One of the most popular and widespread mycotoxin is aflatoxin $B_1$ ($AFB_1$). Its name came from originally its produced by *Aspergillus flavus* [9], modernly recognized to generated by other genera of *Aspergillus*. The $AFB_1$ is generated by *Aspergillus flavus*, though it might also create by *Aspergillus terreus* moreover *Aspergillus oryzae*. The $AFB_1$ is considered as carcinogenic, most toxic and most predominant of the several aflatoxins. Mostly, mycotoxins were involved as occasional agents of several animal and human health disorders[10]. Jointly toxigenic fungi with mycotoxins its produce are tolerable problems of both health and economic viewpoint.

Mycotoxin production by fungi in stored cereals, subsequently actually creating a health problem in animals and human. In a phyto-hygienic-toxicological region, the paramount interest was forced to aflatoxins and trichothecenes to be studied. Their happening is often greater in the humid region or in years have rainy weather. Another hazard factor is the cropping system where previous crop, soil plowing and varietal resistance play the major role. Groth *et al.*[11], declared that the solitary efficient conservation was the plowing method that would lay organic matter residues-potential infection sources and high resistance of host variety under a layer of soils. Krauthausen *et al.*[12] were reported highest incidence of *Fusarium*, according to that there was the highest content of mycotoxins. This study aims to identify the extent of the actual contamination of barley grain by fungi producing-toxin under the conditions of the Egyptian environment, along with estimating the quantity and quality of mycotoxin located on that grain under the current climatic conditions. Also, it aims to compare the effect of different storage places on the quality of fungi that grow on stored grain as well as its impact on the amount of mycotoxin produced.

## MATERIALS AND METHODS

**Apparatus:** Stoppard conical flask, measuring cylinders 25, 50 and 250 mL, chromatography columns 25×300 mm length, high speed blender, buchner funnel with Whatman No. 1 filter paper, wrist action shaker, rotary evaporator, UV light chamber, micropipette 5-100 μL, adjustable and borosilicate-screw cap vials lined with teflon.

**Medium preparation:** Forty one grams of commercially produced Potato Dextrose Agar (PDA) was dissolved in 1 L (1000 mL) of distilled water and allowed to homogenize in a water bath. The medium was later sterilized by autoclaving in and autoclave at 121°C for 15 min. Chloramphenicol/tetracycline (0.1/0.05 mg) was added to the medium, before pouring the melted medium into sterile petri plates and cool at room temperature to solidify.

**Mycological analysis:** Thirty barley grain samples for feeding usage collected from six regions, each region was represented as governorate (five samples collected from different locations in governorate then mixed well to have a representative sample), 12 barley samples for human consuming also were collected (two samples for each governorates to made representative sample). The regions that samples collected from were North, Northwest, middle, middle of Delta, East and upper Egypt, representative as Alexandria, Matrouh, Cairo, Gharbia, Ismailia and Assiut respectively. All samples were used for isolation and detection of seed-borne fungi. Fungi were isolated and cultured according to the method described by Singh and Smith[13].

Ten grams of grain samples were used after being surface-sterilized (using 2% sodium hypochlorite solution and washed 3 times with sterile distilled water). Ten grains were placed randomly on the surface of petri dishes containing Potato Dextrose Agar with Chloramphenicol (PDAC) in triplicate. Plated grains were incubated at 25±2°C and examined daily for 5 days, after which the colonies developing from the grains were counted. Isolated fungi were purified either by single spore or hyphen tip methods and then transferred to slanted PDA. Isolates identification was carried out based on morphological and microscopic characteristics in the Mycological Laboratory, Alexandria University, Egypt.

### Determination of mycotoxin on barley

### Determination of total aflatoxins

**Extraction:** Fifty grams of barley sample were placed into ultra-turrax and then 100 mL of 60% acetonitrile/water (v/v) were added. The mixture was stirred for 2 min at high speed. The extract was filtered through a Whatman No. 3 filter paper and then through a microfiber filter.

**Preparation of standards for aflatoxin:** For aflatoxin standards received as dry films: Volume of benzene-acetonitrile (98+2) was added to container of dry aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$, calculated to give a concentration of 8-10 μg $mL^{-1}$. Label statement of aflatoxin weight was used as a guide. Solution was vigorously agitated for 1 min on vortex shaker and transferred without rinsing to convenient sized glass flask.

