FUNGI OF STORED WHEAT GRAINS FROM VARIOUS AREAS OF PAKISTAN

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Samples of wheat grains were collected from 15 different locations in Lyallpur, Jhang, Multan & Bahawalpur districts, 3 months after harvest. The grains were plated on different media, with and without surface sterilisation with Calcium hypochlorite. The number of different fungi appearing on grains was recorded and they were identified after obtaining pure cultures. *Alternaria* and *Fusarium* species were most abundant when the grains were planted on Malt extract Agar (MEA), and Czapek Dox agar (CDA) When Malt Salt Agar (MSA) was used then storage fungi like species of *Penicillium* and *Aspergillus* were maximum in their frequency. Other fungi like *Rhizopus Mucor*, were also isolated.

INTRODUCTION

What grains during storage are subject to spoilage and according to Christensen and Kaufmann (1965) under ordinary aerobic storage conditions, Fungi are the major causal agents of spoilage. Extensive investigations have been carried out abroad on fungi infesting cereal grains during harvest and storage alongwith the effect of various environmental factors like moisture, temperature etc. on the extent of grain spoilage (Sinha and Wallace 1965, Christensen and kaufmann 1969 and Papavizas and Christensen 1960.) In Pakistan, some investigations have been reported on food grain fungi of Karachi area (Hasany et al 1968, Husain and Ahmed 1971, Ahmed and Husain 1971 and Husain and Ahmed 1971).

Fungi which are designated as "Storage Fungi" by Christensen and kaufmann 1965 cause deleterious physical and chemical changes in grains and

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render it unfit for human consumption. Prevalence of certain number and kind of fungi at a specified time after harvest, provides a reliable index of the condition of the grain, whether it is close to spoilage or has spoiled. Furthermore, grain harvested in West Pakistan is stored in godowns, where it is very likely that rain will raise their moisture contents and thus hasten their spoilage. Documentary evidence on the extent of mold spoilage in Pakistan is not available but frequent press reports bear the evidence that wheat grains do get spoiled immediately after harvest in the field, at the railway stations waiting to be transported and finally in Government godowns.

This investigation was, therefore, taken up to examine in detail the number and type of fungi infesting wheat grains. This is the first part of our investigation reporting the fungi isolated from 15 different locations in Lyallpur and Multan regions of Panjab Province.

MATERIALS AND METHODS

The wheat grain samples were collected in polyethylene bags from 15 different govt. godowns located in Lyallpur, Jhang, Multan & Bahawalpur districts of Panjab province. The details are given in table I. The samples were brought to the laboratory and cultured within a period of a week. Three different media were used for culturing and they are: Malt Extract Agar (MEA) Czapek's DOX Agar (CDA) and Malt Agar salt (MSA). The media were prepared according to the standard methods. Two hundred grains were placed on 8 petri-dishes, with 25 grains/plate after random picking from the sample bag with a forcep. Similarly another lot of 200 grains was surface sterilised with 1 % Calcium hypochlorite for one minute followed by 2 washings with sterilised distilled water. The grains subsequently were cultured on the above mentioned media. Incubation temperature was 28°C, and after 2—3 days of incubation, the number of grains showing growth of different fungi was recorded. Direct examination of fungi on the grains was also made to identify them and further isolations were made on MEA slants to obtain pure cultures.

The plates were examined after every 24 hours because of fast growing fungi of *Rhizopus* and *Mucor* species. Final data is presented as percentage of grains showing the growth of various fungi. Moisture contents of all the samples were determined by Air Oven method.

RESULTS AND DISCUSSION

(i) *M E A without surface sterilisation.* (Table 2)

Alternaria tenuis was isolated from almost all the samples examined, with the frequency ranging from 34-100%. This is an important indicator

Table 1

Sample No.	*Location*	*Moisture content*
1	Lyallpur	8.2%
2	Lyallpur	7.9%
3	Chiniot	9.2%
4	Kamalia	8.4%
5	Jhang	8.5%
6	Shorkot	9.1%
7	Bahawalpur	8.4%
8	Bahawalpur	8.9%
9	Jaranwala	8.6%
10	Jaranwala	7.8%
11	Jaranwala	8.2%
12	Sumundri	9.1%
13	Tandlianwala	8.6%
14	Multan	8.9%
15	Lodhran	7.7%

