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Mycoflora of Barley Grains in the Southern Region of Saudi Arabia and Its Control

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ABSTRACT. Twenty-nine species of fungi were isolated from normal and discolored grains of barley. A higher number of species were isolated from discolored seeds and the percentage of occurrence of fungi was higher in non-disinfected seeds as compared to disinfected seeds. The most common genera were *Ulocladium* (four species); *Alternaria, Aspergillus* and *Drechslera* (three species); *Curvularia, Fusarium, Mucor, Penicillium* and *Syncephalastrum* (two species). Diathane was found to be the most effective among the tested fungicide.

Introduction

A knowledge of seed-borne fungi of particular seeds are important because these fungi can determine the healthy condition of the seed and, hence, yield[1]. Seed-borne fungi cause reduction in seed viability and vigour[2,3]. The damage to the germ and also the low percentage of germination and unpleasant odour of spoiled grains are largely caused by fungi[12]. Seeds infected by seed-borne fungi cause mycotoxicoses in poultry, livestock and in humans[5-7]. Shafie and Webster[8] reported that these fungi may invade seed pericarp and embryos. Discoloration of seeds is largely to be caused by seed-borne fungi[9,10] which produce proteolytic enzymes that help in the spread and development of plant pathogens[11]. Post emergence death of grains can also be caused by these fungi[1, 12].

Saudi Arabia has devoted much efforts towards agricultural development and hence the areas for cultivation of cereals increased several fold during the past ten years. The annual growth value of cereals has also increased. Due to this policy,

Saudi Arabia has not only become self-sufficient in cereals and vegetables but also in exporting them to other countries[13]. Global reports are available on the seed-borne fungi of barley because it is an important crop for animal feed[4,14-17]. Seed-borne fungi of wheat, sorghum and maize have been studied in Saudi Arabia[18-22]. Limited work has been reported about the seed-borne fungi of barley in Saudi Arabia[18,21 & 22]. The aim of this present study was to determine the seed-borne fungi of normal and discolored barley seeds and also the effect of fungicides for control.

Material and Methods

Seeds of barley (*Hordeum vulgare* L.) were collected from local growing areas of Jizan and Najran immediately after harvesting. A total number of ten samples, five from each locality were mixed together and then 200 normal and 200 discolored seeds[8] were chosen randomly and 25 seeds/plate (sterilized disposable Petri dishes of 15 cm diam.) were incubated at 25 ± 1°C for one week with 12h light/dark period. These seeds were immediately incubated after collection without storage. Standard blotter and agar plate methods were used for detection of seed-borne fungi[23]. Seeds were surface disinfected by suspending in 1% sodium hypochlorite for 10 minutes and washed three times with sterilized distilled water prior to incubation[10,19 and 23]. A total number of 100 disinfected and 100 non-disinfected seeds were chosen for each experiment. The fungal flora isolated were identified using the texts by Gilman[24], Barnett and Hunter[25], Ellis[26,27], Raper and Fennel[28], Zycha *et al.*[29]. Five different fungicides were used namely Agrosin, Ceresan, Diathane, Bavistin and Vitavax. These fungicides were bought from the local market. The fungicides were applied to the seeds (both disinfected and non-disinfected) for 30 minutes in 0.3% concentration. The treated seeds were plated after 24 hours[30]. A total number of 100 seeds of normal and discolored seeds were chosen for each type of treatment. Sabouraud dextrose agar (Oxoid Ltd., London) was used for agar plate methods in all cases. Penicillin (0.03 g/l) was used as an antibiotic to prevent bacterial growth. The germination potential of normal and discolored seeds was carried out according to Shafie and Webster[8].

Results

The germinability of normal and discoloured, barley grain, with and without seed treatment, when assessed using two method is summarized in Table (1).

Percentage occurrance of fungi on untreated/treated, normal/discoloured barley grain is given in Table (2a).

Number of fungal species isolated from treated and untreated × normal and discoloured barley grains is presented in Table (2b).

Tablel (3a) represent effect of different fungicides in seed-borne fungi.

Mean effects of 5-fungicides on the fungi isolated from normal and discoloured barley grains is summarized in Table (3b).

TABLE The germinability of normal and discoloured, barley grain, with and without seed treatment when assessed using two methods.

Method	% germination of grain		
	Normal grain	Discoloured grain	Mean
1 – Agar Plate Method			
a. Not treated seed	82	45	63.5
b. Treated seed	100	70	85.0 74.3
Mean	91	57.5	
2 – Blotter Method			
a. Not treated seed	62	30	45.5
b. Treated seed	86	36	61.0 53.3
Mean	74	33	

TABLE 2a. Percentage occurrence of fungi on untreated/treated, normal/discoloured barley grain (Readings are the mean of percentage occurrence in agar plate and blotter methods).

