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STUDIES ON STORED FOOD GRAIN FUNGI

Part IV.—Fungi from Pulses

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The present investigation relates to the microorganisms infesting certain pulses like *Cyamopsis psoralioides* DC (Guara) *Phaseolus mungo* L. (Moong), *Phaseolus radiatus* L. (Mash or Urad), *Cajanus indicus* Spreng (Arhar), *Lens esculenta* Moench (Masoor), *Cicer arietinum* L., (Desi chana), and *Cicer arietinum* L. (Kabuli chana). Thirty-six species belonging to sixteen genera were isolated from the above-mentioned pulses. Fungi Imperfecti was the most dominant group with an occurrence of 86%. The genus *Aspergillus* was most frequent in contributing 12 species with a total prevalance of 60%. *A flaws*, among the species of *Aspergillus* accounted for maximum damage 24%. *Cicer arietinum* (Desi chana) was the most heavily infested pulse with an infestation of 98% of the samples investigated while *Phaseolus radiatus* was the least infested pulse with an infestation of 45% of the samples studied.

In our previous communications, we have reported fungi inhabiting and infesting cereals and oil seeds in storage. The above two groups of crops are important from nutrient and industrial point of view as they provide food and oil for cooking and other purposes. Pulses, however, have their own importance as a crop because of their multipurpose usefulness. On the one hand they are a source of food for humans and on the other they have also been used extensively as food for animals. Pulses contain more protein material than any other vegetable product[1] and so are nearer to animal flesh in food value. Considerable amount of carbohydrates and fats are also present in pulses.

Pulses have been cultivated and used for food for centuries all over the world, however poor storage conditions and inadequate crop practices result in heavy losses in Pakistan where storage facilities are poor. As has been mentioned in earlier papers[2,3,4] substantial amount of damage to the grains occurs in storage resulting in shortage of food grains.

The above facts emphasize the need of constant efforts to save crops from deterioration by microorganisms in storage. The present work was, therefore, undertaken to isolate and identify fungi causing damage to pulses and to suggest possible means of control.

Materials and Methods

Random samples of pulses *Cicer arietinum* L. *Phaseolus mungo* L., *P. radiatus* L., *Cajanus indicus* Spreng *Cyamopsis psoralioides* DC., and *Lens esculanta* Moench, were collected from the godowns situated in Karachi at different times of the year during 1969 in order to obtain the most representative picture of the fungi infesting the grains. As mentioned in the earlier communications[2,3,4] the godowns were of various types and the jute bags containing pulses were mostly placed on wooden platforms. The grains stored in these godowns were previously stored in the various parts of West Pakistan, and were transported from time to time to Karachi. The samples were collected directly in the sterilized flasks by inserting the pointed end of the spatula in the jute bags containing pulses and its broad and open end in the flasks so as to get the samples directly in to the flasks. After taking the samples, the flasks were immediately plugged with sterilized cotton to avoid the grains from being contaminated by outside microflora.

The grains were taken out from the flasks randomly in the sterilized atmosphere of the inoculation chamber. Four to five grains each were planted in sterilized petri plates containing Czapek's Dox agar, corn meal agar, corn meal dextrose agar, sabouraud agar and malt agar, autoclved at 15-lb pressure for 20 min. Several sets of petri plates were prepared and incubated at 28°C±1. The experiment was repeated several times at different times of the year.

Another set of pulses under study was surface sterilized with 1:1000 mercuric chloride ($HgCl_2$) solution for 10, 20, 40, 60, 80 and 100 sec. After treatment these grains were thoroughly washed with autoclaved distilled water to remove $HgCl_2$ solution present on the surface of the grain. Four to five grains were then planted in petri plates containing culture media and incubated at 28°C±1. This experiment again was repeated for several times.

Petri plates were kept under study and as soon as any fungus colony emerged from the grains it was isolated in culture tubes containing Czapek's Dox agar slants. Transfer and retransfer technique was applied for obtaining pure cultures.[5]

For identification, several aspects of fungi like colour of colony, growth pattern, size and shape

of conidia and conidiophores and all other type of fruiting bodies etc, were taken into consideration. Cultures were sent from time to time to Commonwealth Mycological Institute for confirmation and identification.