TABLE 2: *Percentage of wheat grains from which various fungi were isolated on Malt Extract Agar (MEA) without surface sterilisation*

SAMPLE No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Name of Fungi *Alternaria tennuis*	67	45	34	100	76	62	73	94	59	78	—	84	55	50	50
Fusarium sp.	56	50	—	47	15	10	33	16	29	18	—	34	10	15	22
Rhizopus sp.	—	—	—	22	16	—	40	—	20	—	100*	—	8	—	—
Mucor sp.	—	—	—	—	—	—	—	—	—	—	—	4	1.0	—	—
Aspergillus niger	—	—	—	2.5	0.5	1	—	—	—	—	5.5	12	1.0	31	4
A. flavus	—	—	—	8	1	3	—	—	1.5	—	5.5	12	0.5	—	—
A. spp.	3.5	—	4	1.0	0.5	2	—	—	—	—	—	—	—	—	—
A. candidus	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
Stemphylium sp.	—	—	—	—	—	—	2	—	—	4	—	—	1	—	—
Penicillium sp.	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
Trichoderma sp.	2	—	—	—	—	—	1	—	—	—	—	—	—	—	—

*Whole Petri-dish was covered.

TABLE 3: *Percentage of wheat grain from which various fungi were isolated on Malt Extract Agar (MEA) with surface sterilisation.*

Sample No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Name of fungi															
Alternaria tenuis	34	28	24	—	19	54	45.5	41.5	12	39.5	0.5	34	33	9	—
Fusarium sp.	8.5	—	6	1.5	2.5	21	16	11	2.5	—	1	6	10.3	2	—
Rhizopus sp.	—		7	7	7	1.5	19	16.5	17.5	—	3.5	—	—	—	—
Mucor sp.	2	—	—	—	—	4	—	2	—	—	—	—	—	—	—
Aspergillus niger	2.5	—	—	13.5	3.5	—	—	—	2	2.5	5	5	5.5	49	49.5
A. flavus	—	0.5	0.5	27.5	4.5	—	—	—	3	—	—	5	4.5	11.5	6.5
A. spp.	2.5	—	4.5	—	—	—	—	—	3.5	0.5	—	—	—	—	3
A. candidus	—	—	—	2.5	—	—	—	2	—	—	—	—	—	—	—
A. terreus	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—
Stemphylium sp.	—	—	0.5	—	—	—	—	—	—	—	—	1	—	—	1.5
Penicillium sp.	—	—	—	0.5	—	—	—	—	—	—	—	1	—	—	—
Syncephalastrum sp.	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—
Curvularia sp.	—	—	—	—	—	—	—	—	—	1.0	—	—	—	—	—
Geotrichum sp.	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—
Neurospora sitophila	—	—	—	—	—	—	—	—	—	—	—	0.5	—	—	—
Zygorhynchus sp.	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—

TABLE 4: *Percentage of wheat grains from which various fungi were isolated on Malt Salt Agar (MSA) without surface sterilization*

Sample No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Name of fungi *Alternaria tenuis*	43.5	3	2	2.5	34	23	30	8	—	—	1	—	5	—	—
Aspergillus niger	38.5	9.5	11.5	11	4	2	—	49.5	38	29.5	23.5	56.5	50.5	77.5	51
A. flavus	42	40	100	66.5	39.5	7.5	0.5	89	49	33.5	32.5	100	22.5	100	20
A. spp.	68	100	14	37	25	100	75	38.5	8.5	33	57.5	47.0	81.5	1.57	61.5
A. candidus	5.5	10	—	—	0.5	—	—	—	—	—	—	0.5	—	—	—
A. nidulans	—	—	0.5	—	—	—	—	—	—	3	—	—	—	—	—
Stemphylium sp.	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Penicillium sp.	—	—	0.5	1	2	0.5	0.5	5.5	—	—	1	15	3.5	—	2
A. terreus	—	—	—	—	—	—	—	—	—	—	—	1.5	—	—	—
Syncephalastum	—	—	—	—	7	—	—	—	—	2	—	—	—	—	—

fungus and its prevalence shows the age of grain. Thus our results indicate that the grain was still fresh. Christensen and Kaufmann (1955) reported that immediately after harvest *Alternaria* should be recoverable from 100% of the grains, while Sinha & Wallace (1965) have observed that it might be less frequent.

