

Occurrence of Hemolytic Fungi Mounted on Wheat Grains in the main Silo of Sakaka, Saudi Arabia

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A lot of countries around the world are seeking to strategic storage of large quantities of wheat grain in giant silos for use in the food industry for humans, animals and poultry. Length of the period of transport and storage, which can be exposed to amounts of moisture qualifies them to serious injury of many types of dangerous fungi. Objectives of this study were to identify fungi mounted on wheat grain stored in the main silo in the city of Sakaka, Saudi Arabia and to study the ability of these fungal spores and conidia to cause damage to people exposed to inhalation of large quantities of the dust of wheat grain in causing lyses of human red blood cells. *Aspergillus* was consistently the most frequent genus (100% of places) and contributed by *A. flavus* and *A. niger* were of high frequency (20 places out of 20) contributing 19.3% and 19% (total 38.3%) of the total count, respectively. *Penicillium* was recovered from 18 samples matching 36.5% of total count fungal isolates. It was represented by *P. frequentans* and *P. islandicum* sharing 18.5% and 18%, respectively. *Ulocladium atrum* gave 15.7% of the total count but with low occurrence. *Circinella umbellata* and *Gliocladium* sp. were less frequently isolated representing 7.4% and 2.1% of total count. The tested 7 fungi proved the ability to break down human red blood cells but with varying degrees in varying significant intensity.

Keywords: fungi, hemolytic activity, Sakaka, silo, spores and conidia.

Nowadays, however SURGE in the number of human beings and the hustle and human congestion, became obsessed with the growing pollution levels on the surface of events. It is worth mentioning that the sources of pollution are many and varied, and worse, is that pollution can be up to any place on the earth's surface. Bio-contamination is a kind of pollution sources, and it passed on vital pollutants from one place to another, causing problems for the place which receives pollution.

Wheat is a major source of carbohydrates in many countries of the world. Some countries produce large quantities of wheat which is enough for domestic use and the rest is exported to other

countries that suffer from a lack of this product. Wheat flour enters in bread production, which is essential in all diets for humans. Wheat flour is also included in candy industry, biscuits and baby food and so on¹. Additionally, wheat grains and flour enter as a key ingredient in feed for poultry and the cattle². Therefore, many countries need massive amounts of grains and wheat flour where store it in huge quantities in places such as silos. There are also some countries that import large quantities of wheat which shipped across oceans in giant ships³.

During storage and transport in wet conditions, those massive stockpiles of grain and flour are affected and encourage growths of various fungi that emit spores and toxins in these foods⁴. It is known that many of fungi have the ability to excrete metabolic mycotoxins within the media that grow in it⁵. Since wheat comes from a

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variety of geographical locations to be stored in silos, it makes sense that they carried with it a variety of types of fungi. Sometimes fungi grow in deep places hidden in piles of grain which created fungal growths extremely dangerous as producers of mycotoxins. Subsequently, it is necessary to know fungi situated on grains stored in silos of Sakaka city, Aljouf, Saudi Arabia and ability of these fungi to be factors of human hazard as hemolytic elements.

One of the main objectives of this study is the isolation and identification of fungi located on wheat grain stored in silos of Sakaka city, Saudi Arabia. Study the effect of spores and conidia of the isolated fungi on the decomposition of human red blood cells is one of the objectives of this research.

MATERIALS AND METHODS

Sampling places

Twenty samples (each 200 gm), were collected from inside the heaps of wheat grains (at a depth of 30 cm under the surface) from the silo of Sakaka city, Aljouf governorate, Saudi Arabia, during October, 2015.

Fungal isolation and identification

In a 300 ml bottle of sterile water-packed, each bottle was opened next to the rallies in wheat grain silos Sakaka city and half of the bottle was discarded and then each bottle was placed filled with wheat grains. Aliquots of one ml water containing washing grains were added to malted (50°C) Potato dextrose agar (PDA), which is most suitable culture media for the isolation of seed borne fungi, with Rose-bengal [1/15000 ml / media / 1 medium]⁶. Plates were incubated at 28°C for 7 days or until emergence of colonies and then purified to be examined. The number of the identified fungi was counted. Fungal genera and species were identified using colonial and microscopical structures with the aid of taxonomic keys of⁷⁻¹².

