第 49 卷 第 4 期
2022 年 4 月

湖南大学学报（自然科学版）
Journal of Hunan University (Natural Sciences)

Vol. 49 No. 4
April 2022

Open Access Article https://doi.org/10.55463/issn.1674-2974.49.4.36

Defining Mycotoxins Associated with Wheat Grains in Mosul Silo by ELISA

Hadeel A. Al-Ameri

Biology Department, College of Sciences, Mosul University, Mosul, Iraq

Abstract: The study aimed to identify fungi-contaminated with some varieties of wheat grains locals, as well as isolate fungi-contaminated with grains imported wheat in Silo of Mosul city, and estimate the level of mycotoxins ochratoxin, zearalenone, and aflatoxin in them by ELISA method. Results showed that the local wheat variety, Ebaa 99, showed the highest percentage of appearance in Aspergillus flavus, was 63.15%, while the lowest appearance of fungi was Mucor spp. With a percentage of 2.47%, the variety Rasheed showed the highest percentage of A. flavus, which was 49.12%, while the lowest appearance was of Rhizopus spp. With a percentage of 2.29%, As well as in the variety Al-Baraka A. flavus showed the highest percentage, which was 5.453%, and the lowest percentage was Cladosporium spp by 9.43%. The contaminated fungi differed from the imported varieties and the percentage of their appearance. In the Canadian variety, the highest appearance of A. flavus was 63.72%, and A. dematatus was the least appearance with 5.42%. The fungus A. flavus also appeared in the American variety, with the highest appearance of 43.17%. The least appearance was Fusarium oxysporum at 5.34%. In the Australian variety, the highest appearance was found in A. flavus, at 54.03%, and the fungus Fusarium spp. was the least appeared fungi, at 1.96%. Estimation of mycotoxins indicates that the aflatoxin amount in the variety Ebaa 99 samples was 34.55 ppm, while its amount of ochratoxin and zearalenone was 21.75 ppm and 0.23 ppm, respectively. Canadian variety grains had the lowest aflatoxin content, with the highest ochratoxin. Australian variety grains had the lowest content of aflatoxin, ochratoxin, and zearalenone amount 12.29 ppm, 0.82 ppm, and 11.51 ppm, respectively.

Keywords: Fusarium oxysporum, Aspergillus flavus, mycotoxins, aflatoxin, zearalenone.

通过酶联免疫吸附测定法确定摩苏尔筒仓中与小麦谷物相关的霉菌毒素

摘要：该研究旨在鉴定当地部分小麦谷物品种的真菌污染，以及分离受谷物污染的真菌摩苏尔市筒仓的进口小麦，并通过酶法估算其中的真菌毒素赭曲霉毒素、玉米赤霉烯酮和黄曲霉毒素水平。联免疫吸附测定法。结果表明，当地小麦品种埃巴99在黄曲霉中的出现率最高，为63.15%，而真菌的出现率最低的是毛霉属。以2.47%的百分比显示，品种拉希德的黄曲霉百分比最高，为49.12%，而出现率最低的是根霉属。百分比为2.29%，在品种祝福黄曲霉中百分比最高，为5.453%，最低百分比是枝孢菌属，为9.43%。受污染的真菌与进口品种及其出现的百分比不同。在加拿大品种中，黄曲霉的出现率最高，为63.72%，德马图斯的出现率最低，为5.42%。真菌黄曲霉也出现在美国品种中，最高出现率为43.17%。最少出现的是尖孢镰刀菌，占5.34%。在澳大利亚品种中，黄曲霉的出现率最高，为54.03%，而真菌镰刀菌。是最少出现的真菌，为1.96%。霉菌毒素的估算表明，埃巴99品种样品中的黄曲霉毒素含量为34.55百万分之几，而其赭曲霉毒素和玉米赤霉烯酮的含量分别为21.75百万分之几和0.23ppm。加拿大品种谷物的黄曲霉毒素含量最低，赭曲霉毒素含量最高。澳大利亚品种谷物的黄曲霉毒素、赭曲霉毒素和玉米赤霉烯酮含量最低，分别为12.29百万分之几、0.82百万分之几和 11.51百万分之几。

关键词：尖孢镰刀菌、黄曲霉、霉菌毒素、黄曲霉毒素、玉米赤霉烯酮。

Received: February 20, 2022 / Revised: March 19, 2022 / Accepted: March 22, 2022 / Published: April 30, 2022
About the author: Hadeel A. Al-Ameri, Biology Department, College of Sciences, Mosul University, Mosul, Iraq Corresponding author Hadeel A. Al-Ameri, hadeelahmed.mu@gmail.com

1. Introduction

Fungi contaminate large numbers of crops worldwide, including wheat, barley, rice, yellow corn, cotton seeds, pistachio, and many derivative and manufactured products included in human and animal food [1]. Wheat is one of the oldest crops known to man, and it is one of the most important crops in terms of food and economy; and more than a third of the world's population depends on it as a staple food [2]. Wheat grains are exposed to many pathogens, whether in the field or during transportation or storage, especially fungi, as they cause damage to the seeds during the storage period. The damages that accompany the storage and transportation conditions that affect the seed coat may lead to the formation of cracks from which the fungi enter the seed's body and work to grow inside rapidly. The fungi carried by wheat grains are among the most important pathogens because they reduce the rate of germination and poor quality. Their ability to produce compounds toxic to humans and animals that cause allergies and cancerous diseases called mycotoxins [3]. These fungi are divided into Field fungi and Storage fungi. Field fungi invade seeds before harvest while the crop is still in the field. While field fungi may affect the appearance and quality of seeds or grains, damage from field fungi usually occurs before harvest can be detected by routine inspection and does not continue over storage if the grain is stored at the appropriate moisture content temperature. Most field fungi are more prevalent when rainfall is higher than normal during grain packing and harvesting. Field fungal invasion may be more severe where insects, birds, or hail have damaged the crop. Common field fungi in corn in Missouri include Genera of Alternaria, Cladosporium, Aspergillus, Penicillium, Fusarium, and Gibberella [4].

