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Challenges to Safe Wheat Storage

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Abstract

There are enormous challenges facing wheat storage, which is the most important crop in existence. Wheat is one of the most famous and important plants in human history. There is no country in the world that does not give up wheat yields. Countries of the world vary and differ in their production and consumption of that important plant. Since ancient times, humans have stored wheat grain in special places. Storage areas were developed until the current silos were reached. With large quantities of wheat stored in silos, there are many challenges to the healthy environment of storage. One of the most important challenges facing quality of wheat stored in silos is the spread of conidia and spores of many dangerous fungi on wheat grains. One of studies conducted by the authors proved presence of some of notorious fungi on and inside wheat mass stored in the silo under study. *Aspergillus flavus*, *A. niger*, *Circinella umbellata*, *Gliocladium sp.*, *Penicillium frequentans*, *P. islandicum*, and *Ulocladium atrum* were isolated from wheat samples. All seven isolated fungi demonstrated their ability to analyze human red blood cells with different strengths. These results are consistent with previous studies that confirm the seriousness of presence of these fungi on the health of dealers and exposers especially with bad storage and humidity.

Keywords: fungi, humidity, silos, storage, wheat

1. Introduction

Wheat is one of the most important crops in the world. It is cultivated on an area of about 2 million square kilometers. Its ease of adaptation to the soil has spread to several areas of the world. Wheat is the mainstay of nutrition for many countries in the world. Based on the latest report

of the Food and Agriculture Organization of the United Nations (FAO), that the global wheat trade in 2017 reached 754.8 million tons; Russia's exports of wheat amounted to 30 million tons, America 26.5 million tons, Europe 25 million tons, Canada 21 million tons, Australia 20 million tons, while exports of Ukraine 15 million tons, Kazakhstan 8.5 million tons, Argentina 8 million tons, the largest countries exporting wheat worldwide (www.fao.org).

Fortunately, global wheat prices have fallen, driven by an increase in global production, which has been affected by good weather and the availability of water to grow in the EU, India, Pakistan, China, and the United States, which was accompanied by a rise in global exports (www.fao.org).

Forecasts indicate that the use of wheat in 2017–2018 will reach 740 million tons. More than 43% of the production is consumed by only six countries: China, India, Russian Federation, the United States of America, and Pakistan. This is because of the population of these countries of nearly three billion people, or half the world's population (www.fao.org).

As many countries in the world do not produce sufficient quantities of wheat for their uses and therefore tend to import this strategic commodity. These wheat-importing countries store large quantities of these grains in storage places called silos. Since ancient times, wheat grains have been stored in huge shipments. Earliest discovery of grain stores dates back to the year 9500 BC [1], and these stores in the settlements of the Neolithic period before the pottery "A" were located in the Jordan Valley, where the first stores were located in places among other buildings. But at the beginning of the 8500 BC, they were moved inside the houses, but with the period of 7500 BC, they were stored in rooms dedicated to it [2]. The area of the first wheat stores was 3 × 3 m from the outside, and had suspended layers in order to protect grain from rodents and insects and provide ventilation [3]. These stores are then located in Mahjara, which is placed in the valley of Sindh since 6000 BC. The ancient Egyptians used to store wheat grains in years of prosperity for use in drought ones. Because Egypt's climate is very drought, Egyptians have been able to store grain in silos without a significant loss of quality. The grain silo, as it is called, is an ideal way to store grains in all lands of the East since time immemorial. In Turkey and Iran, moorings used to buy wheat or barley, which is relatively cheap, and stored it in closed and hidden places in the face of famine. In Malta, relatively large quantities of wheat were stored in hundreds of silos dug into rocks. The silos can store up to 60–80 tons, by taking proper precautions and keeping it in good condition for 4 years or more. By the end of the nineteenth century, stores specifically designed for grain conservation began to spread in Great Britain, but North America was the home for the major stores, called grain levers. There were large-scale climatic obstacles to grain storage in Great Britain on those difficulties significantly. In order to keep the grain in good condition, it should be kept as far away as possible from moisture and heat because new grains tend to release moisture when brought to the store. In this case, microorganisms (mainly fungi) are more active and can heat the grains. If the grain continues to be heated, its quality will be affected. Therefore, effective treatment is to place grains on the ground in the form of non-thick layers, and to keep the place well ventilated. Hence, grain can be configured to store in silos.

In Great Britain, small wheat stores were built on mushroom-shaped logs called stone pillars. It was built on a wooden frame and usually has stone roofs. The large ones resemble the open ceiling from the front, but the upper part is closed. The first floor is usually accessed by a stone