Study the ability of isolated fungi on the hemolytic activity to the human red blood cells

To study the toxicity of different types of fungi that have been isolated used human red blood cells through the method of^{13,14} was followed. Solutions of conidia or spores of the tested fungi were prepared using neutral osmotic solution (0.9

% NaCl). One hundred µl of human blood, after washing with saline solution (0.9 % NaCl) three times, was incubated with 900 µl of each of the tested spore suspension or sodium chloride solution, which represents a negative control sample, as well as, or distilled water (positive control sample), under aseptic conditions, for comparison. After incubation at 27°C, for 24 hours, in the dark, components of the mixture were separated using a centrifuge and absorbance of the supernatant was then measured using UV visdspectrophotometer (spectro uv-2505) at 540 nm to calculate percentage of dissolution and cracking of red blood cells according to the following formula (each treatment was repeated 3 times and subjected to statistical analysis):

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

Statistical analysis

ANOVA was used to assess data using Minitab statistical software (version 12) unless somewhere else mentioned.

RESULTS

Numbers in Table (1) reveal that 7 species belonging to 5 genera were isolated from 20 samples placed in the main silo of Sakaka city, Aljouf governorate, Saudi Arabia. *Aspergillus* was consistently the most frequent genus (100% of places) and contributed by *A. flavus* and *A. niger* were of high frequency (20 places out of 20) contributing 19.3% and 19% (total 38.3%) of the total count, respectively, by the used isolation method (Figs. 1&2). *Penicillium* came second which was recovered from 18 samples matching 36.5% of total count fungal isolates. It was represented by *P. frequentans* and *P. islandicum* sharing 18.5% and 18%, respectively, (Figs. 5&6). *Ulocladium atrum* gave 15.7% of the total count but with low occurrence (Fig. 7). *Circinella umbellata* and *Gliocladium* sp. were less frequently isolated representing 7.4% and 2.1% of total count (Figs. 3&4).

Study the ability of fungi isolated on the hemolytic activity of the red blood cells of the human Hemolytic activity

Results revealed that all of the tested fungi had the ability to analyze and break down

human red blood cells but with varying degrees in intensity, as shown in Figs (8 & 9).

P. frequentans appeared the highest ability to break down human red blood cells (by analyzing 63%), followed by *Gliocladium* sp. (Analysis by 51%), then *A. niger* (analysis by 50%). Spore suspension of *Ulocladium atrum*, *Circinella umbellata*, *Aspergillus flavus* and *Penicillium islandicum* gave effects ratio of 23, 22, 20 and 19%, respectively (Fig. 8).

DISCUSSION

Search results showed, the presence of fungi *Aspergillus flavus*, *Aspergillus niger*, *Circinella umbellata*, *Gliocladium* sp., *Penicillium frequentans*, *Penicillium islandicum*, and *Ulocladium atrum*, mounted on surfaces of wheat grains collected and stored in the main silo of the city of Sakaka, Aljouf governorate, Saudi Arabia, in October, 2015.

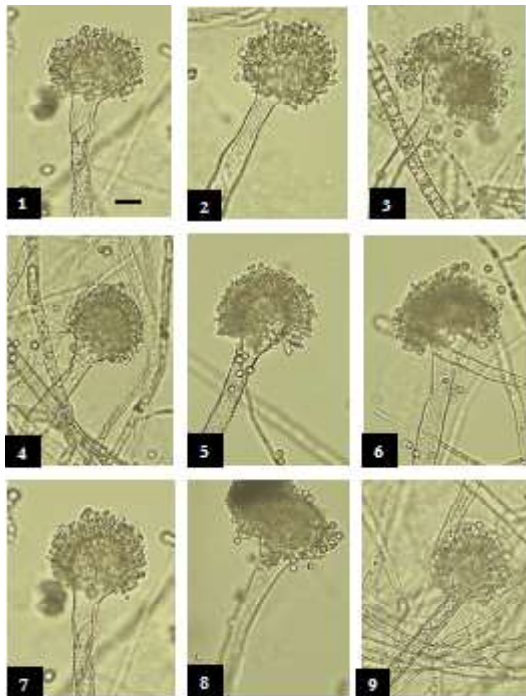


Fig. 1. Mycelial growth of *Aspergillus flavus* on PDA at 28°C in the dark. Images from 1-9 were taken using normal compound microscope. Aseptate conidiophores and mostly radiate heads with mono and biseriate sterigmata mounted on flask shaped vesicles. Bar 10 µm in the pictures (1) is the same for all images

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

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	5L	7.4
<i>Gliocladium</i> sp.	8	4L	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	3L	15.7
Gross total counts	378		
No. of genera	5		
No. of species	7		