Air oven method was applied[6] for determining the moisture content of the stored grains.

Results

During investigation on the above-mentioned pulses a sum total of 36 fungus species were isolated from them (Table 1). These species belonged to 16 genera. It may be noted that some fungi were isolated from all the pulses while others were isolated from one or two pulses alone. Here again, like cereals[2,4] species of *Aspergillus* were most predominant and formed the core of all the isolated microorganisms.

Maximum number of species were isolated from *Cyamopsis psoralioides*. *Aspergillus* dominated the picture with five species, and a total prevalence of 61%. Two species each belonged to the genus *Alternaria*, *Fusarium* and *Penicillium*. *Rhizopus*, *Drechslera*, *Cochliobolus* and *Cladosporium* were represented by one species alone. *Aspergillus niger* was the most prevalent fungus on *C. psoralioides* (26%) while *Cochliobolus spicifer* was the least occurring organism (0.7% only).

Fourteen organisms were isolated from *Phaseolus radiatus*. Half of these belonged to the genus *Aspergillus* with a total prevalence of 58%. Two species belonged to *Fusarium* while *Alternaria*, *Drechslera*, *Cladosporium*, *Curvularia* and *Pleospora* were represented by one organism each. *Aspergillus flavus* with 28% was the most common organism on *Phaseolus radiatus* while *Aspergillus tamarii* was least common with 0.9%. Prevalence of remaining twelve fungi was of intermediate order.

Lens esculenta yielded twelve fungi. Here again, species of *Aspergillus* were dominant and the genus was represented by six species. The prevalence of *Aspergillus* species on *Lens esculenta* amounted to 63%. *Monilia*, *Alternaria*, *Pleospora*, *Drechslera*, *Penicillium* and *Rhizopus* yielded one species each. *Aspergillus flavus* topped the list with 24% while *Pleospora infectoria* was the least common organism. The prevalence of this fungus on *Lens esculenta* was only 2%.

Twelve species of fungi were isolated from *Cicer arietinum* (Desi chana). Species of *Aspergillus* constituted 64% of the organisms. Out of a total of twelve species, five belonged to *Aspergillus*. The genus *Penicillium* yielded two species while *Rhizopus*, *Drechslera*, *Alternaria* and *Paecilomyces* were represented by one species each. The species of *Momilia* could not be determined at present. *Aspergillus miger* was most frequent orange with a frequency of 28%. *Penicillium decumbens* was least frequent with a prevalence of 0.7%.

Table 1.—Fungi Isolated from Pulses Alongwith their % Prevalence.

	Phaseolus mungo L. (Moong)	
1.	*Aspergillus flavus* Link	33.1
2.	*A. niger* van Tiegh	15.0
3.	*A. fumigatus* Fres	7.0
4.	*A. tamarii* Kita	4.0
5.	*A. violaceus* Raper & Fennell	3.0
6.	*Neurospora sitophila* Shear & Dodge	2.0
7.	*Paecilomyces varioti* Bainier	7.0
8.	*Macrophomina phaseoli* (Maubl.) Ashby	9.0
9.	*Rhizopus arrhizus* Fischer	11.0
10.	*Penicillium notatum* Westling	6.0
11.	*Monilia* sp.	3.0
	Phaseolus radiatus L. (Mash or Urad)	
1.	*Aspergillus flavus* Link	28.0
2.	*A. niger* van Tiegh	20.0
3.	*A. violaceus* Raper & Fennell	2.0
4.	*A. sydowii* (Bain & Sart) Thom & Church	3.0
5.	*A. tamarii* Kita	0.9
6.	*A. terreus* Thom	4.0
7.	*A. ochraceus* Wilhelm	0.9
8.	*Fusarium semitectum* Berk & Rev	5.0
9.	*F. moniliforme* Sheld	4.0
10.	*Curvularia lunata* (Wakker) Boedijn	6.0
11.	*Cladosporium sphaerospermum* Penz	7.0
12.	*Alternaria alternata* (Fr.) Keissler	9.5
13.	*Drechslera hawaiiensis* (Bugn.) Subram. & Jain	7.0
14.	*Pleospora infectoria* Fuckel	5.0
	Cajanus indicus Spreng (Arhar)	
1.	*Aspergillus flavus* Link	19.0
2.	*A. niger* van Tiegh	21.0
3.	*A. candidus* Link	4.0
4.	*A. fumigatus* Fres	6.0
5.	*Rhizopus arrhizus* Fischer	15.0
6.	*Alternaria alternata* (Fr) Keissler	11.0
7.	*Drechslera hawaiiensis* (Bugn.) Subram & Jain	2.0
8.	*Penicillium decumbens* Thom	7.0
9.	*Phoma* sp.	3.0
10.	*Humicola* sp.	6.0
11.	*Monilia* sp.	5.0
	Cyamopsis psoralioides DC (Guar)	
1.	*Aspergillus flavus* Link	23.0
2.	*A. niger* van Tiegh	26.0
3.	*A. nidulans* (Eidam) Wint	4.4
4.	*A. fumigatus* Fres	6.0
5.	*A. ochracceus* Wilhelm	1.5

