

## PREVALENCE OF FUNGI ON BARLEY SEEDS

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### Abstract

Ten varieties of barley seeds harvested in 1976 and collected from Tandojam and Faisalabad were studied for the presence of seed-borne mycoflora. Using ISTA technique, 19 species of fungi viz., *Alternaria tenuis*, *Aspergillus niger*, *A. flavus*, *Aspergillus spp.*, *Chaetomium olivaceum*, *Cladosporium cladosporioides*, *Curvularia lunata*, *C. pallescens*, *Drechstera hawaiiensis*, *D. halodes*, *D. papendorfii*, *D. rostrata*, *D. tetramera*, *Fusarium moniliforme*, *F. semitectum*, *Penicillium spp.*, *Rhizopus nigricans*, *Stachybotrys atra* and *Trichothecium roseum* were isolated. Of these *F. semitectum*, *D. papendorfii*, *S. atra*, *F. moniliforme*, *D. rostrata* and *D. halodes* have not so far been recorded on barley seeds from Pakistan. As determined by the blotter and agar plate methods, the average prevalence of fungi in the ten vars. was 87.1% and 73.25%, respectively. Seeds from the same varieties after surface disinfection with 0.1% Hg Cl<sub>2</sub> showed reduction in the prevalence to 34.3% and 24.8%, respectively. *R. nigricans*, *F. semitectum*, *S. atra* and *C. cladosporioides* did not occur after disinfection of the seeds, however, *F. moniliforme*, *D. rostrata* and *D. halodes* showed an increase in prevalence. Barley var. Border Arivate was found heavily infected with most of the isolated species of fungi.

### Introduction

Studies on the mycoflora of barley seeds from different parts of the world have established several species of *Fusarium* and *Helminthosporium* to be pathogenic and occurring at levels which vary from region to region and from year to year (Jorgensen, 1969; Hewett, 1975). Although several fungal species from barley seeds have been reported, however, no data is available regarding their frequency of occurrence (Mulinge & Apinis, 1969). Jorgensen (1969) and Flannigen (1970) have, however, examined the specific composition of the fungus flora of barley seeds. Akhter (1963) and Ahmed & Hussain (1971) have reported some fungi found in association with barley seeds. Data on the seed-borne fungi found in association with 10 different varieties of barley seeds is presented in this report.

### Materials & Methods

Seeds of barley var. B.A. and T. J 70 were collected from Tandojam and B96/76,

Table 1. Percentage incidence of seed-borne fungi of barley on blotters before and after surface sterilization of the seeds and incubation at 25°C.

Fungi	Varieties	B.A.	T.J70	B96/76	B99/76	B161/76	B164/76	B173/76	B184/76	B192/67	B223/76
<b>Before Sterilization</b>											
<i>Alternaria tenuis</i>		32.75	16.75	74.50	76.00	62.25	81.00	60.25	61.25	45.50	42.50
<i>Aspergillus niger</i>		7.75	4.00	4.25	4.75	4.75	3.50	4.25	6.75	6.50	3.00
<i>Aspergillus flavus</i>		5.75	2.50	2.25	3.25	3.75	4.00	3.75	—	—	8.5
<i>Aspergillus</i> sp.		3.00	—	—	—	—	—	—	—	4.50	—
<i>Penicillium</i> sp.		4.50	—	—	—	—	—	—	3.75	—	—
<i>Rhizopus nigricans</i>		—	1.50	1.00	0.50	—	0.75	0.25	—	1.00	0.75
<i>Trichothecium roseum</i>		0.25	—	0.50	—	—	—	5.25	3.00	—	3.50
<i>Curvularia lunata</i>		9.50	3.50	3.50	—	2.25	1.50	1.50	1.25	2.25	—
<i>Curvularia pallescens</i>		2.00	6.50	—	0.75	1.50	—	2.75	—	3.50	—
<i>Fusarium moniliforme</i>		1.75	—	—	—	2.75	—	—	1.75	2.75	—
<i>Fusarium semitectum</i>		1.25	2.00	2.25	1.75	0.50	—	—	1.75	—	1.75
<i>Drechslera tetramera</i>		6.50	5.00	4.50	2.75	6.50	—	6.00	2.75	—	1.75
<i>Drechslera hawaiiensis</i>		6.75	3.25	2.00	1.75	2.00	—	4.00	—	—	4.50
<i>Drechslera rostrata</i>		7.25	—	—	—	—	—	—	—	—	—
<i>Drechslera papendorfii</i>		5.50	—	—	—	2.75	—	—	2.50	—	—
<i>Drechslera halodes</i>		5.00	—	—	—	1.50	—	3.75	—	—	4.50
<i>Stachybotrys atra</i>		—	8.00	0.75	—	1.00	1.25	1.75	1.25	—	—
<i>Chaetomium olivaceum</i>		—	—	0.25	—	0.75	0.50	—	—	1.50	0.75
<i>Cladosporium cladosporioides</i>		—	—	—	—	—	1.00	—	0.75	—	—

