STUDIES ON STORED GRAIN FUNGI

Part 1.—Fungi from Wheat and Rice from Karachi

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Studies to isolate and identify different fungi from seven varieties of rice (Basmati, Joshi, Kangni, Permal, Begmi, Basmati Broken and Sela Basmati) and two varieties of wheat (Red and White) stored in four different godowns in Karachi area were carried out.

A total of 132 samples were screened from which 550 colonies of fungi emerged. Forty-three species and 7 genera belonging to different groups of fungi were isolated. Among rice, Joshi and Basmati were found to be highly susceptible to storage fungi. From Joshi 15 fungi while from Basmati 16 organisms were isolated. In wheat varieties, White was more susceptible than Red variety.

Aspergillus flavus with a percentage of 29.7 was the most prevalent organism on both wheat and rice. The frequency of other *Aspergilli* like *A. sydowi*, *A. tamarii*, *A. versicolor*, and *A. candidus* was low as compared to *A. flavus* and *A. niger*.

Phaeoramularia sp., *Aspergillus nidulans*, *Absidia corymbifera*, *Alternaria tenuissima* and *Circinella* sp. appear to have been isolated for the first time from wheat and rice.

Surface disinfection of seeds at 1:1000 $HgCl_2$ for 1,2,3,4 and 5 min decreased the frequency of fungi and at 5 min interval very few fungi appeared.

Introduction

The storage of food grains in disease-free form is the prime concern of the storage pathologists. According to an international survey report by the Food and Agricultural Organization of the United Nations,[1] about 10% of the total world food production is lost in storage due to pest and fungus infection and infestations. Such great losses through pest deterioration cannot be afforded particularly in the developing countries as there already exists a food shortage due to increased population.

In Pakistan where storage facilities are still in initial stages of development, the losses are approximately 10–18%.[2] It is estimated that annual loss of stored grains in Pakistan is approximately Rs. 10 crores. The damage is caused by insects (5–6%), rats (6–10%) and fungi (1–2%). Different organizations have done some work on the control of insects[3,4] in storage but not much work has been done on the isolation and identification of fungi from stored food grains in this country. Scanty records are available as comprehensive studies have not been carried out so far.

In West Pakistan the losses are highest, about 20% in Hyderabad and Karachi area alone.[1] The losses are much less in former Punjab and Frontier regions.[1] Due to these heavy losses in Hyderabad and Karachi region and the availability of meagre records on the isolation and identification of fungi from stored grains, the authors considered it necessary to survey four different godowns in Karachi in order to study and ascertain the prevalence and frequency of various fungi present on the grains.

The godowns under study store and maintain the imported wheat from U.S.A. and Australia and rice varieties commonly grown in Pakistan.

Material and Methods

Samples were collected twice from four different godowns situated in various parts of the city. The house type godowns were properly maintained and were mostly of concrete. The cereal grains were kept in jute bags placed on wooden platforms. Fumigation with methyl bromide was carried out periodically by the technical staff of these godowns to control insects while fungi and other molds remained active on the grains. All varieties of rice and wheat were not available in the four godowns visited for collecting the samples. Basmati and Joshi were available in all the four godowns except in third, while Permal, Begmi and Kangni were only available in the first godown. Basmati broken and Sela Basmati were present in second and third godown, respectively.

One special spatula was made of hard tin with one end pointed while the other end was kept hollow. The pointed end was inserted into the jute bags in order to obtain the samples directly into the sterilized flasks. The flasks were then

immediately plugged. The samples were collected at random from different bags, heaps and stocks.

The samples were brought in the laboratory for experimentation. Five grains were taken randomly from each flask and planted asceptically, without surface disinfection, in petri plates containing Czapek's–Dox agar medium and 10% phosphoric acid (phosphoric acid was used to control any possible emergence of bacteria). The petri dishes were incubated for 2–7 days at 28°C. The experiment was replicated 5 times.