**Clean-up by immunoaffinity chromatography:** Aflatest immunoaffinity columns (Vicam column®) were used for this step as follow, 2 mL of the final extract was diluted with 48 mL of Phosphate Buffered Saline (PBS, pH 7.3) to give a solvent concentration of 2.5%. The mixture was allowed to pass through column by gravity or at a flow rate of 5 mL $min^{-1}$. The column was then washed with 20 mL of PBS. The elution of aflatoxins was done with 1.5 mL of methanol and 1.5 mL of pure water.

**HPLC chromatography:** Ten microliters of the samples were injected into the HPLC column heated to 40°C. The mobile phase was water:methanol solution (60:40, v/v). To 1 L of mobile phase were added 119 mg of potassium bromide and 350 μL of 4 M nitric acid. The flow rate was 1 mL $min^{-1}$. For fluorescent detection of $AFB_1$ (and also, $AFB_2$) the excitation wavelength was 362 nm and the emission wavelength was 425 nm.

### Determination of ochratoxin A

**Ochratoxin A (OTA) standard:** About 50 μg $mL^{-1}$ (in benzene/acetic acid, 99:1) was purchased from Sigma (Sigma-Aldrich, Bellefonte, USA). A stock solution of about 1 μg $L^{-1}$ was prepared by evaporation of a standard base solution and dissolution in 50 mL of a filtered LC mobile phase. Working standard solutions were prepared by appropriate dilution of this solution with the LC mobile phase. Acetonitrile and methanol (both LC grade), hexane, formic acid and acetic acid were from Merck (Darmstadt, Germany). Phosphoric acid (85%, analytical grade) was from Lachema (Neratovice, Czech Republic). Glass-microfiber filters (GF/A) were from Whatman (Maidstone, UK). Econofilters of regenerated cellulose (0.45 μm) were from agilent (Germany). Ochratest® immunoaffinity columns were from Vicam® a waters business. (Milford, MA 01757 USA).

About 1 kg of corn sample was finely ground by a model 2A Romer mill (Romer, Union, MO, USA) and homogenized. Subsamples (about 100 g) were taken and stored at -20°C until analysis. Ground sample (20 g) were weighed into a blender jar, 2 g KCl added and extracted with 50 mL acetonitrile:water (90:10, v/v) by blending at high speed for 2 min (Sorvall omnimixer). The extract was filtered through filter paper and 10 mL filtrate was collected and mixed with 90 mL distilled water. The diluted extract was filtered through Whatman GF/A glass microfibre filter and the filtrate collected. Ten milliliters volume of diluted extract (equivalent to 0.4 g sample) was passed through Ochratest® immunoaffinity columns were from Vicam®, OTA was then eluted with 2 mL methanol and collected in a clean vial. The eluted extract was evaporated under nitrogen stream at ~50°C and reconstituted with the HPLC mobile phase.

A mixture of acetonitrile and 2% acetic acid (60:40, v/v) at a 1 mL $min^{-1}$ flow-rate was used as the mobile phase. Quantification of OTA was performed by measuring its peak area with the aid of a calibration curve calculated from standard solutions. Excitation and emission wavelengths were 333 and 480 nm, respectively.

**Clean-up with an immunoaffinity column:** Ten grams of a maize sample was extracted with 40 mL of methanol/water mixture (80:20, v/v) in a blender jar at high speed for 1 min. The extract was filtered through a glass-microfiber filter and an aliquot of 4 mL was added to 46 mL of PBS buffer (pH 7.2). The diluted extract was passed through an Ochratest® column. A gentle vacuum or overpressure can be used in all steps involving passage of liquid through the column. The column was washed with 10 mL of distilled water and carefully dried. The OTA was eluted at least 2 times with 1 mL of methanol. The combined solutions were then evaporated to dryness under a gentle stream of nitrogen. The residue was resolved to 500 µL then 10 µL was injected into the HPLC system.

**Zearalenone (ZEA)**

**Sample preparation and immunoaffinity clean-up:** About 1 kg of corn sample was finely ground by a model 2A Romer mill (Romer, Union, MO, USA) and homogenized. Subsamples (about 100 g) were taken and stored at-20°C until analysis. Ground sample (20 g) were weighed into a blender jar, 2 g KCl added and extracted with 50 mL acetonitrile:water (90:10, v/v) by blending at high speed for 2 min (Sorvall omnimixer). The extract was filtered through filter paper and 10 mL filtrate was collected and mixed with 90 mL distilled water. The diluted extract was filtered through Whatman GF/A glass microfiber filter and the filtrate collected. Ten milliliters volume of diluted extract (equivalent to 0.4 g sample) was passed through the ZearalaTest immunoaffinity column at a flow-rate of about 1 drop/s, followed by 235 mL distilled water at 1-2 drops/s flow-rate. Zearalenone was then eluted with 1.5 mL methanol and collected in a clean vial. The eluted extract was evaporated under nitrogen stream at ~50°C and reconstituted with the HPLC mobile phase.