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

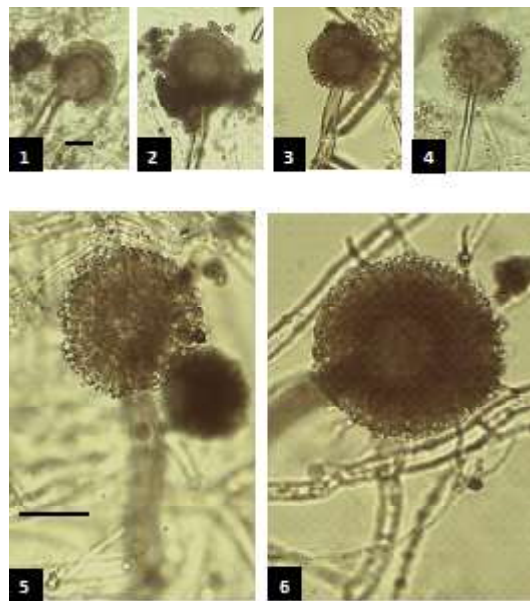


Fig. 2. Mycelial growth of *Aspergillus niger* PDA at 28°C in the dark. Images from 1-6 were taken using normal compound microscope. Aseptate conidiophores and radiate heads with biseriate sterigmata. Bar 10 µm in the pictures (1) are the same for photos 2-4, and in picture 5 is the same as the image of No. 6

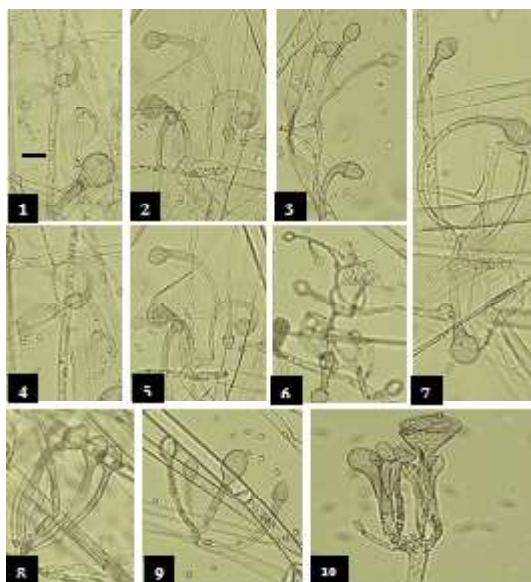


Fig. 3. Mycelial growth of *Circinella umbellate* on PDA at 28°C in the dark. Images from 1-10 were taken using normal compound microscope. 1-7 Branched conidiophores with curved side branches ended by sporangia. 8-10 gatherings tent form sporangiophores. Bar 10 µm in the pictures (1) are the same for all images

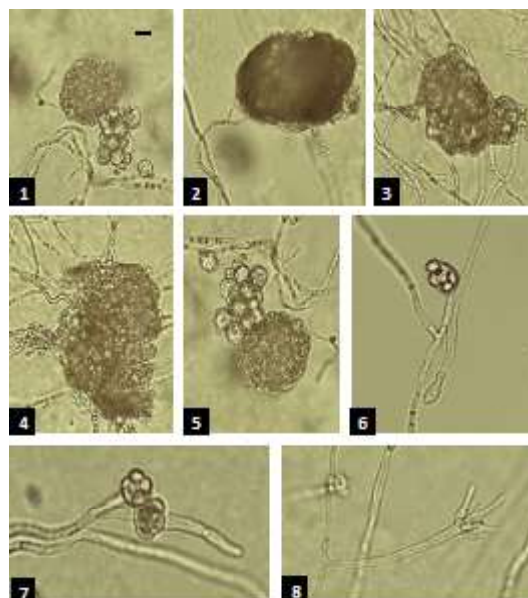


Fig. 4. Mycelial growth of *Gliocladium* sp. on PDA at 28°C in the dark. Images from 1-8 were taken using normal compound microscope. 1-7 gelatinous aggregations of conidia. 8 A conidiophore carries a distinctive sterigmata characteristic of this fungus. Bar 10 µm in the pictures (1) is the same for all images