Grains from the same samples were surface sterilized with 1:1000 mercuric chloride solution for 1,2,3,4, and 5 min in order to eliminate fungi present on the surface. The grains were then washed thoroughly with distilled water. Five grains were planted in sterilized petri plates containing Czapek's–Dox agar medium. Sample number and the name of the variety was marked on the plates which were incubated at 28°C for 2–7 days. Air-oven method[5] was adopted for determining the moisture in the grains.

Mucorales, the fast growing group of fungi were isolated after 2 days on Czapek agar slants. The emerging colonies of other fungi were periodically observed and isolated in culture tubes through repeated transfer and retransfer technique and thus pure cultures were obtained.

For detailed investigations and confirmation of various fungi the cultures were reinoculated in petri dishes in different media, pH and incubated at various temperatures. These factors are generally of great help for correct identification of genera and species. The colour of the colonies, growth pattern, measurements of mycelium, conidia, conidiophore and other fruiting bodies were taken into consideration for specific identification. Mycelium and fruiting bodies were mounted in lactophenol and stained either in cotton blue or acid fuchsin.

Results

Forty-three species and 7 genera belonging to different groups of fungi were isolated from 7 varieties of rice and 2 varieties of wheat collected from four different godowns during the present screening. Figures 1 and 2 show different fungi emerging out of plated wheat and rice seeds, respectively.

From Permal variety 5 different fungi, 4 belonging to Imperfect fungi and 1 to Phycomycetes, were isolated. Begmi variety contained higher number of fungi than Permal. Kangni variety was the least infested and carried only *Aspergillus flavus* and *A. niger*.

Basmati was stored in all four godowns in huge stocks and thus 16 different organisms belonging to fungi Imperfecti and *Phycomycetes* were isolated. The frequency of *Aspergilli* was higher than the members of *Phycomycetes*. Joshi variety, which was available in first, second and third godowns, yielded 15 different fungi. Only 2 fungi from Basmati Broken and 4 from Sela Basmati were recorded.

The two wheat varieties, Red and White, imported from U.S.A. and Australia were stocked in first and second godowns. In the first godown only white (American) variety was available.

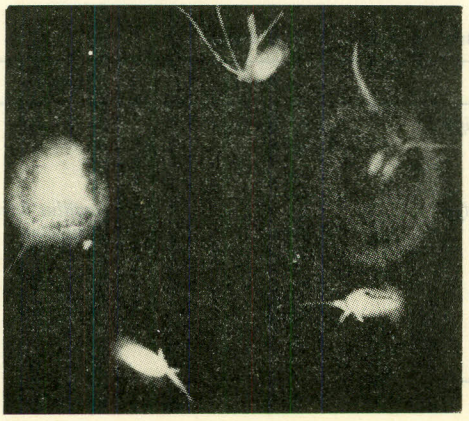

Fig. 1.—Fungi growing out of plated wheat seeds in Czapek medium.

Fig. 2.—Fungi emerging from rice in Czapek medium.

From this variety 18 different genera and species of fungi were isolated while from the Red variety 10 fungi were recorded. *Chaetomium globosum* and *C. minutum* belonging to *Ascomycetes*, were only isolated from White wheat variety during the course of entire experiment.

Field fungi like *Alternaria*, *Cladosporium*, *Epicocum*, *Curvularia*, *Stemphylium* and *Paecilomyces* were abundant on wheat than rice varieties (Tables 1–4).

When the grains were surface disinfected with mercuric chloride 1:1000 at different time intervals, it was observed that 24 different species of fungi appeared from those kept for 1 min but as the time of disinfection increased there was a gradual fall in the frequency of organisms. At 5-min duration, only 4 different species were isolated. There was a significant decline in the frequency of fungi at 1 to 4 min duration but the prevalence of fungi became fairly constant at 4–5 min duration (Fig. 3).

The results of this investigation are based on the screening of 132 samples of wheat and rice. From these samples a total number of 550 fungus

TABLE 1.—FUNGI ISOLATED FROM SEVEN RICE VARIETIES BEFORE SURFACE DISINFECTION.

