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EFFECT OF DIFFERENT MOISTURE AND STORAGE TEMPERATURE ON SEED BORNE MYCOFLORA OF MAIZE

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Abstract

Maize seeds with 8, 12,16 and 20% moisture content stored at 4,25, 35 and 40ºC for 6 months showed heavy (98-100%) fungal infection at 20% moisture after 30 days of storage at all temperatures of 4, 25, 16 and 35°C. Infection of *Aspergillus* species was highest in seeds with 20% moisture level stored at 25 and 40ºC , however lower number of fungi were observed on seed at 8% moisture level stored at 4 and 25ºC. Other fungi included *Absidia hesseltini*, *Alternaria alternata*, *Fusarium* spp., and *Penicillium* spp., in seeds at 8, 12, 16 and 20% moisture levels stored at 25 and 35°C rather than 40°C. Germination of seeds was significantly decreased with the increase in storage time due to fungal infection.

Introduction

Moisture and temperature are the two factors which affect on germination of seed, beside affecting mold growth and mycotoxin production (Bullerman *et al.,* 1984). Tariq *et al.,* (2005) also reported that high moisture and temperature increase the infection of *A.flavus* and decrease the germination of soy been seed. High moisture increased the incidence of *A.flavus* and *aflatoxin* B_1 production on sunflower seeds (Dawar & Ghaffar, 1992). Maize (*Zea mays* L.) is an important crop of Pakistan. It is grown over 0.9355 million hectare and production is 1.7371 million ton annually with an average yield of 1857 Kg / hectare (Anon, 2005). A large number of pathogenic fungi of maize grain causes combined world wide annual losses of 9.5% (Shurtleff, 1980). In Pakistan fungal infection on maize seed was recorded upto 66-90% with an average of 71.7% from sound seeds and 83.7% from damaged grains (Hafiz, 1986). Maize is multipurpose crop used for food, fodder and grains as well as production of oil, starch and glucose. In Pakistan, smuts, stalk rots, *Helminthosporium* blight, seedling blight, kernel and ear rot are considered economically important diseases of maize. However, rust, downy mildews and leaf spots are of minor importance (Hafiz, 1986). Most of the fungal pathogens of maize crop are seed borne. During prolonged storage of grains decrease in field fungi and increase in storage fungi has been reported (Sinha, 1979). *Alternaria*, *Helminthosporium Fusarium* and *cladosporium* are major fields fungi, *Aspergillus* and *Penicillium* are storage fungi. *Aspergillus flavus* and *Penicillium* species were found to be the most predominant fungi on maize seed (Hafiz, 1986) which is known to produces aflatoxin B_1, B_2, G_1 and G_2 in food and feed stuff (Diener & Davis, 1969; Peskta & Bonday, 1990). Many workers have found that poor harvesting practices, improper, storage and drying leads to fungal growth and toxin proliferation (Reddy *et al.,* 2001; Bankole & Adebanjo, 2003; Ravikiran *et al.,* 2005). The present investigation was carried out to the study effect of moisture and temperature on seed borne mycoflora of maize.

Materials and Methods

Seed sample of maize was collected from Peshawar variety kisan. Moisture content of seed was determined by oven dry method. Seed with an original moisture content of about 8% was adjusted at 12, 16 and 20% moisture levels by adding the required amount of sterilized distilled water to seed and kept in glass jars. The seeds were incubated for 24 hr in a refrigerater at 4-5 ºC with frequent shaking to facilitate the uniform distribution of moisture throughout the seedling (Lutey & Christensen, 1963; Dawar & Ghaffar, 1992) Seeds sample with moisture content of 8, 12, 16 and 20% were stored at 4, 25, 35 and 40ºC. Samples were removed at 0 and after 30, 60, 90, 120,150 and 180 days of incubation to study the germination and seed borne mycoflora, using blotter method as recommended by ISTA (Anon, 1993). Fungi growing on seeds were identified after reference to (Ellis, 1971). Nelson *et al.,* (1983); Domsch *et al.,* (1980); Raper & Fennel (1965). The data were subjected to analysis of Variance (ANOVA) following the procedure as given by Costat program (Gomez & Gomez, 1984).