Fungi	Untreated grain		Treated grain	
	Normal	Discoloured	Normal	Discoloured
Alternaria alternata (Fr.) Keissler	9	17	5	12.5
A. chlamydospora Mouchacca	10.5	14	4.5	5
A. padwickii (Ganguly) M.B. Ellis	0	22	0	10
Aspergillus flavus Link	15	44	12.5	33.5
A. niger van Tieghem	33	56	23	42.5
A. terreus Thom	24	16.5	21	11.5
Cephalosporium acremonium Corda	0	3.5	0	2.5
Choanephora cucurbitarum (Berk. & Rav.) Thaxter	0	9.5	0	6
Cochliobolus heterostrophus Drechsl., eF.	0	11.5	0	7
Curvularia intermedia Boedijn	10.5	28	4.5	17.5
C. verruculosa Tandon & Bilgrami	4.5	3	2	3
Drechslera australiensis (Bungnicourt) Subram. & Jain ex M.B. Ellis	14.0	25	9	22
D. maydis (Nisikado & Miyake) Subram. & Jain	7.5	9	6	10.5
D. setariae (Sawada) Subram. & Jain	0	2.5	0	1.5
Fusarium oxysporium Schlecht.	52.5	66	39	61
F. solani (Mart.) Sacc.	22.5	62.5	18.5	47.5
Mucor circinelloides van Tieghem	31	75	32	54.5
M. racemosus Fres.	0	14.5	0	19
Nigrospora sp.	0	0	0	0
Penicillium notatum Westling	24.5	34	16	25
P. sp.	0	4	0	6
Rhizopus stolonifer (Ehrenb. : Fr.) Vuill	28	42.5	34	38.5
Setosphaeria rostrata Leonard	0	0	0	10
Stemphylium sp.	0	4.5	0	2
Syncephalastrum verruculosum Misra	0	28	0	24
Ulocladium atrum Preuss	7.5	8	5.5	7.5
U. chartarum (Preuss) Simmons	32.5	22	27.5	9.5
U. septosporum (Preuss) Simmons	0	5.5	0	3.5
U. tuberculatum Simmons	4	4	4	2.5

Total number of species 29.

TABLE 2b. Number of fungal species isolated from treated and untreated × normal and discoloured barley grain. Two methods of assessment were used.

Method	No. of fungal species isolated		
	Normal grain	Discoloured grain	Mean
1 – Agar Plate			
a. Untreated grain	17	26	21.5
b. Treated grain	17	29	23.0
Mean	17	27.5	22.3
2 – Blotter			
a. Untreated grain	16	21	18.5
b. Treated grain	16	21	18.5
Mean	16	21	18.5

TABLE 3a. Effect of different fungicides on seed-borne fungi. (Total number of seeds incubated was 200 for each treatment).

Fungi	Normal seeds												Discolored seeds											
	Disinfected						Non-disinfected						Disinfected						Non-disinfected					
	C	Ag	Dia	Cer	Bav	Vit	C	Ag	Dia	Cer	Bav	Vit	C	Ag	Dia	Cer	Bav	Vit	C	Ag	Dia	Cer.	Bav	Vit
Alternaria alternata	10	4	–	6	3	4	16	3	–	2	2	3	25	5	–	6	8	6	28	6	–	2	6	5
A. chlamydospora	6	–	–	–	–	1	6	–	–	–	1	1	5	–	–	–	–	–	9	–	–	–	1	3
A. padwicki	–	–	–	–	–	–	–	–	–	–	–	–	8	1	–	2	–	1	12	–	–	–	3	1
Aspergillus flavus	21	5	1	7	9	5	25	7	–	8	8	9	49	16	2	12	10	6	61	10	2	15	18	9
A. niger	19	12	–	15	5	4	44	7	–	3	6	3	56	21	3	10	8	6	63	15	–	15	10	7
A. terreus	20	5	–	5	7	2	26	4	–	7	6	4	16	3	–	2	4	1	10	2	–	4	3	–
Cephalosporium acremonium	–	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	10	–	–	–	2	–
Choanophora cucurbitarum	–	–	–	–	–	–	–	–	–	–	–	–	15	–	–	–	2	–	16	–	–	–	1	–
Curvularia intermedia	5	–	–	–	–	–	10	–	–	–	–	–	26	–	–	3	–	–	35	2	–	–	1	–
C. verruculosa	7	–	–	–	–	–	8	–	–	–	–	–	10	–	–	–	–	–	12	–	–	–	–	–
Drechslera australiensis	16	–	–	–	–	4	21	–	–	–	–	3	30	–	–	–	2	4	39	–	–	–	4	2
D. maydis	–	–	–	–	–	–	–	–	–	–	–	–	11	–	–	–	–	–	15	–	–	–	–	–
D. seteriae	–	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	7	–	–	–	–	–
F. oxysporum	46	4	–	2	5	1	53	9	–	4	6	2	62	–	–	12	10	5	71	–	–	16	15	8
E. solani	29	–	–	7	7	2	35	–	–	12	8	4	49	–	–	18	15	9	62	–	–	25	16	17
Mucor circinelloides	39	10	–	15	19	7	49	15	–	21	15	16	55	20	–	29	32	19	76	35	–	22	26	22
M. racemosus	–	–	–	–	–	–	–	–	–	–	–	–	15	–	–	–	–	–	19	–	–	–	–	–
Nigrospora sp.	–	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	–	–	–	–	–	–
Penicillium notatum	21	4	–	1	2	–	25	6	–	3	–	3	30	10	–	6	9	5	43	15	–	10	8	2
Rhizopus stolonifer	39	18	4	21	18	16	45	21	6	22	25	15	42	29	6	23	25	18	53	25	9	18	21	12
Syncephalastrum verruculosum	–	–	–	–	–	–	–	–	–	–	–	–	30	6	–	8	12	7	42	6	–	16	19	5
Stemphylium sp.	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	5	–	–	–	–	–
Ulocladium atrum	9	1	–	2	–	1	8	–	–	–	–	–	15	2	–	1	1	1	16	2	–	1	–	–
U. chartarum	18	5	–	–	–	–	21	7	–	3	2	3	25	5	–	3	4	3	27	3	–	2	2	2
U. tuberculatum	4	–	–	–	–	–	–	–	–	–	–	–	6	–	–	–	–	–	5	–	–	–	–	–