**HPLC determination and confirmation of ZEA:** One hundred milliliters of reconstituted extract (equivalent to 0.16 g of sample) was injected into ZEA at levels of 0.1, 0.5, 1.0, 2.0 and 4.0 and chromatographic apparatus by full loop injection system. The mobile phase consisted of a mixture of acetonitrile: water: methanol (46:46:8, v/v) eluted at a flow-rate of 1.0 mL $min^{-1}$. Quantification of ZEA was performed by measuring peak areas at ZEA retention time and comparing them with the relevant calibration curve. The identity of ZEA was confirmed in all positive samples by injecting sequentially sample extracts using 274 and 236 nm excitation wavelengths (440 nm emission wavelength) and comparing the peak area ratio (236/274 nm) with that of zearalenone standard.

## RESULTS AND DISCUSSION

In comparing of metrological data in Egypt in last 30 years, there were great changes and this period had the 10 hottest years, these changes in climate reflecting on the changes in types and amounts of mycotoxins appeared on food crops. In these results, the present data was reviewed in comparing with the past one to explain the climate changes effects on food safety and food security.

United Nation Commissioned International Panel on Climate Change (IPCC) issued the fourth report that was published on November, 2007 by Van der Fels-Klerx *et al.*[14]. This report highlighted on the generic scientific assent that climate of the world was changed quickly by high averages in the past years. That report refers to several climatic changes are imminent all over the world, for instance, the predictable rising in the global surface temperatures and sea levels rises, as well, changes in precipitation. Influence of this shift on numerous aspects of human and animal health and revival is a subject vastly discussed. Nevertheless, the outcome of climate change for food system security, that included all the phases from farm to fork was received a weakly concern contrast to other human and animal health matters.

Table 1: Fungi isolated (on PDAC agar medium) from post-harvest barley area collected from different Egyptian governorates season 2014

| Governorates | Infection (%) | Total fungi colonies | Number and type of isolated fungi (CFU mL$^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|
| | | | *Aspergillus* | *Fusarium* | *Penicillium* | *Alternaria* | *Rhizopus* |
| **Marketing samples (Used for feeding)** | | | | | | | |
| Alexandria | 83 | 65 | 27±4.2 | 11±2.1 | 22±1.7 | 3±1.4 | 2±2.2 |
| Cairo | 47 | 59 | 22±2.5 | 16±3.1 | 19±4.1 | - | 2±1.7 |
| Matrouh | 31 | 27 | 13±2.2 | 4±1.6 | 10±1.3 | - | - |
| Gharbia | 64 | 77 | 31±2.6 | 2±3.3 | 27±3.2 | 6±1.7 | 11±5.1 |
| Ismailia | 49 | 53 | 11±3.7 | 5±3.8 | 25±4.3 | 10±5.4 | 2±2.6 |
| Assiut | 71 | 62 | 29±3.5 | 4±2.1 | 26±2.3 | - | 3±1.7 |
| **Samples from herbal shop (Used for human consumptions)** | | | | | | | |
| Alexandria | 8 | 14 | 9±3.4 | 4±3.7 | 1±2.1 | 2±1.5 | - |
| Cairo | 15 | 29 | 16±8.0 | 5±2.1 | 8±1.53 | - | - |
| Matrouh | 2 | 8 | 5±1.2 | - | 2±1.7 | 1±2.3 | - |
| Gharbia | 11 | 18 | 11±2.6 | 2±2.2 | 5±1.9 | - | - |
| Ismailia | 3 | 11 | 7±2.5 | 2±3.4 | 2±1.4 | - | - |
| Assiut | 16 | 31 | 17±2.4 | - | 11±2.2 | - | 3±2.8 |

Data are expressing as Mean±SD, LSD: 3.19

With special focus, the aforesaid IPCC report visualizes a fundamental come down in African crop output consequent to climate change. A comparatively novel object is symbolized by the hazards of inverse effects of climate change on food safety. Presently, food safety susceptibility to climate change is rarely deemed both at European and international scale. Food safety and food security are notwithstanding concerning in order to inadmissible standards of food safety that recognize food nonsuitable for human consuming with deterioration in food security, probably forcing world to consume foods that are contaminated, otherwise possibly decrease bio-accessibility of important minerals as well as nutrients. The potential opponent effect of climate change on food safety could therefore as well be tacit, inclusive in the objectives of United Nation agreement.