Overall incidence in different var. = 87.1%

Table Continued

After Sterilization	9.75	1.25	25.50	22.50	21.50	22.50	21.75	19.00	12.50	16.50
<i>Alternaria tenuis</i>	0.75	0.75	—	—	2.00	—	—	2.00	1.50	—
<i>Aspergillus niger</i>	3.50	0.25	2.50	1.25	3.00	0.75	1.50	—	—	3.25
<i>Aspergillus flavus</i>	0.75	—	—	—	—	—	—	—	0.75	—
<i>Aspergillus</i> sp.	0.50	—	—	—	—	—	—	—	—	—
<i>Penicillium</i> sp.	—	—	—	—	—	—	—	—	—	—
<i>Rhizopus nigricans</i>	—	—	—	—	—	—	—	—	—	—
<i>Trichothecium roseum</i>	—	—	—	—	—	—	0.75	0.50	—	0.25
<i>Curvularia lunata</i>	8.75	0.75	—	—	1.25	—	0.50	—	2.00	—
<i>Curvularia pallescens</i>	3.00	3.75	—	—	0.50	—	1.75	—	2.50	—
<i>Fusarium moniliforme</i>	4.50	—	—	—	4.50	—	—	3.75	3.00	—
<i>Fusarium semitectum</i>	—	—	0.25	—	—	—	—	—	—	—
<i>Drechslera tetramera</i>	3.50	1.50	—	1.50	0.50	—	4.50	0.75	—	1.50
<i>Drechslera hawaiiensis</i>	5.50	2.75	0.75	1.25	2.75	—	2.50	—	—	3.50
<i>Drechslera rostrata</i>	12.75	—	—	—	—	—	—	—	—	—
<i>Drechslera papendorfii</i>	3.75	—	—	—	1.75	—	—	2.25	—	—
<i>Drechslera halodes</i>	5.75	—	—	—	—	—	—	—	—	—
<i>Stachybotrys atra</i>	—	—	—	—	—	6.75	3.50	—	—	6.50
<i>Chaetomium olivaceum</i>	—	—	—	—	—	0.75	0.50	—	—	—
<i>Cadosporium cladosporioides</i>	—	—	—	—	—	—	—	—	—	—

Overall incidence in different var. = 34.3%

Table 2. Percentage incidence of seed-borne fungi of barley on PDA before and after surface sterilization and incubation at 25°C.