Results and Discussion

Results showed that initial germination of the seeds was 90 to 96% and it decreased little in seeds containing 8 and 12% moisture levels ($p<0.05$). At 25ºC the germination rate decreased correspondingly with the increase in moisture levels and storage period. It was noted that at 40ºC, the germination of seed was zero, after 30 days of storage, having moisture levels 12% and above. At 35ºC the pattern was the same as at 40ºC except that with 12% moisture level and germination did not decrease to zero percentage. Qasem & Christensen, (1958) incubated corn samples containing 17-18% moisture content with *Aspergillus* species and found that the germination of the seeds was reduced to zero percent during storage. The lower rate of germination in seed with original moisture level of 8% and during storage at 4ºC and 25ºC could be due to the growth of insects which had infested the grain.(Moreno *et al*., 1994; Kabeere *et al*., 1997; Joao & Lovato 1999).Germination of maize seed

decreased with the increase in storage time. Low temperature and moisture were better for germination of seed ($p<0.001$) where as high temperature and moisture decreased the germination of seed. Surface sterilization of seeds showed an increase in seed germination (Table 1).

Sixteen fungal species viz., *Absidia hesselitini* Vam Tieghem, *Alternaria alternata* Nees., *Aspergillus condidus* Link., *A. flavus* Link es Gary., *A.fumigatus* Fres., *A.niger* Van Tieghem, *A.versicolor* Vuill., *A.wentii* Wehmer. *Curvularia lunata* Wakker., *Drechslera maydis*, *Fusarium equiseti Corda Sacc., F.moniliforme Sheld., F.semitectum* Berk & Rev., *Penicillium funiculosum* Thom., *P.oxalicum Currien & Thom, Rhizopus oligosporum Saito.,* were isolated from maize seed stored at different moisture and temperature Lowest number of fungi were reported in seeds with 8% moisture content followed by 12, 16 and 20%, stored at 4 and 25ºC. Infection of fungi increased with the increase in storage time ($p<0.001$) (Table 2) .However, germination of seed decreased with the increase in storage period (Table 2) There are reports that infections by storage fungi is a primary cause of loss of germination (Barton, 1961; Harrington, 1963; Mills & Frydman, 1980; Reddy *et al.*, 2001; Tariq *et al.*, 2005). Storage fungi attack the embryo of seed causing discolouration and finally outright decay (Golumbic & Laudani, 1966). Heavy fungal infections (98-100%) were reported on maize seed after 30 days of storage when moisture level was 20% at all temperatures (25, 35 and 40ºC). It was also noted that at 20% moisture the percentage of fungal infection start decreasing after longer storage period (after 120 days). This may due to the exhaustion of nutrients or the accumulation of toxic metabolites produced by the fungi themselves. Infection of all *Aspesgillus* species was highest at 20% moisture stored at 25 and 40 ºC. However, the lowest number of fungi was reported on seed with 8% moisture stored at 4 and 25ºC. Fungi like *Absidia* sp, *Alternaria* sp., *Fusarium* sp and *Penicillium* spp. were mostly recorded on seed at 8, 12, 16 and 20% moisture stored at 4, 25 and 35 than 40ºC (Table 2). Present results suggested that the 8% moisture and 4, 25°C temperatures would be better for the storage of maize seeds for decreasing the chances of mold growth and mycotoxin production.

Table 1. Effect of different moisture levels on germination (%) in maize seed stored at different temperature.

Storage period (Days)	4ºC				25ºC			
	Moisture levels (%)				Moisture levels (%)			
	8	12	16	20	8	12	16	20
0	47	40	35	25	97	93	91	92
30	35	30	20	11	89	89	53	17
60	33	22	18	0	85	65	41	0
90	29	20	15	0	29	25	13	0
120	20	15	7	0	37	13	12	0
150	17	9	0	0	25	9	0	0
180	0	0	0	0	0	0	0	0
Storage period (Days)	**35ºC**				**40ºC**			
	Moisture levels (%)				Moisture levels (%)			
	8	12	16	20	8	12	16	20
0	97	93	91	92	97	93	91	92
30	91	86	33	0	85	81	21	0
60	81	21	0	0	81	0	0	0
90	89	29	0	0	77	0	0	0
120	76	13	0	0	73	0	0	0
150	73	9	0	0	61	0	0	0
180	57	17	0	0	53	0	0	0

Table 2. Effect of different moisture levels on fungal infection (%) of maize seeds stored at different temperatures.