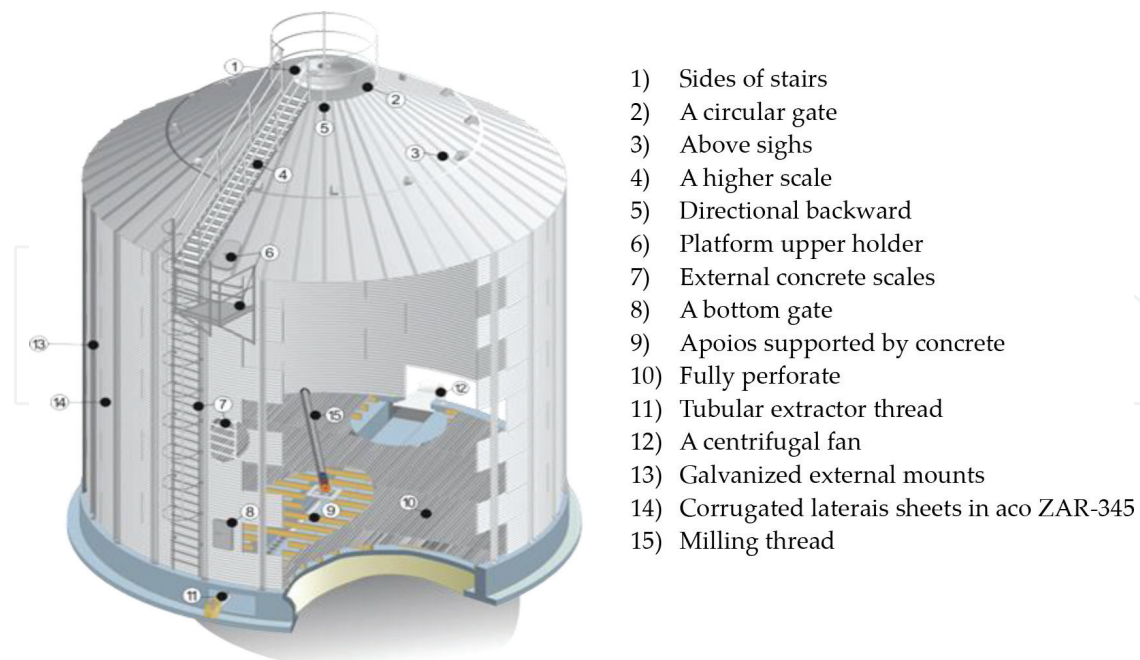


Figure 1. Internal structure of a steel silo as in [3].

staircase on the outer wall. With tremendous advances in engineering technology in the construction of silos, a new generation of forms and possibilities of silos has begun. High-density indoor silos are found in farms, mills, and harbors and can reach a height of 30 m. Largest silos are located in North America at major shipping centers such as Chicago, Kansas city, and Missouri in the United States, and Thunder Bay, Ontario, and Canada. Many cylindrical silos are equipped with a perforated metal floor that allows the air to pass through to keep grain free from moisture. Cylindrical silos are often adjacent, with high mechanical devices nearby.

Generally, there are three types of silos, the first type is made of wood, the second type is made of concrete, and the third type is made of metal (steel and galvanized). Wheat is as barley, oat, rye, and so on, used to store suitably both in concrete and metal silos [3] (**Figure 1**).

Large silos have many appliances including dryers, detergents, workpieces, cranes, and conveyors. Dryers leave grain free from moisture. The air is heated in the dehydrator and directed over the grains and then used air that has not been heated to cool the grains. The use of detergent ensures grain cleanliness. The absorption of dust, husk, or straw is obtained and screening and vibration leads to the elimination of grains that are not of the appropriate size and density. However, some machines use photovoltaic cells to isolate rotten grains.

Cranes and conveyors are used to transport grains. Vertical grain movement is obtained by cranes. Among the most used cranes is a crane with a large conveyor belt and a bundle of buckets. Wheat grains in the bucket are transported to high altitude and poured into storage baskets. Tankers move the grains horizontally across the silo, called a grain lift. It is a silage storage place as well.

Large quantities of wheat are delivered to the silos. Of course, the huge quantities received by silos are coming directly from farms without any transactions. Wheat is then loaded with all the residues of process of flail, agricultural soil, and contaminants of harvesting tools during the

separation of grains from the ears. It is perhaps of standard importance to prepare wheat grains for the flour process to explain how grinding of wheat grains is done by the following procedures. The grain of wheat consists of three main parts: grain coats, embryos, and endosperm. The purpose of the milling process is to separate as much of the endosperm content as possible from the wrappers. The ratio of flour produced to the percentage of grain used in production is known as extraction. The weight of flour produced from 100 g of grain, and depends on this percentage on several factors, the most important; extraction method used, type of mills used, nature, and specific weight of wheat grains. In general, extraction rate is between 70 and 72% in excellent white flour, and this percentage is 90–95% in brown flour. What is left behind from milling, so-called bran, is used to feed animals. Wheat milling is done by the following subsequent serial steps [4]:

1.1. Receiving

Grain coming from fields or silos is received in the mill after sampling and examined to ensure that it meets specifications set by the mill. Grains are received and emptied in conical tanks, each of which can reach 10 tons. Of which is covered with a fixed metal mesh for impurities when unloading grain. The crane and aspirator pull the grain out of the tanks for delivery to the cleaning equipment.

1.2. Cleaning

The cleaning equipment consists of two main units: dry-cleaning unit known as black cleaning unit and the wet-cleaning unit known as the white cleaning unit.