Fungi	Varieties	B.A.	T.J75	B96/76	B99/76	B161/76	B164/76	B173/76	B184/76	B192/76	B223/76
<b>Before Sterilization</b>											
<i>Alternaria tenuis</i>		24.00	11.75	62.50	66.00	55.50	73.25	52.50	59.50	36.00	31.00
<i>Aspergillus niger</i>		6.50	1.50	4.00	—	2.00	—	4.00	2.00	1.50	—
<i>Aspergillus flavus</i>		9.75	0.75	3.75	—	1.50	—	2.00	—	—	—
<i>Aspergillus</i> sp.		2.00	—	—	—	—	—	—	—	2.50	—
<i>Penicillium</i> sp.		—	—	1.50	—	—	—	—	1.75	—	—
<i>Rhizopus nigricans</i>		—	0.50	0.75	—	—	—	1.00	—	0.25	1.00
<i>Trichothecium roseum</i>		—	0.75	1.25	—	—	—	0.75	2.25	—	2.00
<i>Curvularia lunata</i>		3.50	4.00	2.50	—	1.50	0.25	1.00	1.75	—	4.00
<i>Curvularia pallenscense</i>		2.50	2.50	—	2.50	0.75	0.50	2.50	—	2.75	1.50
<i>Fusarium moniliforme</i>		3.75	—	—	—	6.00	—	—	4.25	4.00	—
<i>Fusarium semitectum</i>		2.50	4.50	3.50	4.00	1.75	4.50	3.75	6.00	2.00	4.75
<i>Drechslera tetramera</i>		4.00	3.75	1.25	—	1.00	—	4.25	2.00	2.50	0.75
<i>Drechslera hawaiiensis</i>		5.50	2.00	1.25	—	3.00	—	2.25	—	—	1.50
<i>Drechslera rostrata</i>		3.75	—	—	—	—	—	—	—	—	—
<i>Drechslera papendorfii</i>		6.50	—	—	—	—	—	—	—	1.50	—
<i>Drechslera halodes</i>		4.75	—	—	—	0.75	—	1.27	—	—	1.00
<i>Stachybotrys atra</i>		—	—	—	—	—	—	—	—	—	—
<i>Chaetomium olivaceum</i>		—	—	—	—	—	—	0.50	—	1.75	—
<i>Cladosporium cladosporioides</i>		—	—	—	—	—	—	—	—	0.50	—

Overall incidence in different var. = 68.6%

Table Continued.....

After Sterilization	2.75	0.25	19.75	21.50	24.00	22.50	16.25	2.75	14.50	9.25
<i>Alternaria tenuis</i>	1.00	3.50	—	—	2.00	2.00	—	—	1.00	—
<i>Aspergillus niger</i>	—	0.25	—	—	—	—	—	—	—	—
<i>Aspergillus flavus</i>	1.00	—	—	—	—	—	—	—	0.75	—
<i>Aspergillus</i> sp.	0.25	—	0.50	—	—	—	—	—	—	—
<i>Penicillium</i> sp.	—	—	—	—	—	—	—	—	—	—
<i>Rhizopus nigricans</i>	—	—	—	—	—	—	—	—	—	—
<i>Trichothecium roseum</i>	—	—	—	—	—	—	—	—	—	—
<i>Curvularia lunata</i>	3.00	—	—	—	0.50	—	—	—	3.00	—
<i>Curvularia pallescens</i>	0.75	2.00	—	—	—	—	—	—	2.00	—
<i>Fusarium moniliforme</i>	6.50	—	—	2.00	6.50	2.00	—	6.75	4.75	—
<i>Fusarium semitectum</i>	0.25	1.50	—	—	0.75	—	—	—	0.50	—
<i>Drechslera tetramera</i>	3.50	—	—	1.75	—	—	—	0.75	2.00	—
<i>Drechslera hawaiiensis</i>	3.50	4.50	—	—	1.50	—	—	—	—	—
<i>Drechslera rostrata</i>	4.75	—	—	—	—	—	—	—	—	—
<i>Drechslera papendorfii</i>	4.00	—	—	—	—	—	—	—	1.25	—
<i>Drechslera halodes</i>	5.00	—	—	—	2.25	—	4.75	1.00	—	3.75
<i>Stachybotrys atra</i>	—	—	—	—	—	—	—	—	—	—
<i>Chaetomium olivaceum</i>	—	—	—	—	—	—	—	—	—	—
<i>Cladosporium cladosporioides</i>	—	—	—	—	—	—	—	—	—	—