4°c																											
Moisture (%)																											
	8							**12**							**16**							**20**						
Days																											
Fungi	**0**	**30**	**60**	**90**	**120**	**150**	**180**	**0**	**30**	**60**	**90**	**120**	**150**	**180**	**0**	**30**	**60**	**90**	**120**	**150**	**180**	**0**	**30**	**60**	**90**	**120**	**150**	**180**
A.hesseltini	12	-	4	-	-	-	-	12	-	-	-	-	-	-	12	-	-	-	-	-	-	12	-	-	-	-	-	-
A.alternata	20	10	-	-	-	-	-	20	-	-	-	-	-	-	20	-	-	-	-	-	-	20	-	-	-	-	-	-
A.candidus	7	2	3	12	20	10	10	10	-	20	-	5	27	35	8	22	16	12	21	25	4	4	16	8	10	7	5	8
A.flavus	9	-	9	-	4	20	12	5	-	-	-	3	18	16	20	40	12	16	16	12	9	3	12	10	9	6	7	2
A.fumigatus	6	4	2	4	7	17	14	4	10	-	-	7	21	12	12	21	13	10	14	2	8	7	12	12	8	10	3	10
A.niger	9	-	8	10	9	10	10	-	12	-	-	-	-	14	12	14	14	20	22	10	2	5	10	14	15	10	16	4
A.wentii	4	6	-	2	6	4	2	2	17	10	5	-	4	18	10	20	24	14	30	10	6	8	6	8	20	8	21	2
A.versicolor	-	-	2	-	-	-	-	-	4	-	-	-	-	-	-	-	2	-	-	-	-	-	-	2	2	-	-	-
C.lunata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	4	4	-	-	-	-	10	4	2	-	-	-
D.maydis	6	-	-	-	2	-	-	6	12	8	6	4	-	-	6	16	-	-	-	-	-	6	14	-	-	-	-	-
F.equiseti	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	10	-	-	-	12	14	-	16	-	-	-	-	-
F.moniliforme	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	10	-	12	-	-	-	-	-
F.semitectum	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-
P.funiculosum	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P.oxalicum	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R.oligosporum	3	-	-	-	-	-	-	3	-	-	-	10	-	-	3	-	-	-	-	-	-	3	-	-	-	-	-	-
25°C																												
A.hesseltini	6	-	-	-	-	2	-	6	3	-	-	16	16	8	6	6	10	6	-	-	-	6	1	1	10	10	-	-
A.alternata	12	-	4	-	-	4	-	12	16	14	20	14	24	10	12	12	10	10	4	-	-	12	69	8	82	99	-	-
A.candidus	-	-	16	-	-	-	-	-	4	-	17	-	-	-	50	70	50	38	58	60	76	58	69	82	90	99	10	6
A.flavus	8	-	-	4	-	-	-	-	-	-	12	-	-	-	60	84	25	70	84	29	69	64	88	76	87	96	30	4
A.fumigatus	50	-	-	15	-	5	-	4	3	12	-	-	-	-	36	48	47	80	76	76	85	65	98	80	64	59	15	2
A.niger	20	-	-	-	-	-	-	-	10	-	-	24	-	-	14	48	88	30	48	58	49	21	88	68	88	69	-	-
A.wentii	-	-	-	-	4	-	-	5	-	20	2	-	-	-	35	66	88	88	62	26	46	41	82	69	82	76	-	-
A.versicolor	-	-	-	10	-	-	-	3	-	-	-	-	-	-	22	12	-	7	-	-	-	12	76	24	30	22	4	6
C.lunata	-	-	-	-	-	2	-	-	-	14	-	-	-	-	-	-	-	-	14	-	-	16	48	28	22	28	-	-
D.maydis	-	-	-	-	3	-	-	-	12	-	24	16	8	-	-	-	6	6	-	-	-	13	59	40	48	42	2	4
F.equiseti	-	-	-	6	-	-	-	-	15	-	-	-	-	-	10	-	-	-	16	10	4	-	61	-	40	22	-	-
F.moniliforme	8	6	-	-	-	-	-	4	-	21	-	-	-	6	-	4	-	-	-	-	-	-	30	-	32	-	4	-
F.semitectum	-	2	12	-	-	12	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	4	42	35	30	-	-	-
P.funiculosum	2	-	-	-	10	-	-	-	14	-	-	20	-	-	-	-	4	6	10	-	-	-	48	40	38	26	-	-
P.oxalicum	-	-	-	2	-	-	-	10	-	-	-	16	4	-	-	-	-	-	-	-	-	-	30	-	-	30	-	-
R.oligosporum	2	-	-	-	-	8	-	5	-	3	-	-	-	-	-	-	-	-	-	4	-	-	12	-	-	12	-	-

Table 2. (Cont'd.).