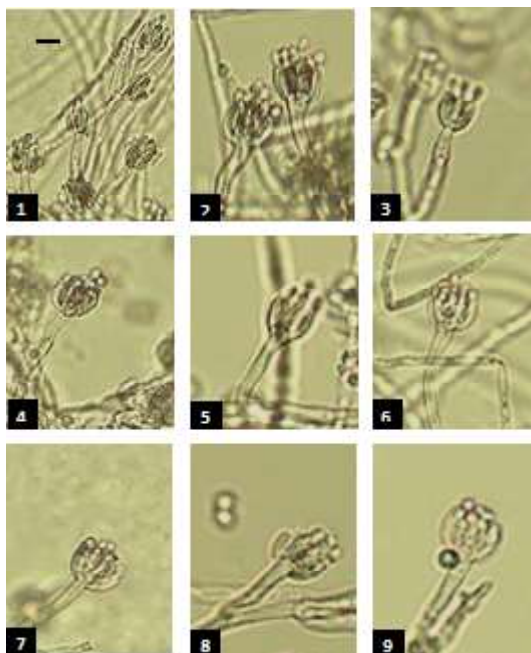


Fig. 5. Mycelial growth of *Penicillium frequentns* on PDA at 28°C in the dark. Images from 1-9 were taken using normal compound microscope. 1-9 septate conidiophores mounted monoseriate sterigmata. Bar 10 µm in the pictures (1) are the same for all images

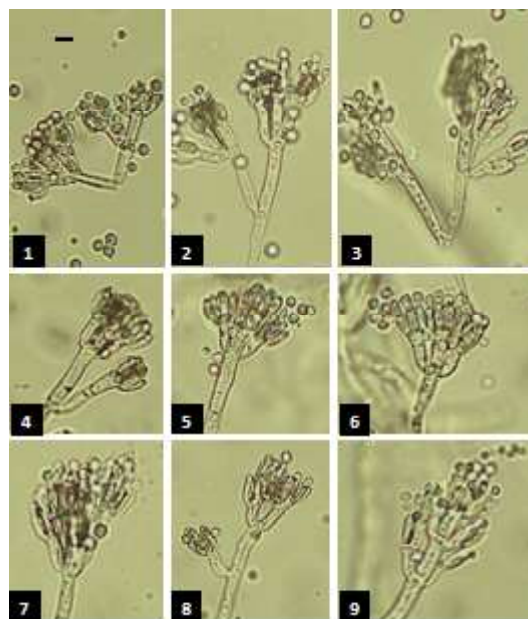


Fig. 6. Mycelial growth of *Penicillium islandicum* on PDA at 28°C in the dark. Images from 1-9 were taken using normal compound microscope. 1-9 septate conidiophores mounted biseriate sterigmata and symmetrical. Bar 10 µm in the pictures (1) are the same for all images

It is known that fungi isolated in this study have precedents in the cause of serious diseases to humans and animals¹⁵⁻²⁷. Furthermore, many previous studies have demonstrated is no doubt that exposure to grains agricultural dust was one of the important causes of many of respiratory and allergic diseases for people exposed to the dust²⁸⁻³¹. In detailed terms, it found that penicillin, such as, *Penicillium frequentans* and *Penicillium*

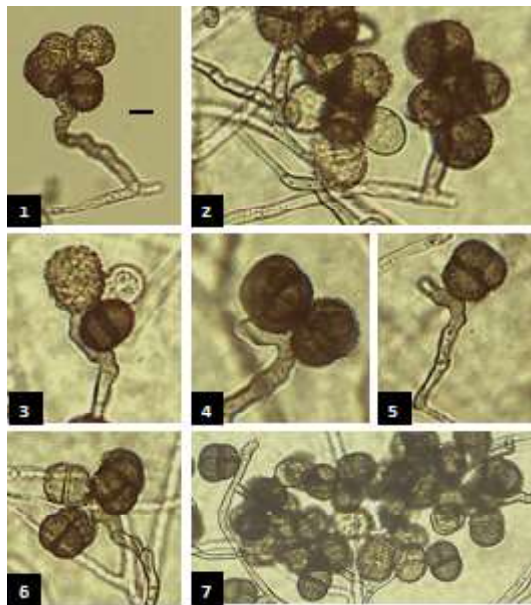


Fig. 7. Mycelial growth of *Ulocladium atrum* on PDA at 28°C in the dark. Images from 1-7 were taken using normal compound microscope. 1-7 septate conidiophores mounted solitarily broad conidia with rough wall has transverse septa (1-3 septa) and longitudinal septum (one or more), somewhat narrow at the base and broad at the top. Bar 10 µm in the pictures (1) are the same for all images

islandicum were the cause of pathogenesis of pulmonary disease, hypersensitivity, alveolitis allergic (alveoli), skin allergies and emphysema, as well as the production of dangerous toxins^{32,33}. *Aspergillus niger* is also the huge producer of conidia was the biggest causes of disease resembling tuberculosis and called Aspergillosis, which is spread in poorly ventilated and heavy pollution and dust places³⁴. Previous studies have also postulated the involvement of *Ulocladium atrum* and *Circinella umbellate* in chest allergy^{35,36}. It is worth mentioning that *Aspergillus flavus* is a type of saprophytic and parasitic factor, with the global distribution of its presence. Colonization of many grains, legumes, and nuts by this fungus, may cause rot and maceration of grains during the harvest season, storage or transport, especially in the slow ships that travel long distances through