Storage Fungi (also called storage molds) are fungi that invade grains or seeds during storage. Storage fungi are not usually found to any extent dangerous before harvest. However, small amounts of storage fungi spores may be present on grain entering storage or on spilled grain in harvesting, handling, and storage equipment or structures. Under improper storage conditions, this small amount of spores can rapidly increase, which leads to major problems. The development of storage fungi in stored grain is influenced by the content of moisture in stored grain, temperature of stored grain, condition of stored grain, the length of time the grain is stored, and the number of insects and moths in cereals [5]. Grain damage caused by storage fungi takes many forms, including biochemical changes. For example, there are contamination of oilseeds with fungi accelerating the segregation of fatty acids (rancidity) [6]; the color change of the embryo or the whole grain; its acquisition of an unpleasant taste [7]; reduced germination strength; heat and rotting of grains; loss of weight per grain; secretion of toxic substances to humans and animals known as mycotoxins, mainly during consumption of contaminated food [8].

The water content of the grain is one of the conditions predisposing to infection with fungi. It affects the quality and number of fungi that grow on it, as wheat, barley, and corn grains are capable of invading certain types of fungi. The number of mushroom colonies increases as the water content of the grain rises. Storage fungi grow on the water content of grains balanced with the degree of relative humidity of the atmosphere [9]. Therefore, there may be clear differences in the moisture content within a stack of stored grain. It is absurd to know the average moisture content of the mass of grains inside a silo if there is a focus or more in which the water content is much higher than the average. In the case of overheating and rotting, the problem of heterogeneity in moisture distribution in the grain mass remains in the silos unless there is a ventilation system [10].

Some types of Aspergillus fungus need to grow in wet grains to an optimum high temperature ranging between 35-40°C and at a moisture content of the grains between 15-16°C. However, most storage fungi grow very slowly in the cereal grains at 12-15°C; some grow at 5-8°C [3, 11]. Therefore, it was possible to keep wheat and corn grains uninfected after harvesting for two years at a temperature of 5°C; the grains retained their germination vitality by 100% and were completely free of storage fungi. However, suppose the grains were infected with fungi from stores, even to a moderate degree. In that case, they continue to grow at high temperatures the low temperature, even if it is at 0°C or less [8, 12, 13]. In addition, mechanical damage to grains represented by cracks, scratches, and fractures in the pod or seed coat of grain affects the incidence of storage fungi [12].

1.1. Aim of the Study

The stated problems are the contamination of wheat grains and food products with mycotoxins, which negatively affect human health and the occurrence of large economic losses, and because our country imports wheat from different origins that differ in the possibilities of controlling pathogenic pathogens. Therefore, the study aimed to isolate and identify fungi-contaminated varieties of local wheat grains and imported wheat in the silo of Mosul city and estimate the level of mycotoxins ochratoxin, zearalenone, and aflatoxin in them by the ELISA method.

2. Methods of Work and Materials

2.1. Collection of Samples

The samples of grains of wheat varieties were collected from Silo at Mosul City/ Iraq. These samples are submitted for examination in the quality control laboratory in the silo, which includes three varieties of local wheat grains (Ebaa 99, Al-Baraka, Rasheed) and three varieties of imported wheat grains, which are American, Canadian, and Australian. Randomly, 3 kg of each type were placed in polyethylene bags and sealed with holes for ventilation to avoid the death of the embryos. Then they were transferred to the laboratory for isolation.

2.2. Isolation

Surface sterilization of the seeds was carried out by immersing them in ethanol for 3 minutes and then washing them by placing them in distilled water for another 3 minutes and drying them using a No. 1 type filter paper. Whatman, 10 seeds of each variety were planted in a 9 cm diameter Petri dish container on medium Potato Dextrose Agar (PDA), with antibiotic Chloramphenicol, at a 0.05 mg/liter concentration, in three replications. The dishes were incubated upside down in an incubator at 28^{o}C for a week [14].

2.3. Identification

After the growth of the fungal cultures isolated from wheat grains from the varieties of study, they were examined microscopically by taking part in the fungal growth with a sterile inoculation needle and placing it on a glass slide containing a drop of Lactophenol. The sample was spread in the loading drop and then put on the cover of the slide and examined microscopically to identify the characteristics of mycelium and conidia, according to the approved classification keys [15-19].

2.4. Purification of Fungi by Single Spore Technique

By mixing a sample of 0.5 cm in diameter from the fungal colony with 10 ml of sterile distilled water and continuing the dilution until the number of spores ranged between 1 - 10 spore/microscopic field (at 10 X). Then 0.5 ml of the spore suspension was taken and spread on the surface of a plate containing a medium consisting of 2% agar and water. Next, the dishes inoculated with the fungi were incubated at 28° C for 48 hours in the incubator. Finally, the fungal colonies resulting from the growth of the single spore were transferred to a new PDA medium by sterile needle [17].

2.5. Calculating the Percentage of Fungi Appearing

The percentage of fungi appearance was calculated as follows:

$$\text{Percentage of fungi appearing} = \frac{\text{How often does the fungus appear in the samples}}{\text{The number of replications}} \text{ X } 100 \qquad (1)$$

2.6. Mycotoxins Analyses of Wheat Grains Varieties

Surface sterilization of the seeds was done by immersing them in ethyl alcohol with 70% alcohol. For mycotoxins analysis, 20 g. of wheat grains were pulverized and extracted. Zearalenone, ochratoxin, and aflatoxins were all investigated using ELISA in a veterinary/ Erbil/Iraq laboratory.