Predictions indicate that Africa with a special focus on North and East of Africa areas will face several effects instead of climate change and its effects on agriculture and food safety differ for the various geographical areas. These changes will have a violent effect on the farming system such as changes in crops seasons and amendment in the land and crop yields, altars of soil quality (like increases in soil mineral losses, change in an ecosystem of soil microorganisms). Other changes may be incurred, like alters in numbers and types of plant pests, as well as the stinging insects, also many animal diseases affecting domestic animals and human consumers of plant and animal products.

In Table 1, the total fungal infection ratio of toxigenic fungi were varied from 83% as the highest infection in Alexandria sample used for feed on 2014 to 31% as the lowest infection in Matrouh feed sample on 2014. These values was changed to 92% as the highest infection in Alexandria feed sample on 2015 and 54% as the lowest infection in Matrouh feed sample on 2015. For Total Fungi Colony (TFC), barley feed sample in the year 2014, Gharbia and Matrouh were the highest and the lowest governorate in TFC by 77 and 27 TFC, respectively. In feed samples of 2015, Gharbia was the highest governorate in TFC by 98 fungal colonies and Ismailia was the lowest governorate by 67 fungal colonies. But in barley samples for human consumption, Assiut and Cairo were the highest governorates in TFC by 31 and 29 fungal colonies for years 2014 and 2015, respectively. Otherwise, Ismaillia was the lowest governorate by 11 and 12 fungal colonies in 2014 and 2015, respectively.

The dominant fungus in the presence of barley samples was *Aspergillus* sp. in the 2 years of the study. *Aspergillus* sp. was also the dominant fungi on barley samples used for human consumptions but with low rates compared to feed samples. For *Aspergillus* sp. of barley feed samples, in the first year Assiut was the highest governorate in fungal count and Ismailia was the lowest one, otherwise in the 2nd year Alexandria and Gharbia were higher governorate in *Aspergillus* fungal infection and Matrouh was the lowest one (Table 1, 2).

For *Aspergillus* sp. of barley used for human consumptions samples, in the first year of the experiment Cairo was the highest governorate in fungal count and Ismailia was the lowest one, otherwise in the 2nd year Alexandria and Gharbia were higher governorate in *Aspergillus* fungal infection and Matrouh was the lowest one (Table 1, 2).

In comparison to the previous study, Abdel-Kader *et al.*[15] estimated the fungi of 40 samples of barley grains collected from upper Egypt. In this study two varieties of *Aspergillus nidulans* were identified, along with one variety of *Aspergillus flavus* and one variety of *Penicillium cyclopium*. The common frequent species in this survey varied as

Table 2: Fungi isolated (on PDAC agar medium) from post-harvest barley area collected from different Egyptian governorates season 2015

| | | | Number and type of isolated fungi (CFU $mL^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|
| Governorates | Infection (%) | Total fungi colonies | *Aspergillus* | *Fusarium* | *Penicillium* | *Alternaria* | *Rhizopus* |
| **Marketing samples (Used for feeding)** | | | | | | | |
| Alexandria | 92 | 86 | 37±6.1 | 21±3.0 | 28±3.5 | 3±2.1 | 4±3.1 |
| Cairo | 61 | 77 | 29±8.0 | 19±5.1 | 25±1.53 | - | 6±4.7 |
| Matrouh | 54 | 68 | 24±7.02 | 18±4.2 | 24±3.61 | - | 5±1.5 |
| Gharbia | 72 | 98 | 37±6.7 | 24±14.1 | 29±2.52 | 4±2.5 | 8±4.5 |
| Ismailia | 80 | 67 | 26±2.5 | 14±2.2 | 17±2.52 | 7±3.8 | 7±4.58 |
| Assiut | 86 | 79 | 35±3.5 | 11±3.5 | 26±4.2 | - | 7±3.8 |
| **Samples from herbal shop (Used for human consumptions)** | | | | | | | |
| Alexandria | 11 | 22 | 13±2.1 | 2±2.5 | 6±1.5 | 1±2.1 | - |
| Cairo | 16 | 29 | 16±8.0 | 5±2.1 | 8±1.53 | - | - |
| Matrouh | 7 | 13 | 8±3.3 | 1±2.3 | 4±1.5 | - | - |
| Gharbia | 10 | 18 | 9±6.7 | 3±2.2 | 4±3.6 | - | 2±1.4 |
| Ismailia | 4 | 12 | 7±2.5 | 2±3.4 | 3±1.4 | - | - |
| Assiut | 9 | 17 | 15±3.5 | - | 2±1.7 | - | - |