Fusarium sp. was isolated but not from all the samples. Its frequency ranged between 10% to 56%. Other fungi isolated from different samples included species of *Rhizopus* isolated from six samples and *Mucor* and *Trichoderma* from two only.

Storage fungi like species of *Aspergillus* and *Penicillium* were frequently isolated from these grains, *A. niger* and *A flavus* being the most frequent. Thus less recovery of these storage fungi is again an indication that the grain was freshly harvested.

(ii) *MEA with surface sterilisation.* (Table 3)

Alternaria tenuis was isolated from almost all the samples with a frequency range of 0.5 to 54%. Surface sterilisation appeared to have exerted this eliminative effect. Similarly recovery of *Fusarium* sp. was reduced and its frequency ranged between 1%—21%. Among the storage fungi, *Aspergillus* species increased in number after surface sterilisation. *A. niger* was isolated up to 49.5% in sample No. 15. Frequency of isolation of *A. flavus* was also increased. Many other fungi were isolated, which were not obtained in the previous treatments. They included *Syncephalastrum* sp. *Curvularia* sp. *Geotrichum* sp. *Neurospora sitophila*, and *Zygorynchus* sp.

The frequency of isolation of *Rhizopus* and *Mucor* has also been reduced and they were not isolated from all the samples.

(iii) *MSA without surface sterilisation* (Table 4)

Alternaria tenuis was isolated with a frequency ranging from 1—43% but it could not be isolated from all the samples. This particular strain appeared to be salt-tolerant, although the growth was very slow when compared with the non-salt-media. Species of *Fusarium, Rhizopus* and *Mucor* could not be isolated from any of the samples.

Aspergillus niger, A. flavus and *Aspergillus* sp. were isolated from all the grains, and as this medium is selective for these molds, hence in some cases these could be recorded from 100% of the grains. *Aspergillus nidulans, A. terreus,* and *A. candidus,* were also isolated but only from 1-2 samples. Species of *Trichoderma, Curvularia* were not isolated from any of the samples.

TABLE 5. *Percentage of wheat grains from which various fungi were isolated on Malt Salt Agar (MSA) after surface sterilisation*

Sample No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Name of fungi *Alternaria tenuis*	10	0.5	1.0	7.0	12.5	12	27	21.5	1.6	40.5	1	39	47.5	—	4.8
Fusarium sp.	—	—	—	—	—	—	—	—	—	8.5	—	8.5	2	—	—
Aspergillus niger	1	4	3	5	4.5	2.0	9	16	11	7	3	2.5	3.5	—	—
A. flavus	4.5	12	67	40	28	55	2.5	15.5	30.5	4.5	3.5	8	11	38.5	18
A. spp.	53.5	100	50.5	34.5	18.5	96	86	26.5	48.5	12.5	8	3	16.5	27	33
A. candidus	—	—	—	—	0·5	—	—	—	—	—	—	—	—	—	—
A. nidulans	—	—	—	—	—	—	—	—	—	0.5	—	0.5	—	—	—
Penicillium sp.	8	1.5	0.5	0.5	4	3	2.5	6	—	3	1	0.5	0.5	—	1
Curvularia sp.	—	—	—	—	—	—	—	—	—	—	—	5	—	—	—
Syncephalastrum	—	—	—	—	0.5	—	—	—	—	—	—	—	—	—	—

TABLE 6: *Percentage of wheat grains from which various fungi were isolated in Czapek Dox Agar (CDA) without surface sterilisation*

Sample No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Name of fungi															
Alternaria tenuis	70	52.5	49	51	63	65.5	63.5	52.0	39	48	—	55.5	39.5	96	17
Fusarium sp.	29	37.5	12.5	12.5	19	1.5	11	16	12	24	—	21	11.5	8	4
Rhizopus sp.	—	37.5	6.5	11	7.5	24	8	—	5	1	27	12.5	18.5	3	—
Mucor sp.	31	—	—	—	1.5	0.5	1.0	1.0	3	2	5.5	—	12	—	—
Aspergillus niger	0.5	—	1.5	2.5	3.5	—	—	—	0.5	3	12.5	16	2.5	23	15
A. flavus	—	—	34.5	8	6.0	—	—	—	—	1	29.5	9.5	0.5	100	21.5
A. sp.	—	—	—	1.0	—	—	—	—	—	—	—	—	—	—	—
Curvularia	—	—	—	—	—	—	—	5	—	—	—	—	—	—	—