35°C																												
Moisture (%)																												
	8							**12**							**16**							**20**						
Days																												
Fungi/Days	**0**	**30**	**60**	**90**	**120**	**150**	**180**	**0**	**30**	**60**	**90**	**120**	**150**	**180**	**0**	**30**	**60**	**90**	**120**	**150**	**180**	**0**	**30**	**60**	**90**	**120**	**150**	**180**
A.hesseltini	-	2	6	4	20	-	-	12	20	12	16	14	-	-		-	14	-	-	-	-		10	10	10	68	2	-
A.alternata	-	12	-	-	20	-	-		24	12	10	6	2	-		6	2	4	2	10	6		-	-	-	-	-	-
A.candidus	-	30	14	6	4	-	-		16	4	12	10	6	2		16	14	22	16	4	2		46	30	40	24	4	4
A.flavus	-	12	10	6	2	-	-		6	8	6	6	10	-		12	18	16	14	10	2		50	40	14	22	2	-
A.fumigatus	-	10	6	4	2	2	-		10	2	4	10	6	-		14	20	12	12	10	4		40	22	20	12	-	4
A.niger	-	14	10	8	6	4	8		10	4	6	6	8	10		20	14	12	20	16	10		26	36	32	24	-	-
A.wentii	-	16	14	14	10	6	4		8	6	12	8	4	4		22	10	10	10	4	-		30	58	14	24	-	-
A.versicolor	-	2	-	-	-	-	-		6	6	-	-	-	6		8	4	-	-	-	-		10	34	20	16	-	-
C.lunata	-	4	6	-	-	-	-		12	-	6	-	16	-		6	-	-	-	-	-		24	14	14	20	4	4
D.maydis	-	-	-	-	-	-	-		-	-	-	-	-	-		6	-	-	-	-	-		32	16	32	32	10	2
F.equiseti	-	-	-	-	-	-	-		10	2	6	8	-	-		14	-	-	-	-	-		12	26	34	6	-	-
F.moniliforme	-	12	3	-	-	-	-		16	-	-	2	-	-		20	14	10	8	4	2		10	20	26	8	-	-
F.semitectum	-	4	2	2	-	8	-		6	-	2	4	-	-		14	12	4	-	-	-		16	12	10	16	-	-
P.funiculosum		4	-	-	10	-	-		-	-	2	-	-	-		12	-	-	-	-	-		8	10	16	10	-	-
P.oxalicum		2	-	-	-	-	-		8	6	2	-	-	-		-	-	-	-	-	-		24	20	14	8	-	-
R.oligosporum		-	-	-	-	-	-		4	2	-	-	-	-		-	-	-	-	-	-		22	16	16	6	-	-
40°C																												
A.hesseltini	2	-	6	-	-	-	-	2	12	-	-	-	-	-	2	-	-	-	-	-	-	2	10	10	50	26	4	6
A.alternata	4	-	12	-	-	-	-	4	-	-	-	-	-	-	4	-	-	-	8	-	-	4	-	-	48	40	-	-
A.candidus	-	10	6	14	4	10	16	-	12	4	12	2	4	-	-	20	16	10	4	-	-	-	39	61	50	26	-	-
A.flavus	-	2	4	-	-	-	16	-	20	6	-	2	-	-	-	20	10	12	6	-	-	-	42	49	48	40	-	-
A.fumigatus	-	6	-	16	6	4	4	-	10	8	-	-	8	-	-	16	4	2	10	10	-	-	60	38	60	46	-	-
A.niger	-	4	2	2	10	8	-	-	6	-	-	-	4	14	-	20	16	20	14	6	10	-	42	30	40	16	-	-
A.wentii	-	8	10	14	8	-	-	-	12	4	-	6	-	10	-	10	12	14	-	-	6	-	48	24	32	20	-	-
A.versicolor	-	-	-	5	-	10	-	-	-	-	-	2	-	-	-	-	-	-	8	-	-	-	46	28	28	28	10	6
C.lunata	-	-	-	-	4	-	-	-	-	2	2	-	2	-	-	-	3	-	-	-	-	-	22	20	40	16	-	-
D.maydis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	2	-	-	-	48	32	26	28	-	-
F.equiseti	-	-	-	-	-	-	-	-	4	-	-	6	-	-	-	-	-	-	-	-	-	-	22	10	34	16	-	-
F.moniliforme	-	-	-	-	-	-	-	-	-	2	6	-	-	-	-	-	2	-	-	2	-	-	26	16	32	38	-	-
F.semitectum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	24	28	14	-	-
P.funiculosum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	2	26	10	24	-	-
P.oxalicum	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	8	22	30	-	-
R.oligosporum	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	4	12	16	30	-	-

Absidia hesseltini, Alternaria alternata, Aspergillus candidus ,Curvularia lunata, Drechslera maydis, Fusarium equiseti, Penicillium funiculosum, Rhizopus oligosporum.

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