2.7. The Technique of Mycotoxins Extraction

5 g. of wheat grains samples were poured into conical flasks. 25 ml of methanol were added at 70% concentration (extraction of zearalenone and aflatoxin) and 50% concentration (extraction of ochratoxin). After three minutes of shaking, the samples were filtered through filter paper (No.1). A test tube was filled with 5 ml of filtrate. However, 1 ml of the filtrate was diluted four times with sterile distilled water in the case of zearalenone (1:4). All samples were kept refrigerated before being analyzed.

The study used kits of Neogene to analyze samples by ELISA technique the following processes: In the red plate wells, 100 µl of samples and control were pipetted, followed by 100 µl of Enzyme conjugate, then mixed 2-3 times for homogenization. Next, the mixtures were transferred to white wells in another plate and kept for 10-20 seconds at room temperature. Next, the plate was washed five times with distilled water. Then 100 µl of the substrate was added and stirred for 10-20 seconds before being kept at room temperature for 3 minutes. Finally, 100µl of stop solution was added. By using Neogen Vertex software, Vera tax ELISA Reader obtained the results.

3. Results

The isolation results showed that fungi had contaminated all studied samples. The percentage of appearance of the contaminated fungi differed in the instances according to the local and imported wheat varieties. Fig. 1 for the local grains wheat variety Ebaa 99 indicated that the highest percentage of appearance of Aspergillus flavus was 63.15%, followed by Aspergillus niger with an incidence of 10.18%. Then A. fumigatus, which recorded 17.96% appearance, while Cladosporums pp. 3.24% and Fusarium solani 3.00%, respectively, was Mucor spp. The least percentage of formation was 2.47%.

The local variety Rasheed of grain wheat showed different percentages of appearance of the fungi, as shown in Fig. 2. Aspergillus flavus showed the highest appearance was 49.12%, followed by A. niger with

22.70%, A. fumigatus with 22.48%, and Mucor spp. The less growth rate was 3.41%, while the least growing fungus was Rhizopus spp. and 2.29%.

The local cultivar Al-Baraka showed different incidence rates of fungi, as shown in Fig. 3. The highest incidence of A. flavus was 35.45%, followed by A. niger with an appearance rate of 28.37%, and A. candidus at 15.91%. The Fusarium solani appeared with a percentage of 10.84%, while the lowest percentage of appearance was for Cladosporium spp. by 9.43%.

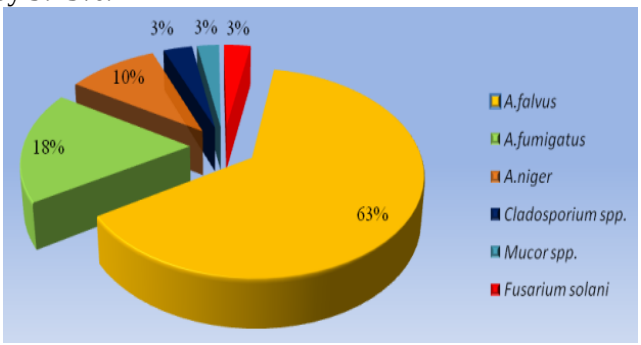

Fig. 1 Fungi isolated from the grains of local wheat variety Ebaa 99, and their appearance percentage

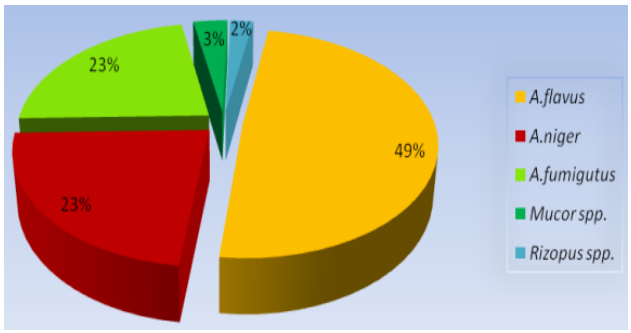

Fig. 2 Fungi isolated from the grains of local wheat variety Rasheed, and their percentage of appearance

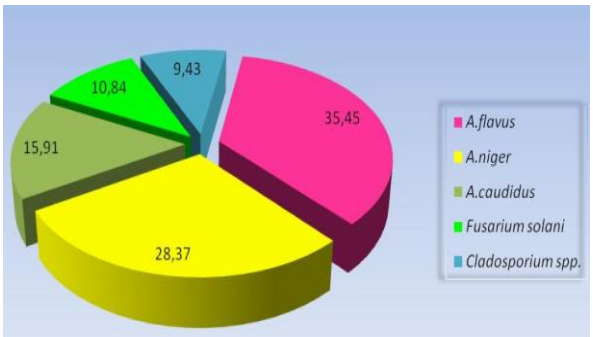

Fig. 3 Fungi isolated from the grains of local wheat variety Al-Baraka, and their percentage of appearance

Fungi isolated from imported wheat grains differed from local wheat varieties in their appearance percentages. In the Canadian imported wheat, the fungi are shown in Fig. 4; the highest incidence was for A. flavus with a rate of 63.72%, followed by A. niger with a rate of 17.35%, and it appeared The fungus A. dematatus with 5.42% was the least appearance, and Cladosporium spp. also appeared in percentage 13.51%.

As for the American imported wheat grains, isolation of the fungi shown in Fig. 5 appeared. A. flavus appeared with the highest growth rate of 43.17%, followed by A. niger with an emergence rate of 30.13%, Fusarium solani With a percentage of 15.90%, the fungus Aspergillus spp. By 5.46%, the least growing fungus was Fusarium oxysporum, 5.34%.

As for the Australian imported wheat grains, the percentage of appearance of the fungus was as shown in Fig. 6. The highest percentage showed the fungi A. flavus at 54.03%, followed by the fungus A. niger with a percentage of 28.37%, then the fungus A. candidus with an appearance rate of 15.91 %, and the fungus Fusarium spp. with 10.84%, and Cladosporium spp. was the least growing fungus, as it appeared with a percentage of 9.43%.