1.2.1. Black cleaning

This unit consists of the following equipment:

- A. Compound separation device: It is composed of three wire screens installed and portable on a metal frame suspended by pulley rods spring to generate vibration movement. Sieves are arranged on top of each other so that they have wide holes at the top followed by center holes and small holes. As a result, impurities can be eliminated according to their size. The device is fitted with a fan mounted at the top of the frame to generate a stream of air that helps to breakdown light impurities.
- B. Separation device according to the specific weight: This device consists of a metal box revolving inside the fan and a strong aspirator and sieve mounted 12% slope from the horizontal level, working on the suction of grain and the deposition of heavy impurities such as stones and pieces of glass.
- C. Magnetic separation device: It is a death pass inside the grain, and is equipped with magnetic plates electric work to attract pieces of iron nails and clumps which are collected in a special drawer.
- D. Vibrating machine for the separation of impurities: It is a composite of a serrated cylinder from the inside to be grooves or round pockets to settle round impurities similar to the diameter of the grain and different in terms of form, where they gather in special ditches.

1.3. White cleaning

This unit consists of a peeler and stalker.

- A. The peeler consists of a cylinder with rough internal surfaces or coated with a precision metal bed that scrapes centrifugal grains. Shells are separated and pulled by an air stream generated by an electric fan installed at the top of the peeler.
- B. The asset consists of a rectangular basin with a nozzle to feed it with grains, as well as an appropriate water source that can be controlled as needed, and ends with a drainage basin topped by an appropriate filter. Inside cylinder with two helical separators working in opposite directions that wash the grains and push them forward and the light impurities on the surface of the water. With the rush of grain forward, it passes over a vibrator strainer that has been removed from the water and then to a rotary roller dryer to complete removing water droplets from the grain. The question now arises: Is the process of drying wheat grains sufficient to remove all moisture from the grain mass? Can wheat grains be washed before entering the silos? What is the cost for this? What are possible risks of increasing humidity and possibility of conditions for infection of fungal spores and conidia?

Perhaps, it is possible in this book to put forward some ideas as follows:

- Presence of units within silos for washing and drying of grains by successive processes of passage of warm air currents, and the source of energy units of solar cells installed one way or another on sides of the silo exposed to sunlight.
- Putting desiccant materials that absorb air moisture inside silos such as sodium chloride and silica gel.
- Exposure of wheat mass to ultraviolet rays for superficial sterilization with constant flipping, taking into account the non-exposure of direct workers to those rays.
- Air fumigation of silos with volatile oils that have the potential to sterilize the air.

Despite all precautions taken in modern silos, many studies have shown that fungi are flourishing on wheat grains and in the air of these reservoirs [5]. Several research findings have confirmed the presence of harmful and toxic fungi in many silos, not only on stored grains but also on wheat flour derived from those silos. Wheat grains are harvested in agricultural fields so they are subjected to contamination with soil particles as well as germs adhered on wheat plant itself. For these reasons, the mass of wheat stored in silos contains large quantities of dust packed with fungal spores. It is worth mentioning that any defect in the system of grain conservation inside silos is followed by the growth of fungal spores among wheat grains, and these molds may be not visible to the eye, which ultimately leads to the arrival of consumers.

The problem seems more complicated if wheat is stored in poor conditions, due to the ability of wheat to imbibe the air humidity of the silos. A study conducted in Zimbabwe indicated that the storage of red wheat in many silos led to a decline in the commercial level of this commodity and an increase in the level of fungal toxins [6].

In one of the literatures of the previous research, presence of fungi was tested in 34 samples collected from 3 silos. Results of this experiment proved that presence of fungi produced aflatoxins in majority of tested samples [5]. It is worth mentioning that fungi represent the main factors of starchy grain contamination (mycotoxigenic). Therefore, it has been found logical to review a study conducted by the authors on presence of fungi that have serious precedents as causes of diseases of respiratory system in humans. We will discuss the danger of these fungi to people dealing with wheat grains from the beginning of harvest until the entry into silos.

This part of the book will present results of a study conducted in one of the giant silos in Sakaka city, Al-Jouf region, Saudi Arabia, in the autumn of 2015. Our results will be discussed with results of previous studies in some countries in order to highlight some of the challenges facing safe storage of wheat grains inside silos.

2. Materials and methods a research on the presence of fungi in wheat stored in a silo in Sakaka city, Saudi Arabia

Twenty samples of wheat grains stored in the large silo in Sakaka were tested for the presence of fungi (part of this work has been reported elsewhere [7]). This was done by placing a known quantity of wheat in a bottle of sterile water next to the sampling area, taking care to ensure that one source of fungus (wheat grains) reaches the collection container. When returning to the laboratory, fungi were isolated by placing 5 ml of water mashed with fungal spores, coming from grain surfaces, in a 9-cm Petri dish and then adding 15 ml of rose-Bengal Potato Dextrose Agar (PDA) medium, all in isolation cabinet under aseptic conditions. Dishes were closed and sealed with parafilm to ensure full closure and placed upside down in plastic bags (previously sterilized by radiation) and incubated at 28°C, and then followed up until appearance of fungal colonies. After emergence of fungal colonies, each colony was purified on its own, to obtain pure single fungal isolate. Pure fungal samples were subjected to ophthalmic and microscopic examinations with imaging and arranged of figurative plates [8, 9]. Risk of isolated fungi has been tested on human health. Hemolytic ability of the isolated fungi to human red blood cells was tested following the method of [10, 11]. Fungal spore suspension (in 0.9% NaCl) was used. Washed (using 0.9% NaCl) 100 µl of blood, plus 900 µl of spore suspension was incubated, sodium chloride solution, which represents a negative control sample, and distilled water (positive control sample), under aseptic conditions, for comparison at 28°C, for 24 h, in the dark. Reaction mixtures were separated with the aid of a centrifuge and absorbance of supernatant was measured using UV-Vis spectrophotometer (spectro uv-2505) at 540 nm to calculate percentage of hemolysis of red blood cells following formula of the equation:

$$\% \text{Hemolytic activity} = \frac{\text{absorbance of sample} - \text{absorbance of saline}}{\text{absorbance of dist. water}} \times 100.$$

3. Results and discussion

Fungi of *Aspergillus flavus*, *A. niger*, *Circinella umbellata*, *Gliocladium* sp., *Penicillium frequentans*, *P. islandicum*, and *Ulocladium atrum* were isolated from wheat samples. **Table 1** shows prevalence of each fungus.

OR = Occurrence remarks; H = 60% -100.0%, M = 33 - 59.0%, L = 20–32%, and R = 7–19%.

Genera and species	Wheat grains		
	NCI	OR	TC (%)
<i>Aspergillus</i>	145	20H	38.3
<i>A. flavus</i>	73	20H	19.3
<i>A. niger</i>	72	20H	19.0
<i>Circinella umbellata</i>	28	5 L	7.4
<i>Gliocladium</i> sp.	8	4 L	2.1
<i>Penicillium</i>	138	18H	36.5
<i>P. frequentans</i>	70	18H	18.5
<i>P. islandicum</i>	68	17H	18.0
<i>Ulocladium atrum</i>	59	3 L	15.7
Gross total counts	378		
No. of genera	5		
No. of species	7		

Table 1. Gross counts of fungal genera and species derived from 20 samples of wheat grains collected from the main silo, Sakaka, Al-Jouf, Saudi Arabia by germs came from soaked grains in sterilized H₂O, number of cases of isolation (NCI; out of 20 cases), occurrence remarks (OR), percentage of total counts (TC%) on PDA agar at 28°C.

4. Fungal identification

It is worth mentioning that all fungi isolated from wheat surfaces stored in the silo, produces huge amounts of spores and conidia. For example, *Aspergillus flavus* is a fungus of a bad reputation which produces the most dangerous toxin called aflatoxin (AFs). This instinct fungus is famous for corruption and damage of many seeds, grains, and nuts [12]. *A. flavus* produces large quantities of conidia bearing on biseriate sterigmata. *A. niger* produces large quantities of conidia that are carried on two-row stregmata; these conidia are black, in long chains. Conidia of *A. niger* cause respiratory problem in people exposed to inhalation of such germs, such as people working in poultry farms, and so on. *Circinella umbellate* is a fungus belonging to zygomycotina that has huge amounts of spores in many sporangia (**Figures 2–8**). Previous

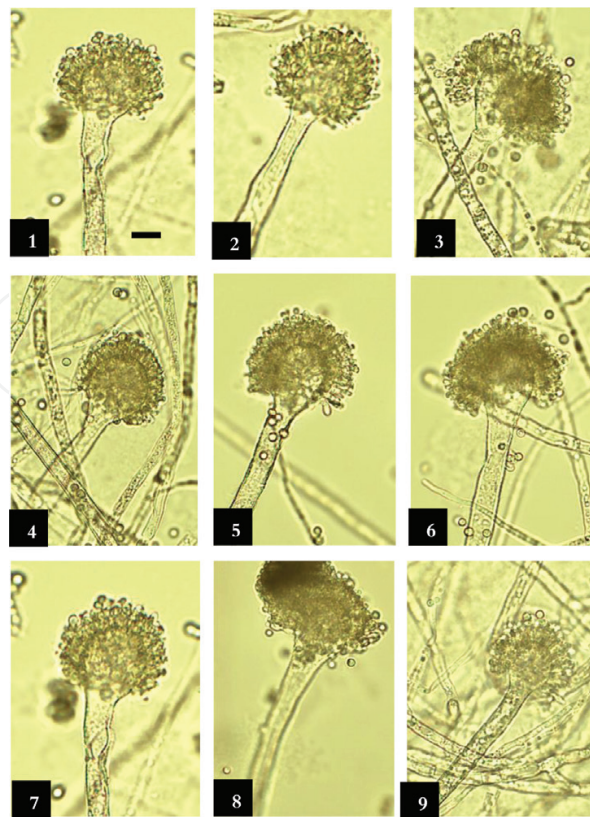


Figure 2. Hyphal growth of *Aspergillus flavus* on PDA at 28°C. Photos 1–9 were shot using ordinary compound microscope. Non-septate conidiophores and foremost radiate heads with mono- and biserial sterigmata carried on conical-shaped vesicles. Bar 10 µm in the photo 1 is the same for rest of the photos.