(Continued)

(Table 1 continued)

6.	*Rhizopus arrhizus* Fischer	17.0
7.	*Drechslera hawaiiensis* (Bugn) Subram & Jain	3.0
8.	*Penicillium notaum* Westling	3.7
9.	*P. islandicum* Sopp	2.0
10.	*Fusarium chlamydosporum* Wr. & Rg	5.0
11.	*F. acuminatum* Ell & Ev	3.7
12.	*Cochliobolus spicifer* Nelson	0.7
13.	*Cladosporium sphaerospermum* Penz	1.5
14.	*Alternaria altarnata* (Fr) Keissler	15.0
15.	*Alternaria* sp.	3.0

Lens esculenta Moench (Masoor)

1.	*Aspergillus flavus* Link	24.0
2.	*A. niger* van Tiegh	12.10
3.	*A. versicolor* (Vuill) Tiraboschi	3.0
4.	*A. sydowii* (Bain & Sart) Thom & Church	14.0
5.	*A. fumigatus* Fres	4.5
6.	*A. violaceus* Raper & Fennell	5.7
7.	*Penicillium chrysogenum* Thom	9.6
8.	*Rhizopus arrhizus* Fischer	13.0
9.	*Drechslera hawaiiensis* (Bugn) Subram & Jain	5.0
10.	*Pleospora infectoria* Fuckel	2.0
11.	*Alternaria alternata* (Fr.) Keissler	11.5
12.	*Monilia* sp	2.5

Cicer aeritinum L. (Desi Chana)

1.	*Aspergillus flavus* Link	25.0
2.	*A. niger* van Tiegh	28.0
3.	*A. fumigatus* Fres	3.0
4.	*A. violaecus* Raper & Fennell	1.4
5.	*A. sydowii* (Bain & Sart) Thom & Church	5.7
6.	*Rhizopus arrhizus* Fischer	18.7
7.	*Paecilomyces varioti* Bainier	3.0
8.	*Drechslera hawiiensis* (Bugn) Subram & Jain	5.0
9.	*Penicillium citrinum* Thom	3.0
10.	*P. decumbens* Thom	0.7
11.	*Alternaria triticina* Prasada & Prabhu	4.0
12.	*Monilia* sp.	2.0

Cicer aeritinum L. (Kabuli Chana)

1.	*Aspergillus flavus* Link	29.0
2.	*A. niger* van Tiegh	26.0
3.	*A. amstelodami* (Mangin) Thom & Church	3.7
4.	*A. midulans* (Eidam) Wint var *echinulatus* Raper & Fennell	12.0
5.	*A. fumigatus* Fres	4.6
6.	*Rhizopus arrhizus* Fischer	17.6
7.	*Cladosporium sphaerosperum* Penz	5.5
8.	*Monilia* sp.	2.0

Phaseolus mungo (Moong) yielded eleven fungi. Five species of *Aspergillus* were dominant and their total frequency of occurrence was 62%. One species each belonged to *Macrophomina*, *Paecilomyces*, *Penicillium*, *Neurospora*, *Rhizopus* and *Monilia*. Here again like *Cicer arietinum* (Desi Chana) species of *Monilia* could not be determined.