Overall incidence in different var. = 28.8%

B99/76, B161/76, B164/76, B173/76, B184/76, B192/76 & B223/76 were collected from Faisalabad. For the isolation of seedborne fungi, the Blotter (Muskett, 1938) and Ulster (Muskett & Malone, 1941) methods as suggested by International Seed Testing Association (ISTA) were used. The blotter method deals with the growing of seed-borne fungi on wet blotting papers and Ulster method with the development of fungi on PDA medium. In each method, one series of seeds was plated directly and another series was treated for 5 minutes with 0.1% Hg Cl<sub>2</sub> before plating. The plates were incubated at 25°C ± 1 and examined after 7 days. Discernible fungal growths emerging from the seeds were subcultured. The macro and microscopic characteristics of each fungal growth were studied in detail for identification.

## Results

A total of 19 species of fungi viz., *Alternaria tenuis*, *Aspergillus niger*, *A. flavus*, *Aspergillus spp*, *Chaetomium olivaceum*, *Cladosporium cladosporioides*, *Curvularia lunata*, *C. pallescens*, *Drechslera hawaiiensis*, *D. halodes*, *D. papendorfii*, *D. rostrata*, *D. tetramera*, *Fusarium moniliforme*, *F. semitectum*, *Penicillium spp.*, *Rhizopus nigricans*, *Stachybotrys atra*, *Trichothecium roseum* were isolated during the course of the work.

Using blotter technique the frequency of occurrence of the isolated fungi from 10 varieties of barley seeds both before and after the sterilization is given in Table 1. *A. tenuis* occurred in all the varieties from 16.75 to 81%, though its incidence declined from 32.5 to 1.25% after seed disinfection. *C. olivaceum*, *C. cladosporioides*, and *R. nigricans* were totally eliminated after the treatment of the seeds. *F. moniliforme*, *D. halodes* and *D. rostrata* showed an increase in numbers after the disinfection of seeds. The remaining isolates were also affected by 0.1% Hg Cl<sub>2</sub>.

Incidence of seed-borne fungi developed on PDA before and after surface disinfection of the seeds is given in Table 2. *Stachybotrys atra* was the only fungus not isolated on PDA. The prevalence of fungi on PDA was more or less similar to that on blotter except that the percentage of the isolated fungi here reduced comparatively. Species of *Fusarium* only showed better growth in terms of percentage on PDA. *F. moniliforme*, *D. halodes* and *D. rostrata* increased with the seed disinfection.

## Discussion

Comparative study made indicated that the blotter method yielded quantitatively more fungi than Ulster method. *A. tenuis* was the most common fungus occurring in all the barley varieties, even after the seed disinfection. This would suggest that mycelial fragments remain either present in the crevices of seed coats or inside the pericarp of the seeds. Comprehensive studies carried out by Jorgensen (1969) also showed the presence of this fungus after seed disinfection.

*D. rostrata*, *D. halodes* and *F. moniliforme* showed an increase in their percentage after sterilization of the seeds. These fungi have been reported to be pathogenic (Luttrell, 1955; Mitra, 1931; Bugnicourt, 1952). It can be said that the increase in percentage after disinfecting the seeds may be due to the elimination of superficial fungi which remain as saprophytes on seeds thus providing an opportunity to the deeply seated pathogenic fungi to grow out from inside of the seeds. It is to be noted that *D. rostrata* was found only from the seed var. B.A. and was altogether absent from other varieties. The remaining species of fungi showed a decrease in their occurrence after sterilization and this is in conformity with the result of Ahmed & Hussain (1971) and Akhter (1963). *Fusarium* species showed better growth on PDA, which may be due to the better nutrient status of the medium. Moreover this medium has been recommended by the ISTA for the isolation of *Fusarium* species from seeds.

*F. moniliforme*, *F. semitectum*, *D. halodes*, *D. rostrata*, *D. papendorfii* and *S. atra*, are being reported for the first time as seed-borne fungi of barley seeds from Pakistan.

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