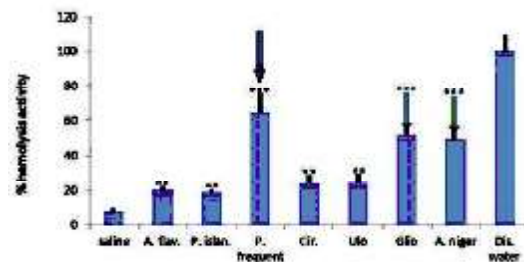


Fig. 8. Effect of spores suspension of 7 tested fungi on decomposition of the red blood cells of a human sample. Bars above all drawing column, represent the standard error of the average data from three replicates and reflect differences between averages of the samples compared to the control sample. Significant values against control represent: ** = highly significant at $p < 0.01$, *** = very significant at $p < 0.001$

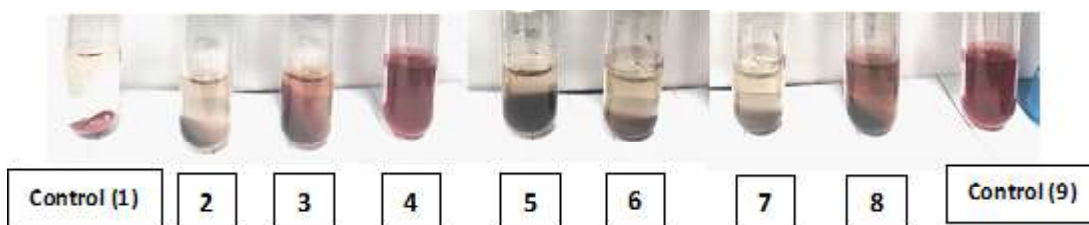


Fig. 9. Effect of fungal spores suspension of each of *Circinella umbellata* (sample No. 2), *Gliocladium* sp. (sample No. 3), *Penicillium frequentans* (sample No. 4), *Ulocladium atrum* (sample No. 5), *Penicillium islandicum* (sample No. 6), *Aspergillus flavus* (sample No. 7), and *Aspergillus niger* (sample No. 8) on the breakdown of red blood cells of a human sample. Sample No. 1, representing the negative control sample [human blood+saline solution (0.9% NaCl)], and Sample No. 9, representing the positive control sample [human blood+distilled H_2O]

humid environments. And for sure, it was found that many of strains of this type produce large amounts of known biological toxins called mycotoxins, which when consumed becomes very toxic to mammals, causing very serious diseases such as liver cancer and diseases of the respiratory system to humans and animals especially for people with weakened immune systems³⁷. Although many previous studies have put *Gliocladium* in a rank of “non-pathogenic fungi”, but recent studies have shown the involvement of this organism in the secretion of a kind of fungal toxins called Gliotoxin³⁸.

And where the fungus, mentioned above, have been isolated in this study, so their presence in wheat grains stored in the main silo in Sakaka city, constitutes a serious source of pollution, especially when there is moisture and the growth of this fungus inside piles of wheat grains, without appears to the consumers, causing a disaster disturbing the excretion of mycotoxins and produced huge amounts of spores and conidia that can cause diseases of the respiratory system of mammals consuming those foodstuffs. The exposure of workers in the storage areas and silos to dust raised from grains movement poses a significant risk to public health. Breathing in wheat grains dust can affect the health and overall comfort for workers who work in silos.

Results of this study showed that fungi of *Penicillium frequentans*, *Gliocladium* sp. and *Aspergillus niger* were caused severe decomposition of red blood cells in humans at high rates and impressive, and the rest of tested fungi gave significant impact, too, which cast a stern warning on exposure to these fungi. These results are harmonious with previous studies documenting a relationship that fungal disease to humans, although previous studies have not carried out the experiment of Hemolysis that conducted in our research.

Therefore, we recommend the reality of the results of this research to wear masks covering the nose and mouth such as influenza masks in order to reduce the entry of dust laden with fungal germs and conidia to the respiratory tract for those people. Additionally, it is recommend making sure to dry the environment of stored grains to prevent encouraging fungal growth by placing dissecting materials in places of conservation.

Grain dust is a complex soup that is made up of both organic and inorganic particles. Some of these can be inhaled easily, and depending on their size, can find their way deep into various parts of the respiratory system causing a range of adverse health effects. Entry of Fungal spores to the inside of the human body and the possibility of interaction with the blood can not be given by this study and needs further investigation.

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