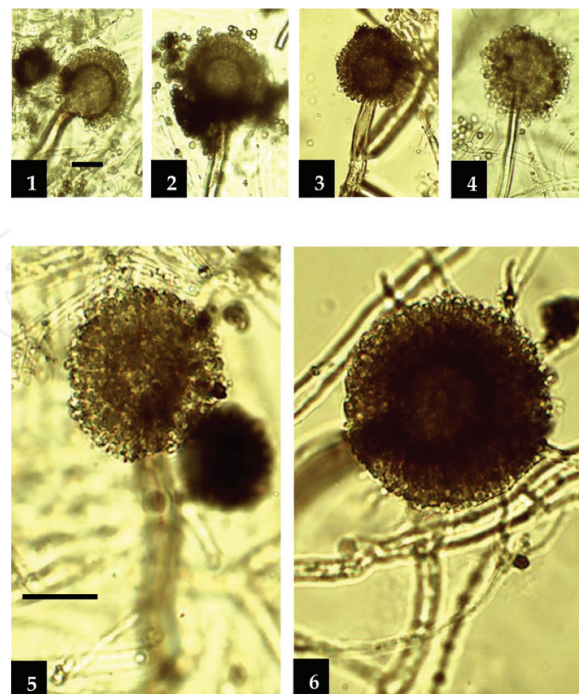


Figure 3. Hyphal growth of *Aspergillus niger* PDA at 28°C. Photos 1–6 taken by ordinary compound microscope. Non-septate conidiophores and radiate heads with biserial sterigmata. Bar 10 µm in the photo (1) is the same for photos 2–4, and in photo 5 is the same as photo 6.

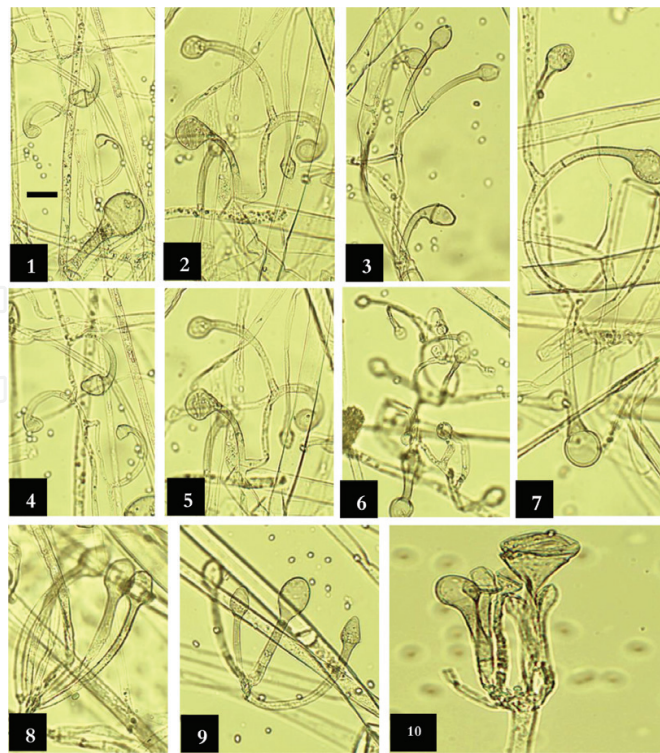


Figure 4. Hyphal growth of *Circinella umbellate* on PDA at 28°C. Photos 1–10 are taken by ordinary compound microscope. 1–7 branched conidiophores with curved side branches cut off by sporangia. 8–10 gatherings tent form sporangiophores. Bar 10 μm in Photo 1 is the same for rest of the photos.

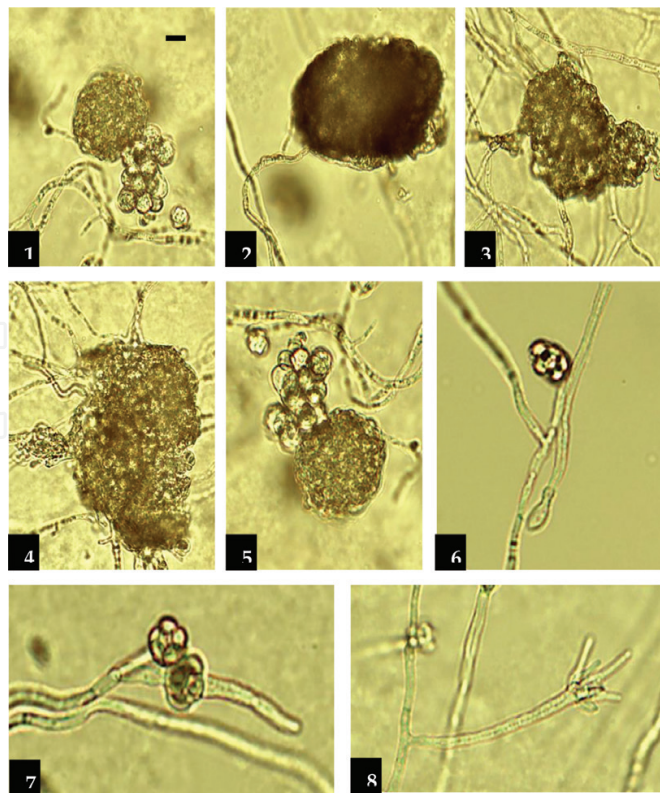


Figure 5. Hyphal growth of *Gliocladium* sp. on PDA at 28°C. Photos 1–8 shot by ordinary compound microscope. 1–7 gelatinous grouping of conidia. 8 A conidiophore loads a distinguishing sterigmata peculiar of this fungus. Bar 10 μm in Photo 1 is the same for the rest of the photos.