Data are expressing as Mean±SD, LSD: 2.71

*Aspergillus*, *Penicillium*, *Rhizopus*, *Alternaria* and *Fusarium* were represented (as checked by the seed plate method) in 60.4, 10.2, 7.7, 3.7 and 5% of fungi total count, respectively. The most predominant fungi were *A. flavus*, *A. niger*, *A. sydowii*, *A. fumiqatus*, *F. moniliforme*, *F. solani*, *P. citrinum*, *P. notatum*, *F. oxysporum*, *A. alternata* and *R. stolonifer*[16]. Other study reported that, *Aspergillus* was the most predominant fungi and it appeared in 1% of the samples and also this fungi accustomed 40.8% of total fungi[17], they also declare that *Aspergillus* followed by *Penicillium* and *Fusarium* were the most widespread fungus in wheat, barley, sorghum and corn grains in Egypt.

In the study of Soliman[18], five cereals samples were collected from three regions in Egypt that were Daqahlia, Gharbia and Kafer El-Sheikh. The cereals samples of wheat, barley, rice, corn and sorghum were tested for mycotoxigenic fungal infection. That study recorded that *Alternaria*, *Aspergillus*, *Fusarium*, *Rhizopus* and *Penicillium* were isolated from cereals seeds, the occurrence of those fungi and its intensities was controlled by that study. All isolated fungi have been tested for toxin production in media and the results appeared positive.

In a state of barley seeds, the generality widespread fungi isolated was *Rhizopus* in Gharbia and Daqahlia, recording percentages of total count 46.6 and 47.0%, respectively. Whilst, *Aspergillus* widely predominant (53.5%) and exceedingly frequent (100%) on Kafer El-Sheikh barley seeds. *Fusarium* appeared as important fungi on barley, it comes ordered as the second fungus in dominance and recurrence of isolation from barley seeds collected from the three governorates. In all cases, *Alternaria* and *Penicillium* were least prevalent on barley seeds. *Aspergillus*, *Penicillium*, *Rhizopus* and *Fusarium* were found to be the general fungal flora on barley seeds collected from different regions all over the world[19,20].

*Penicillium* was the second most common fungus and was represented as 82.5% of the samples frequent, 11.4-27.7% of fungi total count on samples that used for human consumption and 21.3-34.4% of the total count of fungi on samples that used for animal feeding. Two genera were low occurrence namely *Rhizopus* and *Alternaria*. They occurred in 4.6-18.8 and 4.5-14.3% as *Alternaria* of the total count of fungi on samples that used for animal feeding and human consumption, respectively. For *Rhizopus* it's occurred as 3.1-14.2 and 9.2-11.1% of the total count of fungi on samples that used for animal feeding and human consumption, respectively.

With regard to barley for feeding use, 50% of samples in the 1st and 2nd years were free of *Alternaria* sp., infection. In barley samples that use for human consumption on 2014, about 66.7% was *Alternaria* free of the infection but on 2015 the ratio of *Alternaria* free samples were upgraded to 83.4% of samples. On the opposite side, for *Rhizopus* fungi infection on barley feeding samples it was found that 84% of samples on 2014 were infected, but on 2015 the ratio of infection was 100%. On barley that was use for human consumption, about 84% of samples were free of *Rhizopus* infection on 2014 and 2015.

In Fig. 1, the fungal growth of toxigenic fungi appeared in barley grains samples of some governorates that were plated on potato dextrose agar media with chloramphenicol (PDAC). In Fig. 1, different fungi species appeared on the plate count methods, Fig. 1a and c represented the barley human consumption samples and Fig. 1b and d represented the barley samples that were used for feeding.