Maximum number of colonies were that of *Aspergillus flavus* in the petri dishes planted with *Phaseolus mungo*. The prevalence of this fungus was 33%. *Neurospora sitophila*, however was scanty and only three colonies representing a prevalence of 2% emerged.

Eleven species of fungi were isolated from *Cajanus indicus*. The four species of *Aspergillus* were again dominant and had a prevalence of 50%. *Rhizopus*, *Alternaria*, *Penicillium*, *Drechslera Humicola*, *Phoma* and *Monilia* were represented by one species each.

Aspergillus niger once again was the predominant organism as compared to other fungi with a frequency of 21%. *Drechslera hawaiiensis* was the least prevalent fungus and its prevalence was only 2%. The rest of the organisms ranged between the above two mentioned percentages with respect to their prevalence frequency.

Cicer arietinum (Kabuli chana) was the lowest pulse with respect to the number of species isolated. A total number of eight species were identified, five of which belonged to *Aspergillus*. The overall frequency of *Aspergillus* species on this pulse was 75%. *Cladosporium*, *Monilia* and *Rhizopus* were represented by one species each.

Aspergillus niger dominated the scene with a prevalence of 29%. *Monilia* sp. however was the least prevalent organism as it had a frequency of occurrence of 2% only.

As has been observed in our previous communication, the surface sterilization of grains decreased the number of organisms (Table 2).

Moisture content of the grains was also determined. Maximum moisture, 17.01%, was determined in *Phaseolus mungo* while minimum, 9.45%, in *Phaseolus radiatus*. Moisture percentage of rest of the pulses was in the following order. *Cyamopsis psoralioides* 10.3%, *Cajanus indicus* 10.9%, *Lens esculenta* 11.23%, *Cicer arietinum* (Kabuli chana) 13.51% and *Cicer arietinum* (Desi chana) 14.17%.

Discussion

The species of *Aspergillus* cause substantial amount of damage to the grains in storage. The overall prevalence of *Aspergillus* species on these legumes was calculated to be 60% which amounts to 3/5 of the total fungi isolated. It may be observed that among *Aspergillus* species it is *A. flavus* and *A. niger* which cause maximum damage (Fig. 1). The total prevalence of these two

Table 2.—Fungi Isolated After Surface Disinfection with 1:1000 Mercuric Chloride ($HgCl_2$)

Time (sec)	*Phaseolus mungo*	*Phaseolus radiatus*	*Lens esculanta*	*Cyamopsis psoralioidis*	*Cajanus indicus*	*Cicer aeritinum*	*Cicer aeritinum*
10	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus niger*	*Aspergillus niger*	*Aspergillus niger*	*Aspergillus niger*
		A. niger	*Alternaria alternata*	*Rhizopus arrhizus*	*A. flavus*	*A. nidulans*	
			Penicillium chrysogenum			*Rhizopus arrhizus*	*A. flavius*
20	*A. flavus*	*A. niger*	*Alternaria alternata*	*A. flavus*	*A. niger*	*Aspergillus niger*	*A. Fumigatus*
	A. niger	*Alternaria alternata*		*A. niger*			*A. Flavus*
			Aspergillus flavus			*Rhizopus arrhizus*	
40	—	*Aspergillus flavus*	*A. niger*	*A. flavus*	*A. niger*	*Aspergillus flavus*	*A. niger*
					A. flavus	*A. niger*	
60	*Aspergillus flavus*	—	—	—	*A. niger*	*A. niger*	*A. niger*
80	—	*A. flavus*	—	—	*A. niger*	—	*A. niger*
100	—	—	*Aspergillus flavus*	—		*A. niger*	

organisms was about 45%. The remaining 10 species of *Aspergillus* accounted for 15% infection. *A. flavus* however, was the most frequent fungus among the 36 species isolated in this investigation with a prevalence of 24%.