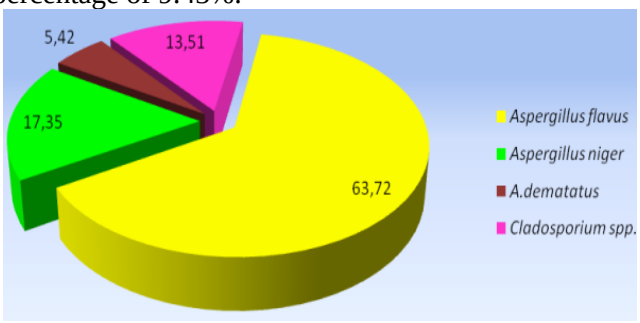

Fig. 4 Fungi isolated from imported Canadian wheat grains and their percentage

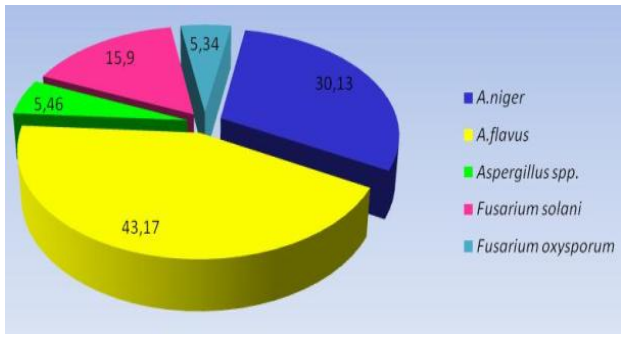

Fig. 5 Fungi isolated from American imported wheat grains and their percentage of appearance

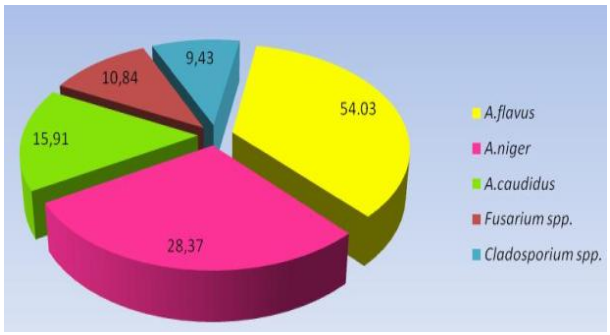

Fig. 6 Fungi isolated from Australian imported wheat grains and their percentage of appearance

3.1. Determination of Mycotoxins Associated with Difference Wheat Grains

The results of estimation of mycotoxins in Fig. 7 indicate that the aflatoxin amount in the grains of the variety Ebaa 99 was 34.55 ppm. In comparison, the

amount of ochratoxin and zearalenone was 21.75 ppm and 0.23 ppm, respectively, while the aflatoxin amount in the American variety was 33.10 ppm, and its amount of ochratoxin was 35.14 ppm, and zearalenone was 9.14 ppm. The variety Canadian had the lowest variety amount reaching 11.9 ppm.

The Canadian wheat grains variety had the lowest aflatoxin content, with an amount of 11.9, while the highest amount of ochratoxin was in the Canadian variety, which amounted to 41.32. Without any content of 0.0 in the Baraka variety, which recorded the highest range in zearalenone, with an amount of 21.03 ppm, while the variety Ebaa 99 had the lowest amount of zearalenone, with an amount of 0.21 ppm, and in general, the Australian type had the lowest content of aflatoxin, ochratoxin, and zearalenone, as it amounts 12.29 ppm, 0.82 ppm and 11.51 ppm, respectively.

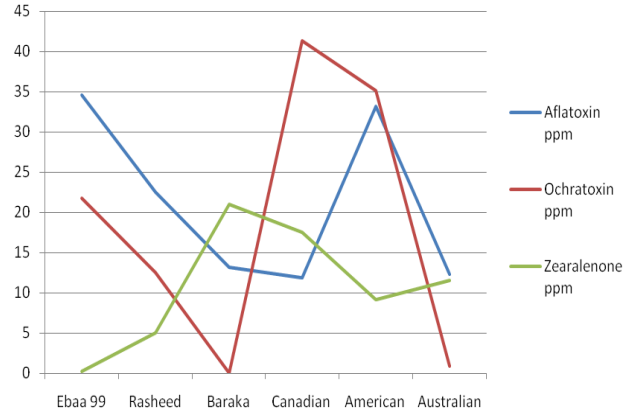

Fig. 7 Determination of mycotoxins associated with difference wheat grains

4. Discussion

Most of the storage fungi can grow in the absence of free water. For example, 85 storage fungi of wheat, barley, sorghum, and maize follow the following eight genera arranged according to the most common being Curvularia, Alternaria, Mucor, Fusarium, Rhizopus, Penicillium, Aspergillus. There are no accurate statistics on global losses in food grains and others due to storage fungi. However, according to the estimates of the FAO in 1973, this loss is at least 5%. This loss in some countries such as India, some African countries, and South America may reach 30% of the annual crop. It has been shown that storage fungi get large rates in grains with high water content. They do not usually infect before harvest, but they may be found on seeds in small numbers, and they may exist as a dormant fungus spinning inside short-grain tissues [3].