C = control
Ag = agrosin
Dia. = diathane
Cer. = ceresan
Bav. = bavistin
Vit. = vitamax
– = absent or not growtl

Results are presented as percentage of a particular species found in the total seeds incubated.

TABLE 3b. Mean effects of 5-fungicides on the incidence of fungi isolated from normal and discoloured barley grains.

	Controls	Grain treated with				
		Ag	Dia	Cer	Bav	Vit
Treated Normal grains	319/16	68/16	5/16	81/16	75/16	47/16
Mean	19.9	4.3	0.3	5.1	4.7	2.9
Untreated Normal grains	392/15	79/15	6/15	85	79/15	66/15
Mean	26.1	5.3	0.4	5.7	5.3	4.4
Treated Discoloured grains	595/25	118/25	11/25	123/25	142/25	91/25
Mean	23.8	4.7	0.4	4.9	5.7	3.6
Untreated Discoloured grains	736/24	121/24	11/24	146/24	156/24	95/24
Mean	30.7	5.0	0.5	6.1	6.5	4.0

Discussion

The fungal genera (not species) found in the present study were almost the same as reported earlier from barley seeds elsewhere[4,14-17] and in Saudi Arabia[18,21 and 22]. The discolored seeds yield higher number of fungal species and higher number of different genera than in normal grains. This conforms the finding of Shafie and Webster[8]. Therefore, discolored seeds are not good for cultivation as this will not only give the low yield but also contaminate other seeds[8]. Generally, non-disinfected seeds yields higher number of fungal species and percentage occurrence as compared to disinfected seed. This confirmed the findings of Shafie and Webster[8] and Singh and Singh[30]. Among the fungicides (Table 3a) diathane was most effective for fungal eradication. Singh and Singh[30] was also reported diathane as a most destructive fungicide while working on seed-borne fungi of broad-bean. Disinfected seeds gives higher percentage germination (Table 3b) as compared to non-disinfected seeds in both normal and discolored seeds as found by Shafie and Webster[8].

Conclusion

Results of present study suggest that normal disinfected seeds are good for planting. Diathane is the best fungicide for the control of seed-borne fungi. Therefore, it is recommended that discolored seeds should not be used for planting and normal seeds should be disinfected and treated with diathane prior to planting for a good harvest.

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الفلورا الفطرية لحبوب الشعير في المنطقة الجنوبية للمملكة العربية السعودية ومقاومتها

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المستخلص . أجريت هذه الدراسة بغرض التعرف على الأنواع الفطرية المصاحبة لحبوب الشعير ، ولقد تم في هذه الدراسة عزل ٢٩ نوعًا من الفطريات من الحبوب السليمة وغير السليمة . كما دلت النتائج على أن معظم الأنواع الفطرية عزلت من الحبوب غير السليمة . كما أظهرت نتائج هذه الدراسة أن معظم الأجناس المعزولة كانت كالتالي :

Ulocladium (أربعة أنواع) ، *Alternaria, Aspergillus* and *Drechslera* (ثلاثة أنواع) ، *Curvularia, Fusarium, Mucor, Penicillium* and *Syncephlastrum* (نوعين) .

كما وجد أن المبيد الفطري Diathane من أفضل المبيدات الفطرية المستخدمة في هذه الدراسة .