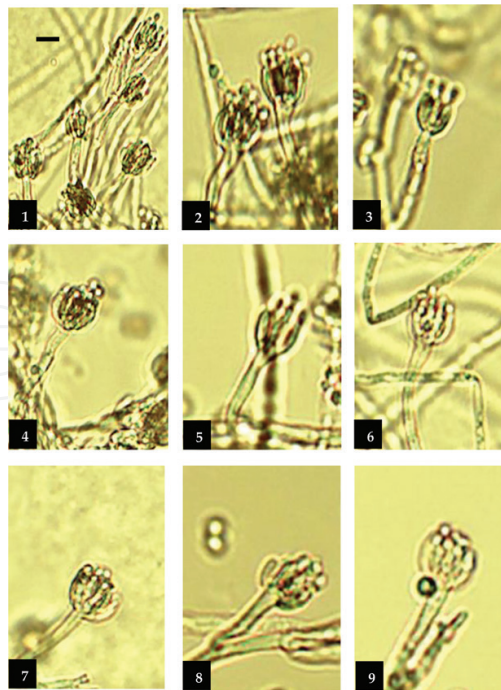


Figure 6. Hyphal growth of *Penicillium frequentans* on PDA at 28°C. Photos 1–9 shot by ordinary compound microscope. 1–9 septate conidiophores carried monoseriate sterigmata. Bar 10 μm in Photo 1 is the same for the rest of the photos.

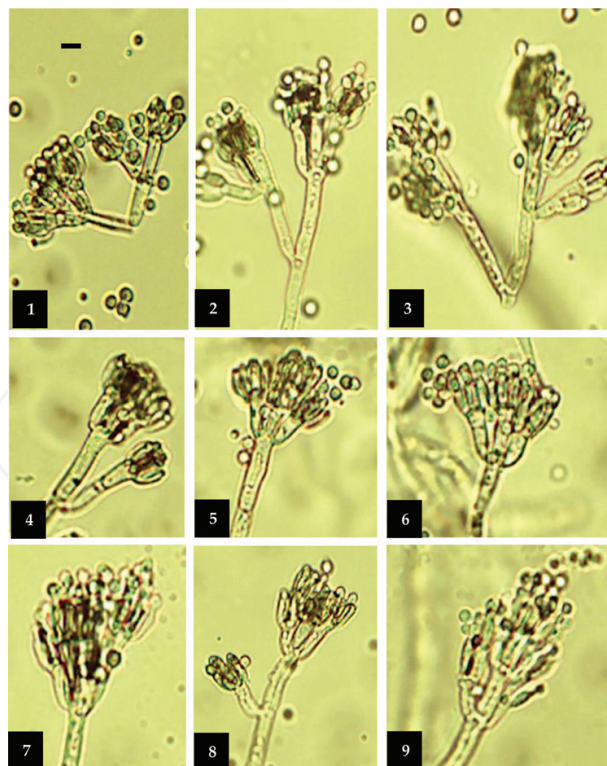


Figure 7. Hyphal growth of *Penicillium islandicum* on PDA at 28°C. Photos 1–9 shot by ordinary compound microscope. 1–9 septate conidiophores carried biseriate sterigmata and symmetrical. Bar 10 μm in the Photo 1 is the same for of the rest of the photos.

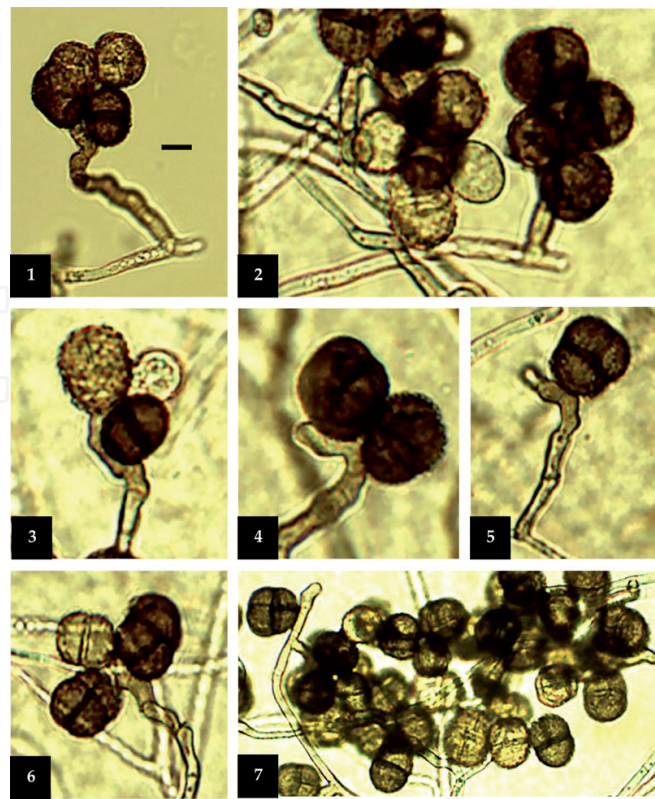


Figure 8. Hyphal growth of *Ulocladium atrum* on PDA at 28°C. Photos 1–7 shot by ordinary compound microscope. 1–7 septate conidiophores carried singularly broad conidia with rough wall has transverse septa (1–3 septa) and longitudinal septum (one or more), comparatively strict at the base and wide at the top. Bar 10 µm in Photo 1 is the same for rest of the photos.

studies suggest that this fungus can cause chest allergic diseases and asthma-like symptoms for people with low immunity who are subject to inhaling its spores [13].