There are several stages of development of different fungi in stored grain. In the first stage, the field fungi of Fusarium, Cladosporium, and Alternaria. In the second stage, the number of fungi decreases. The yeast fungi replace the field fungi with Penicillium in the third stage. Storage fungi appear in the fourth stage, and heat-tolerant organisms flourish with the heating of the grain itself (30-60°C). The control of storehouse fungi increases with the increase in grain temperature and the length of the storage period, which is the fifth stage [20]. Mohammed and Hamid [21] analyzed the isolated stored grains of wheat with the fungus Cladosporium sp., Rhizopus sp., Aspergillus terrus and Diplodia sp. and three different isolated fungus Aspergillus sp. with less frequency. Aspergillus parasiticus topped the list with a frequency of 60.83%, followed by Aspergillus niger with 20%. Also, Srpska and Sad [22] found that A. niger, A. flavus, and Alternaria alternata were the most dominant in the wheat grain samples. At the same time, Enyisi [23] found that A. paraciticus is most dominant in the stored grain of wheat. Chauhan et al. [3] found that Penicillium verrucosum is the most polluting fungus for wheat seeds, variety Gamiza11 in Egypt. In a study aimed at knowing the quality of the storaging process for wheat and corn grains marketed in the markets of Misurata, the fungi Penicillium spp., Fusarium spp., Aspergillus spp., Mucor raceuogenum, Trichoderma spp., Curvularia spp., Rhizopus spp., Ulocladium sporatpp, A. flavus recorded the highest incidence in wheat, reaching 67.6% [24].

Wheat seeds in stores in Bulgaria are infected with a group of fungi belonging to the genera Alternaria, Aspergillus, Mucor, Fusarium, Rhizopus, and Penicillium, and the most visible fungi belong to the genus Aspergillus, especially A. restrictus and A. glaucus [25]. Wheat seeds in the United States found that A. glaucus was dominant in the seeds of wheat samples, followed by Penicillium spp [26].

It's been over 60 years since the discovery of aflatoxins. Since then, numerous other mycotoxins (fungal metabolites toxic) have been discovered, many of which are causes of intoxications (mycotoxicoses). In contrast, others have remained as a laboratory curiosity, The total number of mycotoxins is unknown, but hazardous metabolites of fungi potentially number in the thousands. Studies on mycotoxins, including their detection, biosynthesis, toxicity, epidemiology, and control of the generating fungi, are crucial to maintaining a safe food supply. The mycotoxins number is known to cause disease is far smaller. However, even this figure is difficult to estimate due to the wide range of impacts these distinct substances have on animal systems. Not only are mycotoxins a source of concern for diseases for humans and animals, but many mycotoxin-producing fungi are also plant pathogenic, posing a significant economic threat to crops. Their plant pathogenic activities cause food safety concerns and impact grain trade and food and feed marketing [27].

When different fungi contaminate different foods, the production of mycotoxins varies depending on the fungus, the type of food being consumed, and the location in which it is consumed. Despite the difficulty of eliminating pollutants and mycotoxins and molds,

hard work is required to reduce the harmful effects of these toxins in the most efficient manner possible, whether chemical or physical [28]. The poisonous, mutagenic, and carcinogenic consequences of Aspergillus fungus in food are significant. The International Agency for Research on Cancer has been classified as a first-class carcinogen. Liver poisoning studies in India and Kenya have found that the risk of cancer may increase by hepatitis viral because of the residues of ochratoxin presence in wheat grain samples produced by fungi Aspergillus and Penicillium. Luckily, ochratoxin level was less than 20 ppb in food and less than 5 ppb per Kg of body weight daily. According to data published by the European Commission, the consumption of ochratoxin daily varies from 0.02 to 1.9 ppb /kg weight of body daily [29].

Some Fusarium species (such as F. graminearum, which produces zearalenone) are soil dwellers. However, they have also termed storage fungi since growth and toxin generation can occur under various storage conditions. Therefore, corn and wheat are the most vulnerable to this fungus attack [30].

Various chemical, physical, and biological processing methods have lowered zearalenone levels. Food experienced physical heat treatment procedures in this investigation. Zearalenone's fate is determined by its chemical qualities, such as heat stability and its dispersion in the food matrix [31, 32].

All of the tested residual mycotoxins (ochratoxin, zearalenone, and aflatoxin) were within new regulations specifying that products intended for human consumption or as an ingredient in food must meet the allowed levels for certain toxins in food commodities intended for human consumption. Total aflatoxin, ochratoxin, and zearalenone limit of 4 ppb must comply. The best way to manage the risks associated with mycotoxins contamination is to prevent mycotoxins from forming. In addition, a successful food safety management program must involve preventive, regulatory restrictions, monitoring programs, appropriate agricultural practices, and producer education and consumer [33].

5. Conclusion

All samples of wheat grains studied were contaminated with fungi and mycotoxins, especially variety (Ebaa 99) which showed the highest percentage of appearance in Aspergillus flavus, was 63.15%, and some other fungi. In the Canadian variety, the most elevated appearance of A. flavus was 63.72%, in addition to some other fungi. Also, the study concluded that estimation of mycotoxins indicates that the aflatoxin amount in the samples of the variety Ebaa 99 was 34.55 ppm. In comparison, its amount of ochratoxin and zearalenone was 21.75 ppm and 0.23 ppm, respectively.

References

[1] EL-RAHEEM A. Occurrence of moulds, toxicogenic capability of Aspergillus flavus and levels of aflatoxins in maize, wheat, rice and peanut from markets in central delta provinces, Egypt. *International Journal of Current Microbiology and Applied Sciences*, 2014, 3(3): 852–865. https://www.ijcmas.com/vol-3-3/Abd%20El-Raheem%20R.%20El-Shanshoury,%20et%20al.pdf

[2] HARLAN J. R., & ZOHARY D. Distribution of wild wheats and barley. *Science*, 1966, 153: 1074-1080. https://doi.org/10.1126/science.153.3740.1074

[3] CHAUHAN N. M., WASHE A. P., and MINOTA T. Fungal infection and aflatoxin contamination in maize collected from Gedeo zone, Ethiopia, *SpringerPlus*, 2016, 5(1): 753. https://doi.org/10.1186/s40064-016-2485-x