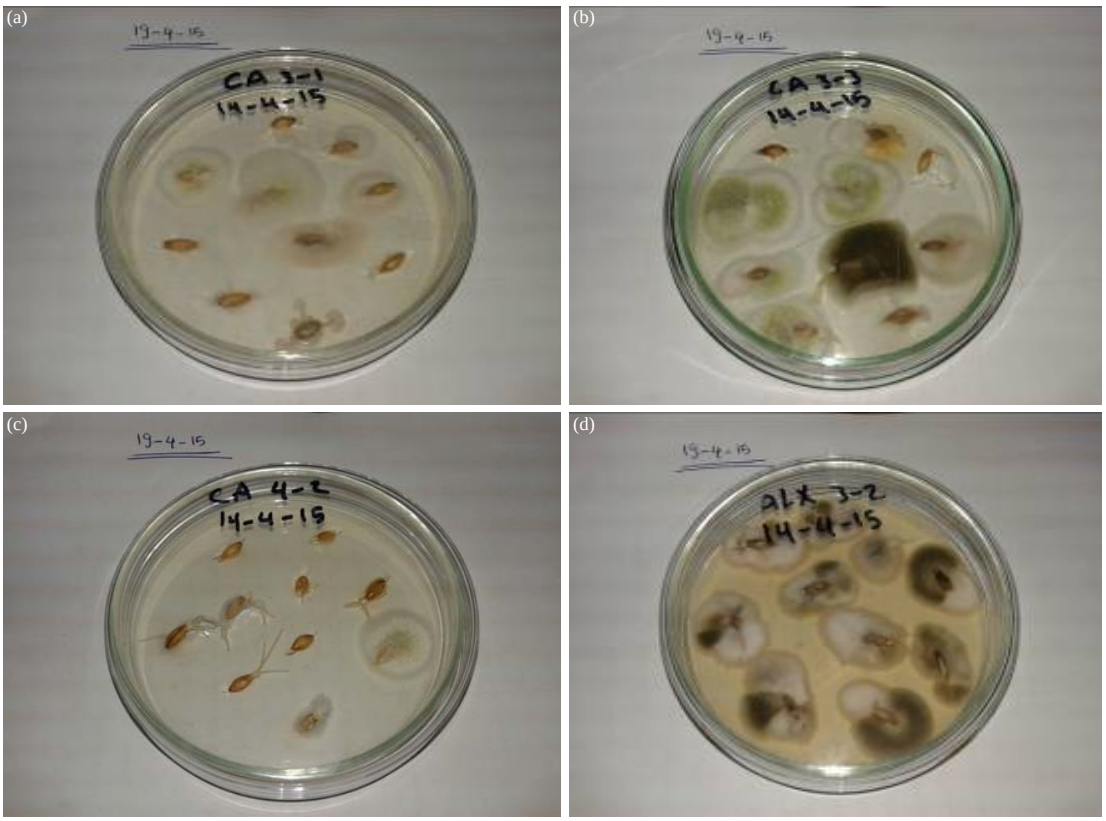


Fig. 1(a-d): Fungal growth of toxigenic fungi in barley grains samples of governorates plated on potato dextrose agar media with chloramphenicol (PDAC)

Table 3: Aflatoxins level in Barley samples from different Egyptian regions in 2014

| | Aflatoxins levels (μg kg$^{-1}$) | | | | |
|---|---|---|---|---|---|
| Governorates | Total aflatoxins | $AFB_1$ | $AFB_2$ | $AFG_1$ | $AFG_2$ |
| **Marketing samples (Used for feeding)** | | | | | |
| Alexandria | 16.60 | 13.40±1.41 | ND | 3.2±1.17 | ND |
| Cairo | 21.70 | 12.90±1.5 | 2.3±1.31 | 5.3±1.54 | 1.2±0.67 |
| Matrouh | 11.60 | 9.50±1.7 | ND | 2.1±1.6 | ND |
| Gharbia | 18.30 | 11.20±1.4 | 1.9±0.91 | 5.2±1.25 | ND |
| Ismailia | 16.30 | 10.30±2.4 | 1.2±1.37 | 3.7±0.31 | 1.1±0.12 |
| Assiut | 26.40 | 17.90±0.37 | 1.7±1.25 | 5.4±1.2 | 1.4±0.81 |
| **Herbal shop samples (Used for human consumption)** | | | | | |
| Alexandria | 11.30 | 7.90±2.1 | ND | 3.4±1.6 | ND |
| Cairo | 20.00 | 14.80±0.89 | 4.1±0.82 | 1.1±0.24 | ND |
| Matrouh | ND | ND | ND | ND | ND |
| Gharbia | 21.90 | 14.50±0.73 | ND | 7.4±1.45 | ND |
| Ismailia | 18.78 | 18.78±2.8 | ND | ND | ND |
| Assiut | 23.20 | 14.80±1.89 | 2.1±1.25 | 4.7±2.3 | 1.6±0.97 |