Basmati	Sela Basmati	Basmati Broken	Begmi	Permal	Joshi	Kangni
Aspergillus flavus	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*
A. terreus	*A. niger*	*A. niger*	*A. terreus*	*A. niger*	*A. niger*	
A. niger	*A. fumigatus*		*A. niger*	*A. syaowi*	*A. terreus*	
A. fumigatus	*A. nidulans*		*A. fumigatus*	*A. fumigatus*	*A. fumigatus*	
A. sydowi	*Circinella* sp.		*A. sydowi*	*Rhizopus nigricans*	*A. versicolor*	
A. tamarii			*A. tamarii*		*A. fumigatus*	
Rhizopus nigricans			*Rhizopus nigricans*		*A. candidus*	
R. oryzae			*Rhizopur* sp.		*Rhizopus oryzae*	
Penicillium islandicum			*Circinella* sp.		*Mucor racemosus*	
Actinomucor sp.					*Paecilomyces vorioti*	
Cladosporium sphaerospermum					*Curvularia verruculosa*	
Absidia Corymbifera					*Penicillium cyclopium*	
					Cladosporium sphaerospermum	

TABLE 2.—FUNGI ISOLATED FROM FIVE VARIETIES OF RICE AFTER SURFACE DISINFECTION.

Two varieties, Sela Basmati and Basmati Broken were free from fungi.

Time (min)	Kangni	Joshi	Permal	Basmati	Begmi
1	*Aspergillus niger*	*Aspergillus niger* *A. flavus* *A. nidulans*	*Aspergillus niger* *A. fumigatus*	*Aspergillus niger* *A. flavus*	—
2	*Aspergillus niger*	*Aspergillus flavus* *A. fumigatus* *A. niger*	*Aspergillus niger* *A. fumigatus*	*A. flavus*	*Aspergillus flavus* *A. fumigatus*
3	—	*A. niger* *A. terreus* *A. fumigatus*	*A. fumigatus*	*A. niger* *A. flavus*	*A. flavus* *A. fumigatus*
4	*A. niger* *A. flavus*	*A. fumigatus*	—	—	—
5	*A. niger*	*A. niger* *A. candidus*	*A. niger*	—	—

TABLE 3.—LIST OF FUNGI ISOLATED FROM TWO WHEAT VARIETIES BEFORE SURFACE DISINFECTION.

White Variety	Red Variety
Alternaria tenuis	*Alternaria tenuis*
A. tenuissima	*Aspergillus fumigatus*
Aspergillus niger	*A. niger*
A. fumigatus	*A. flavus*
A. flavus	*A. sydowi*
A. sydowi	*Rhizopus nigricans*
A. tamarii	*Penicillium islandicum*
Penicillium funiculosum	*Cladosporium sphaerosperumum*
P. islandicum	
P. cyclopium	
Actinomucor sp.	
Paecilomyces varioti	
Absidia corymbifera	
Phaeoramularia sp.	
Curvularia verruculosa	
Epicoccum nigrum	
Chaetomium minutum	
C. globosum	
Stemphylium sp.	

TABLE 4.—LIST OF FUNGI ISOLATED AFTER SURFACE DISINFECTION WITH 1:1000 $HgCl_2$ FROM TWO (RED AND WHITE) WHEAT VARIETIES.

Time (min)	Fungi
1	*Aspergillus fumigatus* *Alternaria tenuis*
2	*Aspergillus niger* *A. fumigatus* *Penicillium viridicatum*
3	*Aspergillus fumigatus* *A. flavus*
4	*Aspergillus niger*
5	*Aspergillus fumigatus* *Alternaria tenius* *Stemphylium* sp.

colonies were isolated. The percentage of prevalence of some common fungi isolated from both kernels was determined. *Aspergillus flavus* dominated on an overall basis (29.7%) followed by other *Aspergilli* except *A. tamarii*. *Penicillium* and *Rhizopus nigricans* showed an equal (6%) percentage of prevalence. *Actinomucor* sp. (3.6%), *Circinella* sp. (3.2%), *Mucor mucedo* (2%) and *Phaeoramularia* sp. (1.1%) show a comparatively less frequency of occurrence in these stored grains (Fig. 4).