A previous study has shown that *Penicillium frequentans* and *P. islandicum* produced a type of fungal toxin called aflatoxin (AFs) [14]. They further caused pulmonary disease, hypersensitivity, allergy (alveoli), a kind of emphysema [15]. As the two fungi produces chains of conidia, which are easy to spread through dusted air with the mass of wheat stored in the silos, therefore, these toxins can cause risk of spread of diseases for dealers within the silos.

While literature treated *Gliocladium* as a class of “useful fungi,” modern research raveled the contribution of it in the production of a toxin named Gliotoxin [16].

Ulocladium atrum is not far from the problems caused by the fungi mentioned above. It was mentioned in some previous studies for its ability to cause allergy in chest and respiratory system in humans [17].

By reviewing the previous narration (above), we can say that the isolated fungi of this study are present in the form of conidia and spores, lying on the surface of wheat grains and between folds of its coats.

There are two types of risks because of the existence of conidia and spores on wheat surfaces. The first risk is the exposure of dealers with large quantities of conidia and spores

inside the silos. The second danger is the possibility of growth of these conidia and spores, with the availability of moisture, to be innate growths producing very dangerous toxins to humans. In order to prove the first risk in a measurable experimental way, an experiment was conducted to determine the damage that could occur as a result of invasion of conidia and spores of the isolated organisms into human lungs and then into the blood via alveoli in one way or another.

5. An experiment showing the effect of inhaling spores and conidia of the isolated fungi on public health of exposed individuals

Biological effect of spores and conidia of *Aspergillus flavus*, *A. niger*, *Circinella umbellate*, *Gliocladium* sp., *Penicillium frequentans*, *P. islandicum*, and *Ulocladium atrum* on the decomposition of red blood cells in humans.

Results of the study showed the ability of each of the tested fungi to analyze the red blood cells in a human blood sample. There was a disparity in the effect of that on the severity of decomposition (hemolytic activity). *P. frequentans* performed highest response to disruption of the human red blood cells (63%), followed by *Gliocladium* sp. (51%), and *A. niger* (50%), respectively. Conidia and spore suspension of each of *Ulocladium atrum*, *Circinella umbellata*, *Aspergillus flavus*, and *Penicillium islandicum* donated sponges of 23, 22, 20, and 19%, respectively (**Figures 9 and 10**).

Despite all evidences from previous studies that confirm the seriousness of isolated fungi on the health of dealers and exposers, we have tested the ability of these fungi on hemolysis

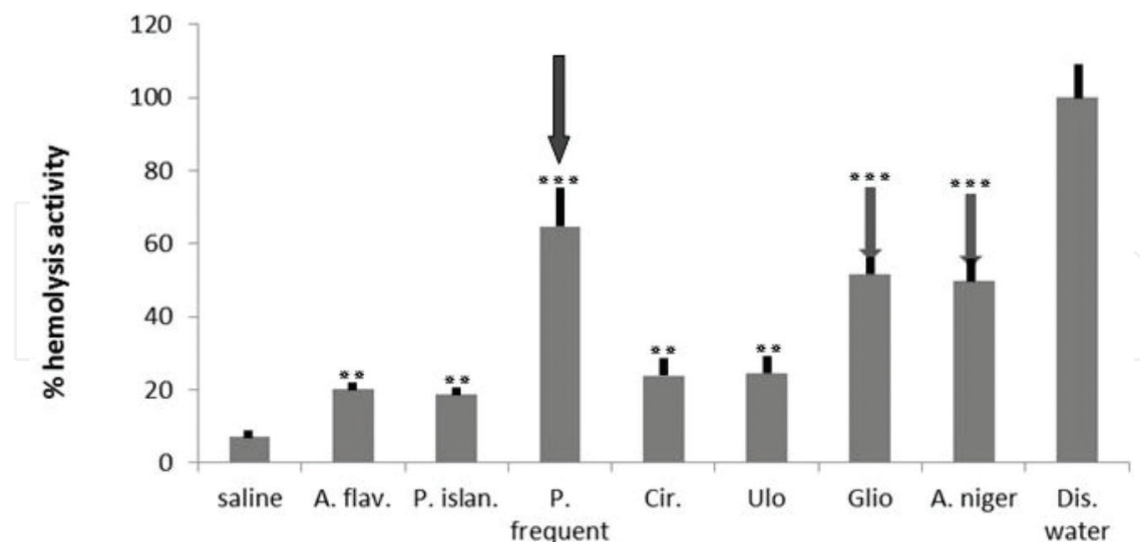


Figure 9. Influence of conidia and spore suspension of *Aspergillus flavus*, *A. niger*, *Circinella umbellate*, *Gliocladium* sp., *Penicillium frequentans*, *P. islandicum*, and *Ulocladium atrum* on breakdown of the human red blood cells. Bars above columns symbolize standard error of average data from three replicates and reveal differences between averages of samples related to control. Significant values against control represent: ** = highly significant at $p < 0.01$, *** = very significant at $p < 0.001$.