All Data are expressed as Mean±SD, ND: Not detected, LSD: 0.47

The total aflatoxins (AFs) in barley for feeding were ranged from 11.6-26.4 μg kg$^{-1}$ of 2014 samples, Assiut was the highest governorate in contaminated samples by AFs and Matrouh was the lowest one (Table 3). Otherwise, total AFs on 2015 barley samples use for feeding ranged from 17.5-34.9 μg kg$^{-1}$, the highest contaminated samples were found in Cairo governorate but the lowest levels of total AFs were found in Gharbia governorate (Table 4). It is worth mentioning that, the barley samples for human consumption were low levels than the samples that used for feeding (Table 3, 4). The levels of AFs in human consumption samples of 2014 were ranged from 11.3-23.2 μg kg$^{-1}$ and it was not detected in samples from Matrouh governorate (Table 3). On 2015, the total AFs was determined in all governorates

Table 4: Aflatoxins level in Barley samples from different Egyptian regions in 2015

| | Aflatoxins levels (μg kg$^{-1}$) | | | | |
|---|---|---|---|---|---|
| Governorates | Total aflatoxins | $AFB_1$ | $AFB_2$ | $AFG_1$ | $AFG_2$ |
| **Marketing samples (Used for feeding)** | | | | | |
| Alexandria | 29.10 | 15.9±0.22 | 4.2±0.32 | 7.9±0.37 | 1.1±0.0 |
| Cairo | 34.90 | 21.8±1.25 | 8.1±0.37 | 4.2±0.45 | 0.8±0.2 |
| Matrouh | 22.30 | 13.6±1.25 | 4.9±0.32 | 3.2±0.86 | 0.6±0.25 |
| Gharbia | 17.50 | 9.4±0.75 | 2.3±0.6 | 5.1±0.25 | 0.7±0.2 |
| Ismailia | 21.90 | 16.2±0.4 | 3.7±0.37 | 1.6±0.31 | 0.4±0.12 |
| Assiut | 20.30 | 16.9±0.37 | 1.7±0.25 | 1.3±0.2 | 0.4±0.0 |
| **Herbal shop samples (Used for human consumption)** | | | | | |
| Alexandria | 14.00 | 11.3±1.68 | 2.7±0.68 | ND | ND |
| Cairo | 19.00 | 11.8±0.67 | 2.1±0.57 | 5.1±0.24 | ND |
| Matrouh | 13.50 | 6.4±0.52 | ND | 7.1±2.5 | ND |
| Gharbia | 22.20 | 11.5±0.73 | 3.4±1.45 | ND | 1.3±0.2 |
| Ismailia | 22.69 | 9.2±0.81 | ND | 6.4±1.29 | ND |
| Assiut | 17.50 | 14.4±1.6 | 2.3±2.6 | 8.1±1.14 | 1.7±0.39 |

All Data are expressed as Mean±SD, ND: Not detected, LSD: 1.17

samples with no absent values, the levels were ranged from 13.5 μg kg$^{-1}$ that was determined in Matrouh as lowest governorate to 22.6 μg kg$^{-1}$ determined in Ismailia as highest governorate.

Notwithstanding that, Ghali *et al.*[21], collected randomly around 209 food samples during 2004 and 2005 from markets all over the Tunisian republic, samples were contained barley and its derivatives products, 25 samples of Tunisian barley grain samples were contaminated by aflatoxin $B_1$ ($AFB_1$), the levels of $AFB_1$ was ranged between 3.5-11.5 μg kg$^{-1}$ by mean levels±SD equal to 18.4±27.3 μg kg$^{-1}$. Anthony *et al.* [22], in a study entitled "Aflatoxin contaminated in foods and feeds: A special focus on Africa", reported that, there was generally low natural incidence of AFs in barley, also Maenetje and Dutton[17], recorded very low levels of AFs observation on barley from South Africa. Otherwise, according to the models that were made by the meteorological data authority which available through the website of the authority" http://www.ema.gov.eg/map and http://www.ema.gov.eg/productview/index/; the temperature, relative humidity, dew point and wind speed differed during the year 2015 than the values of the same factors in the previous years before 2015 and basically on previous studies, temperature and humidity were among the factors which have relative influence on fungi growth and therefore, reflected its effects on the production of mycotoxins.

A recent experiment that was executed to investigate the effect of temperature and water activity on aflatoxin biosynthesis and its production were examined by Medina *et al.*[23] recorded that, there was a direct connection among structural genes under several environmental conditions and relative expression key regulatory, that relation correlate straight to aflatoxin $B_1$ production. There was a model that developed to merge relative expression 10 biosynthetic genes in the pathway, growth and a atoxin $B_1$ production that was supported under highly water stress conditions and temperature. The definitive proposal was, while those favorable environmental conditions have a slight effect on growth, it has a considerable impact on gene expression and could be encouraged aflatoxin $B_1$ production significantly. Whereas, the individual factors had an impact alone, the joint effect of those environmental conditions that had the great impact on mycotoxin production.