It may be observed that certain species were prevalent on one grain only or in other words could only be isolated from a single pulse. *Neurospora sitophila* and *Macrophomina phaseoli* were isolated from *Phaseolus mungo*, *Aspergillus terreus*, *Fusarium semitectum*, *F. moniliforme* and *Curvularia lunata* from *Phaseolus radiatus*, *Aspergillus candidus*, *Phoma* sp. and *Humicola* sp. from *Cajanus indicus*, *Penicillium islandicum*, *Fusarium chlamydosporum*, *F. acuminatum Cochliobolus spicifer* and *Alternaria* sp. from *Cyamopsis psoralioides*, *Aspergillus versicolor* and *Penicillium chrysogenum* from *Lens esculanta*, *Penicillium citrinum* and *Alternaria triticina* from *Cicer arietinum* (Desi chana) and *Aspergillus amstelodami* from *Cicer arietinum* (Kabuli chana). The fact that certain species parasitize certain specific grain may be due to more than one factor. Temperature, moisture content, resistance or susceptibility of a certain pulse to a certain species of fungus, storage conditions including time of storage and a host of other factors can account for this behavior. Of course this aspect needs to be investigated before drawing any definite conclusions.

Fungi *Imperfecti* was the most dominant group with a contribution of 86%. *Phycomycetes* accounted for 13% and *Ascomycetes* for 1%.

As has been observed in our previous studies on stored grain, fungi disinfection of grains with 1:1000 $HgCl_2$ for different lengths of time considerably reduced the number of fungi and only more resistant *Aspergillus* species appeared from the treated seeds (Fig. 2).

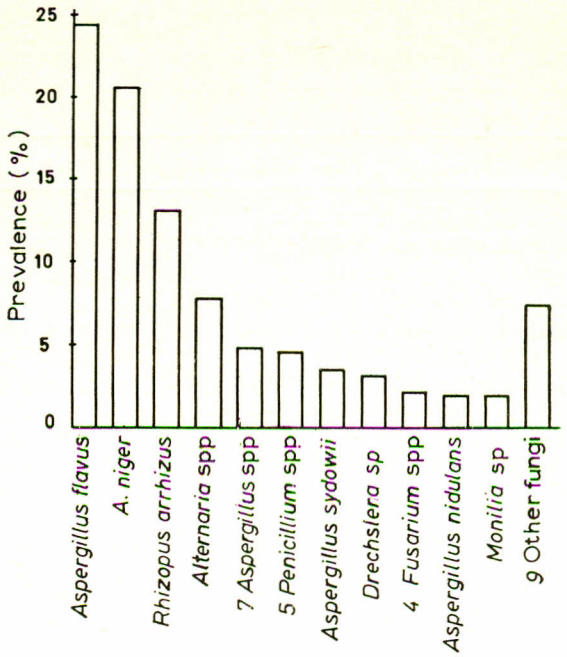

Fig. 1.=Comparative prevalence of some more dominant fungi.

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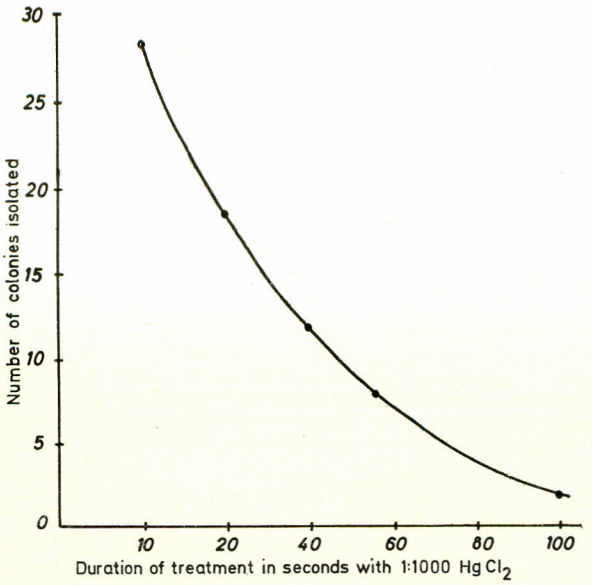

Fig. 2.—The number of colonies isolated after surface disinfection with 1:1000 $HgCl_2$ for different lengths of time.

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