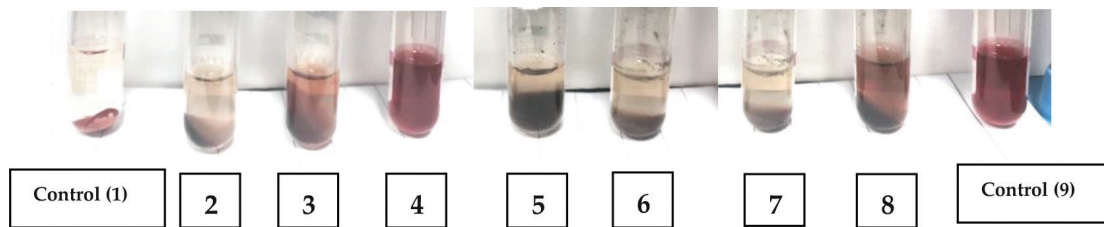


Figure 10. Influence of conidial and fungal spores suspension of *Circinella umbellata* (sample No. 2), *Gliocladium* sp. (sample No. 3), *Penicillium frequentans* (sample No. 4), *Ulocladium atrum* (sample No. 5), *Penicillium islandicum* (sample No. 6), *Aspergillus flavus* (sample No. 7), and *Aspergillus niger* (sample No. 8) on hydrolysis of human red blood cells. Sample 1, is negative control sample [human blood + saline solution (0.9% NaCl)], and Sample 9, is the positive control sample [human blood + distilled water].

of human red blood cells. From our results, *Penicillium frequentans*, *Gliocladium* sp., and *Aspergillus niger* caused significant damage and decomposition of red blood cells at high rates compared to other fungi, while rest of tested fungi also caused damage, but less than the above which highlights the risk of exposure to conidia and spores of those fungi. Our findings here correlate with previous studies on how bad these fungi are, although no data are available on the effect of conidia and spores of some fungi on the decomposition of human red blood cells. All this confirms without any doubt the seriousness of the intense exposure to conidia and spores of fungi and that the huge quantities of wheat stored in silos is dangerous sources for dealers and exposer. We recommend using nasal and oral masks for people working in silos and exposed to dust carrying fungal conidia and spores generated by the movement of wheat grains.

From another dimension, these conidia and spores of fungi are sources of severe contamination to wheat stored in silos. In the case of moisture, wheat becomes an ideal environment for growth, reproduction, and growth of fungi, which may grow inside the wheat mass, producing dangerous fungi that produce toxins.

6. Conclusion

With the steady increase in human numbers and high living and nutrition requirements, it is imperative to increase the production of important cereal crops for large segments of the population. Wheat is a very important component of human needs throughout the world. Therefore, since ancient times people have been interested in working on storing this important and vital commodity to get it in time of need. Granaries and silos were established and they continued to develop until they reached the current structural and architectural design. Nowadays, nearly every country in the world has several silos spread throughout its land to cover the continuing needs of cereals. It should be noted that many countries in the world have a much higher request for wheat than their production. This leads to the import of this important commodity from places of production surplus from the need of producers. Since its harvest, wheat has been subjected to successive steps of transport and conservation, which makes it vulnerable to pollution and damage. As wheat crop is subjected to the sifting process, which removes grain from the harvest residues and soil granules, this factor will be ignored.

Large quantities of wheat during shipping process through giant vessels are exposed to many risks. High humidity of transport containers overseas increases chances of wetting wheat grains and thus chances of increasing the contamination and growth of fungi in wheat. Sometimes conidia and spores of fungi germinate within the mass of wheat grains and producing innate fungi which may not be seen by the naked eye and generating very dangerous toxins. Often, when shipments of wheat arrive via transport to silos, they are loaded with many elements of danger.

In one of the studies conducted by the authors on the presence of harmful fungi in the mass of wheat inside a silo, *Aspergillus flavus*, *A. niger*, *Circinella umbellata*, *Gliocladium* sp., *Penicillium frequentans*, *P. islandicum*, and *Ulocladium atrum* were isolated. Since these fungi have precedents to cause some diseases, an experiment has been conducted to prove this. The test of the ability of the isolated fungi to analyze human red blood cells has been shown to have a high coefficient of effect.

In theory, based on the scientific data and results of the previous studies, we proposed to provide a healthy environment within the silos, which is summarized as follows:

1. Washing wheat grain at the before entering for storage in the silos and then drying with constant exposure and flipping to warm air currents from the sources of solar energy.
2. Exposing air silos to ultra violet light during periods of non-working workers.
3. Use of aerosols and volatile oils to help sterilize air silos.
4. Put moisture absorbent materials such as calcium chloride and silica gel, to ensure dry storage environment.

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