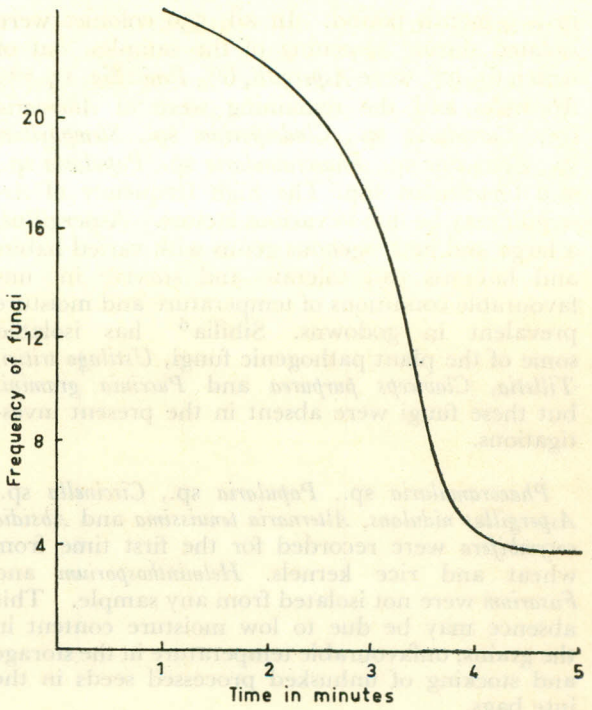

Fig. 3.—Frequency of stored grain fungi after surface disinfection with 1:1000 $HgCl_2$. Note the gradual decrease in the frequency of fungi at longer durations of disinfection.

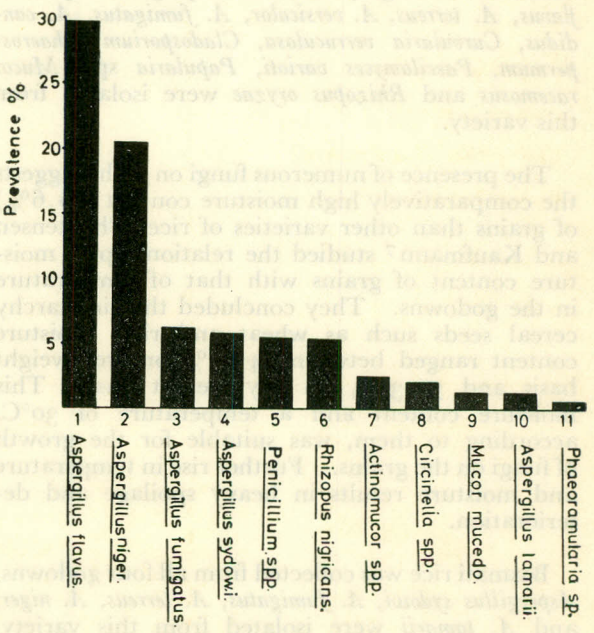

Fig. 4.—Overall per cent prevalence of different fungi in stored rice and wheat grains.

Discussion

In the present studies 132 samples of wheat and rice were collected from four different godowns,

in a 9-month period. In all, 550 colonies were isolated during screening of the samples, out of which 65.9% were *Aspergilli*, 6% *Penicillia*, 15.7% *Mucorales* and the remaining were of *Alternaria* sp., *Curvularia* sp., *Cladosporium* sp., *Stemphylium* sp., *Epicoccum* sp., *Phaeoramularia* sp., *Papularia* sp., and *Chaetomium* spp. The high frequency of *Aspergilli* may be due to various factors. Aspergillus, a large and heterogenous genus with varied habits and habitats can tolerate and survive in unfavourable conditions of temperature and moisture prevalent in godowns. Sibilia[6] has isolated some of the plant pathogenic fungi, *Ustilago tritici*, *Tilletia*, *Claviceps purpurea* and *Puccinia graminis* but these fungi were absent in the present investigations.