[4] SIEBER T., RIESEN T. K., MÜLLER E., and FRIED P. M. Endophytic Fungi in Four Winter Wheat Cultivars (Triticum aestivum L.) Differing in Resistance Against Stagonospora nodorum (Berk.) Cast. & Germ. =Septoria nodorum (Berk.) Berk. *Journal of Phytophathology*, 1988, 122(4): 289-306. https://doi.org/10.1111/j.1439-0434.1988.tb01021.x

[5] VASILIKI H. T., & NICHOLAS S. N. Potentially toxigenic fungi from selected grains and grain products. *Journal of Food Safety*, 2017, 38(1). https://doi.org/10.1111/jfs.12422

[6] MARTIN C. H., SCHÖNEBERG T., VOGELGSANG S., VINCENTI J., BERTOSSA M., MAUCH-MANI B., and MASCHER F. Factors of wheat grain resistance to Fusarium head blight. *Phytopathologia Mediterranea*, 2017, 56(1): 154−166. http://dx.doi.org/10.14601/Phytopathol_Mediterr-20292

[7] LEWIS L., ONSONGO M., NJAPAU H., SCHURZ-ROGERS H., LUBER G., KIESZAK S., NYAMONGO J., BACKER L., DAHIYE A. M., AMBROSE MISORE A., DECOCK K., and RUBIN C. Aflatoxin Contamination of Commercial Maize Products during an Outbreak of Acute Aflatoxicosis in Eastern and Central Kenya. *Environmental Health Perspectives*, 2005, 113: 1763–1767. https://dx.doi.org/10.1289%2Fehp.7998

[8] WALIYER F., CRAUFURD P. Q., PRASAD P .V. V., and TAHERI A. L. Droughpod yield preharvest Aspergillus infection and aflatoxin contamination on peanut in Niger. *Journal of Microbiology*, 2015, 98: 20–29. https://dx.doi.org/10.1016/j.fcr.2005.12.001

[9] MYCOTOXINS INFO BLOG. *European Mycotoxins Awareness Network*, 2012. http://mycotoxinsinfo.blogspot/.com

[10] FLEURAT-LESSARD F. Integrated management of the risks of stored grain spoilage by seedborne fungi and contamination by storage mould mycotoxins – An update. *Journal of Stored Products Research*, 2017, 71, 22–40. https://doi.org/10.1016/j.jspr.2016.10.002

[11] MONKVOLD G. P. Cultural and genetic approaches to managing mycotoxins in maize. *Annual Review of Phytopathology*, 2003, 41: 99–116. https://doi.org/10.1146/annurev.phyto.41.052002.095510

[12] ATANDA O., MAKUN H. A., OGARA I. M., EDEMA M., IDAHOR K. O., ESHIETT M. E. and OLUWABAMIWO B. F. Fungal and Mycotoxin Contamination of Nigerian Foods and Feeds. In: MAKUN H. A. (ed.) *Mycotoxin and Food Safety in Developing*

Countries. IntechOpen, London, 2013: 3–38. https://doi.org/10.5772/55664
[13] KORDAS L., PUSZ W., CZAPKA T., and KACPRZYK R. The effect of low-temperature plasma on fungus colonization of winter wheat grain and seed quality. *Polish Journal of Environmental Studies*, 2015, 24(1): 433-438. http://www.pjoes.com/pdf-89435-23352?filename=The%20Effect%20of.pdf
[14] FANDOHAN P., GNONLONFIN B., HELL K., MARASAS W. F., and WINGFIELD M. Natural occurrence of Fusarium in stored maize in Benin West Africa. *International Journal Food Microbiology*, 2005, 99: 173–183. https://doi.org/10.1016/j.ijfoodmicro.2004.08.012
[15] ELLIS M. B. *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, 1971.
[16] DOMSCH K. H., GAMS W., and ANDERSON T. H. *Compendium of soil fungi*. Academic Press, London, 1980. https://doi.org/10.1111/j.1365-2389.2008.01052_1.x
[17] NELSON P. E., TOUSSOUN T. A., and MARASAS W. F. O. *Fusarium species, an illustrated manual for identification*. The Pennsylvania State University Press, University Park, 1983: 1457-1462. https://www.worldcat.org/title/fusarium-species-an-illustrated-manual-for-identification/oclc/9393324
[18] LESLIC J. F., & SUMMERELL B. A. *The Fusarium laboratory manual*. Blackwell Publishing, Ames, 2006.
[19] PITT J. I., & HOCKING A. D. *Fungi and Food Spoilage*. Academic Press, Sydney, 2007.
[20] SWEETS L. *Fungal spoilage in storage grains*. Agricultural Electronic Bulletin Board, Columbia, 2019.
[21] MUHAMMAD B. T., & HAMID Z. L. Isolation and identification of fungi associated with wheat grains and their chemical resistance. *Journal of Kerbala for Agricultural Sciences*, 2016, 3(2): 100-113. https://journals.uokerbala.edu.iq/index.php/Agriculture/article/view/28/18
[22] SRPSKA M., and SAD N. Molds and mycotoxins in freshly harvested maize. *Matica Srpska Journal for Natural Sciences*, 2013, 124: 111–119. https://doi.org/10.2298/ZMSPN1324111K
[23] ENYISI S. I. Total aflatoxin level and fungi contamination of maize and maize products. *African Journal of Food Science and Technology*, 2015, 6(8): 2141–5455. http://dx.doi.org/10.14303/ajfst.2015.073
[24] SHAABAN M. A., and ABU ZAYAN S. O. Isolation and identification of fungi and estimation of moisture content of wheat and corn grains from some markets of Misurata. Proceedings of the the first annual conference on theories and applications of basic and biological sciences, College of Science, Misrata, 2017.
[25] JOSHAGHANI H., NAMJOO M., ROSTAMI M., KOHSAR F., and NIKNEJAD F. Mycoflora of Fungal Contamination in Wheat Storage, (Silos) in Golestan Province, North of Iran. *Jundishapur Journal of Microbiology*, 2013, 6: 6334-6338. http://dx.doi.org/10.5812/jjm.6334
[26] IN Y., KIM J., KIM H., and OH S. Antimicrobial Activities of Acetic Acid, Citric Acid, and Lactic Acid against Shigella Species. *Journal of Food Safety*, 2013, 33: 79-85. https://doi.org/10.1111/jfs.12025
[27] MILANI C., HEVIA A., FORONI E., DURANTI S., TURRONI F., LUGLI G. A., SANCHEZ B., MARTIN R., GUEIMONDE M., and VAN SINDEREN D. Assessing the fecal microbiota: an optimized ion torrent 16S rRNA gene-based analysis protocol. *PloS one*, 2013, 8(7): e68739. https://doi.org/10.1371/journal.pone.0068739
[28] DO J. H., & CHOI D.-K. Aflatoxins: detection, toxicity, and biosynthesis. *Biotechnology and Bioprocess Engineering*, 2007, 12(6): 585-593. https://doi.org/10.1007/BF02931073
[29] CHRISTENSEN C. M., MIROCHA C. J., and MERONUCK R. A. Molds, mycotoxins, and mycotoxicoses [produced by Fusarium, Aspergillus, contaminated feeds]. *Cereal Foods World*, 1977, n.d.
[30] BAHRAMI-SAMANI O., RAHIMI E., and NILI-AHMADABADI A. Assessment of zearalenone contamination in processed cereal-based foods in Shahrekord, Iran. *Toxin Reviews*, 2017, 36(3): 257-260. https://doi.org/10.1080/15569543.2016.1178148
[31] KRNJAJA V., LEVIĆ J., STANKOVIĆ S. Ž., PETROVIĆ T. S., TOMIĆ Z., MANDIĆ V., and BIJELIĆ Z. Moulds and mycotoxins in stored maize grains. *Biotechnology in Animal Husbandry*, 2013, 29(3): 527-536. http://dx.doi.org/10.2298/BAH1303527K
[32] ABDULRAUF L. *Aflatoxins, Occurrence, Detoxification, Determination and Health Riske*. IntechOpen, London, 2022. https://doi.org/10.5772/intechopen.92518
[33] PARK D. L., & STOLOFF L. Aflatoxin control—How a regulatory agency managed risk from an unavoidable natural toxicant in food and feed. *Regulatory Toxicology and Pharmacology*, 1989, 9(2): 109-130. https://doi.org/10.1016/0273-2300(89)90029-9