TABLE 7: *Percentage of wheat grains from which various fungi were isolated on Czapek Dox Agar (CDA) after surface sterilisation*

Sample No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Name of fungi															
Alternaria sp.	77.5	59	43.5	41.5	59	75	62.5	45.5	46.5	42	10	51	46	44	72
Fusarium sp.	25.5	27	1	9.5	14	9.5	3.5	19.5	19	16	4.5	17	12.3	3	—
Rhizopus nigricans	0.5	10	1.5	1.0	13	5	20	5.5	—	1	3	—	—	—	—
Mucor sp.	—	—	—	—	1.0	—	—	5	—	—	2.5	—	—	—	—
Aspergillus niger	—	—	3	5.5	—	—	0.5	—	0.5	6	7.5	10.5	1.3	38	21
A. flavus	—	1	23.5	2.5	—	—	—	0.5	3.5	15	4	4	3.5	100	21.5
A. sp.	—	—	—	—	5.5	0.5	—	0.5	—	—	—	0.5	—	3	25
A. candidus	—	—	0.5	2	—	—	—	—	—	—	—	—	—	—	—
A. ochraceous	—	—	—	—	—	—	—	—	—	—	—	1.5	—	—	—
Stemphylium sp.	—	—	—	—	—	—	—	1.0	1	—	—	—	—	—	—
Penicillium sp.	—	—	—	—	—	0.5	—	—	—	0.5	—	3.5	—	—	—
Trichoderma sp.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Curvularia	—	—	—	2	—	—	2	—	0.1	—	—	0.5	—	—	—

(iv) *MSA with surface sterilisation* (Table 5).

Alternaria tenuis was recovered from almost all the samples while the other pattern ramained almost the same, as in Table 4. Species of *Aspergillus* were isolated from all the samples. The relative frequency of *A. niger* decreased and the maximum was 38.5% *Aspergillus flavus* showed a frequency range of 2.5 to 67%, while *Aspergillus* sp. showed its association with maximum number of grains. *A. candidus* and *A. nidulans* were recorded only from 1-2 samples. Species of *Rhizopus* and *Mucor* were not recovered from these samples. A species of *Penicillium* was also recorded.

(v) *CDA without surface sterilisation* Table 6)

Alternaria tenuis was isolated from all the samples, with frequency range from 17-96%. Similarly *Fusarium* sp. was recorded from all the samples. *Rhizopus* and *Mucor* species were isolated from most of the samples, and their frequency of isolation was maximum on this medium. Species of *Aspergillus* were recorded from fewer samples, with frequency values much lower than those recorded on MSA (Table 4 & 5), but comparable to MEA (Table 2 and 3). The frequency of isolations were in general less, in comparison with other media.

(vi) *CDA with surface sterilisation* (Table 7)

Surface sterilisation with Calcium hypochlorite did not effect the isolation frequency of *Alternaria tenuis*, It was recorded from all the samples, with a frequency range of 40-77.5%. Recovery of other fungi was not significantly effected when compared to samples without surface sterilisation.

Hasany *et al* 1968 have reported isolation of various fungi from wheat and Rice samples of Karachi area. According to their results *Aspergillus flavus* was the most prevalent (29.7%) on both wheat and rice samples. Surface disinfection with Mercuric chloride decreased the frequency of these fungi.

Our results show that in freshly harvested wheat grains, *Alternaria tenuis* was the predominant fungus. This was a consistent pattern in all the samples collected from various godowns of Lyallpur and Multan area. MEA was found to be most suitable for its isolation.

On MSA, storage molds (*Aspergillus* and *Penicillium species*) were most predominantly isolated. The frequency of *Aspergillus flavus* was maximum, and this fungus is known to produce aflatoxins, a potential health hazard.

We could not find any definite corelation between the moisture content of the grain (Table 1) and the fungi occuring on them. This can most likely

be due to the fact that all the samples studied had low moisture content with a narrow range of variation.

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