Phaeoramularia sp., *Papularia* sp., *Circinella* sp., *Aspergillus nidulans*, *Alternaria tenuissima* and *Absidia corymbifera* were recorded for the first time from wheat and rice kernels. *Helminthosporium* and *Fusarium* were not isolated from any sample. This absence may be due to low moisture content in the grains, unfavourable temperature in the storage and stocking of unhusked processed seeds in the jute bags.

Among the seven varieties of rice, Joshi was found to be highly infested. *Aspergillus niger*, *A. flavus*, *A. terreus*, *A. versicolor*, *A. fumigatus*, *A. candidus*, *Curvularia verruculosa*, *Cladosporium sphaerospermum*, *Paecilomyces varioti*, *Papularia* sp., *Mucor racemosus* and *Rhizopus oryzae* were isolated from this variety.

The presence of numerous fungi on Joshi suggests the comparatively high moisture content (10.6%) of grains than other varieties of rice. Christensen and Kaufmann[7] studied the relationship of moisture content of grains with that of temperature in the godowns. They concluded that in starchy cereal seeds such as wheat and rice, moisture content ranged between 24–25% on wet weight basis and 30–33% on dry weight basis. This moisture content and a temperature of 30°C, according to them, was suitable for the growth of fungi on the grains. Further rise in temperature and moisture results in heavy spoilage and deterioration.

Basmati rice was collected from all four godowns. *Aspergillus sydowi*, *A. fumigatus*, *A. terreus*, *A. niger*, and *A. tamarii* were isolated from this variety. *A. nidulans* was only isolated from Basmati variety of rice. *Cladosporium sphaerospermum* was isolated from Joshi and Basmati varieties.

In wheat varieties most of the field fungi, *Alternaria tenuis*, *A. tenuissima*, *Phaeoramularia*, *Epicoccum nigrum*, *Cladosporium*, *Stemphylium*, *Chaetomium globosum* and *C. minutum* were isolated. Most of these field fungi were absent on rice varieties. The presence of large number of field fungi and molds on wheat was probably due to the presence of high moisture content in grains (26.2%). The storing of early harvested crop at high temperature also helps in the proliferation of fungi and other microorganisms. *Paecilomyces varioti* and *Phaeoramularia* have not been reported to be isolated from wheat. *Penicillium cyclopium P. islandicum*, *P. viridicatum* and *P. funiculosum* were recorded from White wheat variety but were absent in Red variety. The frequency of *Penicillium* species was higher in wheat than in rice varieties.

In surface disinfection technique 24 different colonies were isolated at 1 min time interval but as the time increased, the frequency of occurrence gradually decreased and finally in the seeds treated for 5 min only, four different organisms were isolated. The frequency of *Aspergillus niger*, *A. flavus*, and *A. fumigatus*, was higher at 1–2 min time interval than 3–4 min. *A. niger*, however, was found at 5 min also. *Stemphylium* and *Alternaria* were found at 1 min interval but absent in 2–4 min. Higher concentration of $HgCl_2$ and increase in time of disinfection may eliminate all fungal organisms but effects the germination of seeds. The toxicity of $HgCl_2$ may also poison the food grains.

In the present investigations, the authors isolated many toxic producing fungi. *Aspergillus flavus* dominated in all samples with a percentage of 29.7 in 550 colonies. Aflatoxins secreted by *A. flavus* have some serious deleterious effect on many animals including man. When food and food stuffs are invaded, toxin producing fungi produce some serious health hazards. *Absidia corymbifera* and several species of *Mucor* and *Rhizopus* isolated from rice and rarely from wheat attack human internal nervous system and produce fatal results.[8] *Alternaria*, *Cladosporium*, *Chaetomium globosum* and *C. minutum* which were isolated from wheat produce some substance toxic to animals.[9] The only method to prevent the growth of storage fungi and secretion of toxic substances is to store the stock at low moisture levels and low temperatures in godowns.

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