参考文:

[1] EL-RAHEEM A. 埃及中部三角洲省市场的玉米、小麦、大米和花生中的霉菌发生率、黄曲霉的致毒能力和黄曲霉毒素水平。国际当代微生物学与应用科学杂志, 2014, 3(3): 852–865. https://www.ijcmas.com/vol-3-3/Abd%20El-Raheem%20R.%20El-Shanshoury,%20et%20al.pdf
[2] HARLAN J. R., 和 ZOHARY D. 野生小麦和大麦的分布。科学, 1966, 153: 1074-1080. https://doi.org/10.1126/science.153.3740.1074
[3] CHAUHAN N. M., WASHE A. P., 和 MINOTA T. 从埃塞俄比亚格迪奥地区采集的玉米中的真菌感染和黄曲霉毒素污染，施普林格加, 2016, 5(1): 753. https://doi.org/10.1186/s40064-016-2485-x
[4] SIEBER T., RIESEN T. K., MÜLLER E., 和 FRIED P. M. 四种冬小麦品种(小麦)中的内生真菌对鹿角菌(伯克。)的抗性不同。和细菌。=淋巴结裂孔(伯克。)伯克。植物病理学杂志, 1988, 122(4): 289-306. https://doi.org/10.1111/j.1439-0434.1988.tb01021.x
[5] VASILIKI H. T., 和 NICHOLAS S. N. 来自选定谷物和谷物产品的潜在产毒真菌。食品安全杂志, 2017, 38(1). https://doi.org/10.1111/jfs.12422

[6] MARTIN C. H., SCHÖNEBERG T., VOGELGSANG S., VINCENTI J., BERTOSSA M., MAUCH-MANI B., 和 MASCHER F.
小麦籽粒抗枯萎病的因素。地中海植物病理学, 2017, 56(1): 154−166. http://dx.doi.org/10.14601/Phytopathol_Mediterr-20292

[7] LEWIS L., ONSONGO M., NJAPAU H., SCHURZ-ROGERS H., LUBER G., KIESZAK S., NYAMONGO J., BACKER L., DAHIYE A. M., AMBROSE MISORE A., DECOCK K., 和 RUBIN C.
肯尼亚东部和中部爆发急性黄曲霉毒病期间商业玉米产品的黄曲霉毒素污染。环境健康观点, 2005, 113: 1763 – 1767. https://dx.doi.org/10.1289%2Fehp.7998

[8] WALIYER F., CRAUFURD P. Q., PRASAD P .V. V., 和 TAHERI A. L.
尼日尔花生收获前曲霉感染和黄曲霉毒素污染。微生物学杂志, 2015, 98: 20–29. https://dx.doi.org/10.1016/j.fcr.2005.12.001

[9] 霉菌毒素信息博客. 欧洲霉菌毒素意识网络, 2012. http://mycotoxinsinfo.blogspot/.com

[10] FLEURAT-LESSARD F.
种子传播真菌贮藏谷物腐败和贮藏霉菌毒素污染风险的综合管理——更新。储存产品研究杂志, 2017, 71, 22–40. https://doi.org/10.1016/j.jspr.2016.10.002

[11] MONKVOLD G. P.
管理玉米中霉菌毒素的文化和遗传方法。植物病理学年度回顾, 2003, 41: 99–116. https://doi.org/10.1146/annurev.phyto.41.052002.095510