In Table 5, the levels of the two mycotoxins (Ochratoxin A and zearalenone) on the seasons of 2014 and 2015 were recorded in six governorates, either for feed sample or human consumption samples that were brought from herbal shops. For ochratoxin A, in all samples of governorates the levels of the toxin in 2015 were more than their counterparts. Otherwise, zearalenone values were oscillated between increases and decreases throw the 2 years.

For OTA, levels were ranged from 0.17-0.55 μg kg$^{-1}$ for feed samples on 2014 and 0.3-2.1 μg kg$^{-1}$ for feed samples on 2015. In samples for human consumption, the maximum level was 0.34 and 2.1 μg kg$^{-1}$ in Alexandria on 2014 and 2015. Ochratoxin A wasn't detected in three governorates on 2014 that were Cairo, Matrouh and Ismailia and on 2015 OTA wasn't detected in Matrouh governorate. Otherwise, the maximum levels of ZEA were 3.4 and 1.6 μg kg$^{-1}$ for 2014 and 2015, respectively. The ZEA hasn't detected also three governorates that were Alexandria, Matrouh and Ismailia on 2014 samples but it was not detected in three governorates Cairo, Matrouh and Ismailia on 2015.

Table 5: Ochratoxin A and zearalenone level in barley samples from different Egyptian regions in 2014 and 2015

| | Mycotoxins levels (μg kg$^{-1}$) | | | |
|---|---|---|---|---|
| | OTA | | ZEA | |
| Governorates | 2014 | 2015 | 2014 | 2015 |
| **Marketing samples (Used for feeding)** | | | | |
| Alexandria | 0.47±0.14 | 2.10±0.11 | 7.50±0.55 | 4.50±0.42 |
| Cairo | 0.28±0.22 | 0.62±0.31 | 2.23±0.34 | 1.10±0.44 |
| Matrouh | 0.17±0.11 | 0.80±0.24 | 0.66±0.25 | 0.27±0.62 |
| Gharbia | 0.27±0.37 | 1.70±0.79 | 1.40±0.37 | 1.98±0.71 |
| Ismailia | 0.21±0.31 | 0.30±0.34 | 3.40±0.12 | 2.10±0.37 |
| Assiut | 0.55±0.19 | 0.97±0.42 | 1.54±0.81 | 0.97±0.25 |
| **Herbal shop samples (Used for human consumption)** | | | | |
| Alexandria | 0.34±0.12 | 2.10±0.11 | ND | 1.60±0.76 |
| Cairo | ND | 0.32±0.18 | 0.57±0.18 | ND |
| Matrouh | ND | ND | ND | ND |
| Gharbia | 0.27±0.37 | 0.97±0.43 | 3.40±1.45 | 1.10±0.39 |
| Ismailia | ND | 0.41±0.17 | ND | ND |
| Assiut | 0.16±0.2 | 0.77±0.12 | 0.99±0.6 | 0.27±0.34 |

All Data are expressed as Mean±SD, ND: Not detected, LSD: 0.023

Soliman[18] recorded that, *Fusarium* isolates from wheat seeds appeared to be non-toxigenic. While the toxigenic strains of *Fusarium* were isolated from barley and corn seeds collected from three provinces along with only one sample of rice from one province. Farber *et al.*[24] outstanding that, isolates of *Fusarium* from Canadian cereal seeds showed the ability of *F. moniliforme* and *F. subglutinans* to produce mycotoxins, while strains of *F. graminearum* for Western wheat did not produce toxin. Some *F. moniliform* isolated from barley seeds present significantly very altitude effectiveness to output mycotoxins.

## CONCLUSION

In the last decades, focusing on metrological data, many changes appeared in the global climate, this changes has been affects the safety of agriculture commodities which also effects on raw materials of human food. As changes in climate have impacts on ecosystem, it can reflect on the insects and mites which have a relation with mycotoxigenic producing-fungi. climate factor was also the environment factors that affecting on fungal growth and mycotoxin production; total fungal count, fungal type was differ depending on changes in climate parameters form year to the other. Notwithstanding, dominant fungi differ according to climate, that was reflecting as change in toxins type and amounts. As climate changes not likely from region to the other, changes were not likely either for fungal count, types, mycotoxins type and amounts.

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