[12] ATANDA O., MAKUN H. A., OGARA I. M., EDEMA M., IDAHOR K. O., ESHIETT M. E. 和 OLUWABAMIWO B. F. 尼日利亚食品和饲料的真菌和霉菌毒素污染。在：MAKUN H. A.（编）发展中国家的霉菌毒素和食品安全。英泰克公开赛，伦敦, 2013: 3–38. https://doi.org/10.5772/55664

[13] KORDAS L., PUSZ W., CZAPKA T., 和 KACPRZYK R.
低温等离子体对冬小麦籽粒真菌定殖及种子品质的影响. 波兰环境研究杂志, 2015, 24(1): 433-438. http://www.pjoes.com/pdf-89435-23352?filename=The%20Effect%20of.pdf

[14] FANDOHAN P., GNONLONFIN B., HELL K., MARASAS W. F., 和 WINGFIELD M.
贝宁西非储藏玉米中镰刀菌的自然发生。国际期刊食品微生物学, 2005, 99: 173–183. https://doi.org/10.1016/j.ijfoodmicro.2004.08.012

[15] ELLIS M. B. 暗色丝菌纲。联邦真菌学研究所，邱, 1971.

[16] DOMSCH K. H., GAMS W., 和 ANDERSON T. H.
土壤真菌纲要.学术出版社，伦敦, 1980. https://doi.org/10.1111/j.1365-2389.2008.01052_1.x

[17] NELSON P. E., TOUSSOUN T. A., 和 MARASAS W. F. O.
镰刀菌物种，用于鉴定的插图手册。宾夕法尼亚州立大学出版社，大学公园, 1983: 1457-1462. https://www.worldcat.org/title/fusarium-species-an-illustrated-manual-for-identification/oclc/9393324

[18] LESLIC J. F., 和 SUMMERELL B. A.
镰刀菌实验室手册。布莱克威尔出版社，艾姆斯, 2006.

[19] PITT J. I., 和 HOCKING A. D.
真菌和食物变质。学术出版社，悉尼, 2007.

[20] SWEETS L.
储存谷物中的真菌腐败。农业电子公告板，哥伦比亚, 2019.

[21] MUHAMMAD B. T., & HAMID Z. L.
小麦籽粒相关真菌的分离鉴定及其耐化学性[J].喀巴拉农业科学杂志, 2016, 3(2): 100-113. https://journals.uokerbala.edu.iq/index.php/Agriculture/article/view/28/18

[22] SRPSKA M., 和 SAD N.
新鲜收获的玉米中的霉菌和霉菌毒素。马提卡斯普斯卡自然科学杂志, 2013, 124: 111–119. https://doi.org/10.2298/ZMSPN1324111K

[23] ENYISI S. I.
玉米和玉米产品的总黄曲霉毒素水平和真菌污染。非洲食品科学与技术杂志, 2015, 6(8): 2141–5455. http://dx.doi.org/10.14303/ajfst.2015.073

[24] SHAABAN M. A., 和 ABU ZAYAN S. O.
米苏拉塔部分市场小麦和玉米籽粒真菌的分离鉴定和水分含量估算。第一届基础与生物科学理论与应用年会论文集，科学学院，米苏拉塔, 2017.

[25] JOSHAGHANI H., NAMJOO M., ROSTAMI M., KOHSAR F., 和 NIKNEJAD F.
伊朗北部戈勒斯坦省（筒仓）小麦储藏中真菌污染的真菌群落。容迪沙普尔微生物学杂志, 2013, 6: 6334-6338. http://dx.doi.org/10.5812/jjm.6334

[26] IN Y., KIM J., KIM H., 和 OH S.
乙酸、柠檬酸和乳酸对志贺氏菌的抗菌活性。食品安全杂志, 2013, 33: 79-85. https://doi.org/10.1111/jfs.12025

[27] MILANI C., HEVIA A., FORONI E., DURANTI S., TURRONI F., LUGLI G. A., SANCHEZ B., MARTIN R., GUEIMONDE M., 和 VAN SINDEREN D.
评估粪便微生物群：优化的离子激流16小号核糖体核糖核酸基因分析方案。公共科学图书馆一, 2013, 8(7): e68739. https://doi.org/10.1371/journal.pone.0068739

[28] DO J. H., 和 CHOI D.-K.
黄曲霉毒素：检测、毒性和生物合成。生物技术与生物过程工程, 2007, 12(6): 585-593.

https://doi.org/10.1007/BF02931073

[29] CHRISTENSEN C. M., MIROCHA C. J., 和 MERONUCK R. A. 霉菌、霉菌毒素和霉菌毒素[由镰刀菌、曲霉、受污染的饲料产生]。谷物食品世界，1977，未注明日期。

[30] BAHRAMI-SAMANI O., RAHIMI E., 和 NILI-AHMADABADI A. 伊朗沙赫尔科德加工谷物食品中玉米赤霉烯酮污染的评估。毒素评论, 2017, 36(3): 257-260. https://doi.org/10.1080/15569543.2016.1178148

[31] KRNJAJA V., LEVIĆ J., STANKOVIĆ S. Ž., PETROVIĆ T. S., TOMIĆ Z., MANDIĆ V., 和 BIJELIĆ Z. 储存的玉米粒中的霉菌和霉菌毒素。畜牧业中的生物技术, 2013, 29(3): 527-536. http://dx.doi.org/10.2298/BAH1303527K

[32] ABDULRAUF L. 黄曲霉毒素、发生、解毒、测定和健康风险。英泰克公开赛，伦敦, 2022. https://doi.org/10.5772/intechopen.92518

[33] PARK D. L., 和 STOLOFF L. 黄曲霉毒素控制——监管机构如何管理食品和饲料中不可避免的天然毒物带来的风险。监管毒理学和药理学, 1989, 9(2): 109-130. https://doi.org/10.1016/0273-2300(89)90029-9