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EFFECT OF RELATIVE HUMIDITY ON SEED DETERIORATION OF *SORGHUM VULGARE* PERS.

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The seed samples were stored at different humidities in percentage of 65, 75, 85 and 95 for the study. With advance of storage period, the percentage incidence of molds increased in all the levels of humidities. Storage at higher levels of relative humidity (85% and 95%) showed more total molds counts as compared to lower levels of relative humidity (65% and 75%). The spectrum of storage fungi showed that aspergilli were most dominant followed by *Alternaria, Fusarium, Penicillium* and *Curvularia* sp. The rate of increase and total number of aspergilli seemed to be influenced by high relative humidity levels of 85% and 95% and the seeds were visible moldy. *Alternaria alternata, Aspergillus flavus, Curvularia lunata, Fusarium semitactum* and *Penicillium citrinum* were dominant fungi invading stored seeds of *Sorghum vulgare*. *Alternaria alternata, Curvularia lunata* and *Fusarium semitectum* were prevalent on seeds stored at 65% and 75% RH and in control conditions but at 85% and 95% RH levels Several field fungi including *Alternaria alternata, Curvularia lunata, Myrothecium verrucaria* declined as the storage time of *Sorghum vulgare* seeds extended. The storage fungi attained dominance with increasing storage time at higher humidity levels. Germinability was better retained by seeds with low moisture than those with more moisture and higher relative humidity. After 9 months, the seeds kept at room temperature at different levels of RH lost their germinability. Some germination was recorded in seeds kept at RH 85%. After 12 months of storage, germination was lost in all cases particularly at 85-95% RH.

Key words : Relative humidity, seed deterioration, Sorghum.

Sorghum (*Sorghum vulgare*) is an important grain and fodder crop ranking fourth after paddy, wheat and maize in the world. Sorghum grains are known to be infected with several seed- borne fungi such as *Fusarium moniliforme, Curvularia lunata, Phoma sorghina, Alternaria alternata, Exserohilum turcicum, Macrophomina phaseolina, Drechslera rostrata, Rhizopus stolonifer, Aspergillus* spp., etc. (Patil *et al*. 2008). These seed-borne fungi are responsible for grain discolouration, reduced seed weight, germination, viability and causes seedling mortality (Navi *et al*. 2005, Leslie *et al*. 2005). They also affect the shoot and root length and moisture content of the grains (Kotgire, 2009). Richardson (1990) listed 40 seed-borne fungal pathogens responsible for the low yield of the crop. Seed -borne mycoflora of sorghum reported from different parts of the world include *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium* sp., *Fusarium moniliforme,F. oxysporum , F. pallidoroseum, Drechslera tetramera, Nigrospora* sp., *Phoma* sp.,and *Rhizopus* sp. (Abdullah and Kadhum 1987, Ahmed *et al*. 1992). Species of the genera *Aspergillus, Fusarium, Alternaria* and *Drechslera* have been considered to be major plant pathogens worldwide (Ghafoor and Khan 1976, Mirza and Qureshi, 1978). Important seed-borne fungal diseases recorded on sorghum are stalk rot (*Aspergillus niger*), target spot (*Bipolaris sorghicola*), stalk rot/anthracnose/red leaf (*Colletotrichum graminicola*), seed rot/stalk rot (*Fusarium moniliforme*), seedling blight/charcoal rot (*Macrophomina phaseolina*) and covered smut/grain smut (*Sphacelotheca sorghi*) (Islam *et al.* 2009). Seven species of fungi detected in sorghum seeds obtained from different location of Punjab were—*Aspergillus flavus, A. niger, A. tenuis, Curvularia lunata, Fusarium moniliforme, Helminthosporium* (*Bipolaris*) *sativum* and *Penicillium* sp. *Fusarium moniliforme* was found to be most devastating fungus in seed. Grain mold of sorghum results from colonization of fungi in the developing grains towards the end of the growing season, being often associated with late rains. Grain mold fungal species commonly isolated from sorghum grains include *Fusarium thapsinum, Curvularia lunata, Fusarium semitectum* and *Colletotrichum graminicola, Alternaria alternata, Phoma sorghina* and *Cladosporium* spp. *Fusarium thapsinum* is considered the most prevalent grain mold fungal species infecting sorghum and also produces mycotoxin which is harmful to humans and livestock (Anasari and Shrivastava 1990, Bhat *et al.* 1997). These fungi invade the developing grains but at different stages of maturity (Ratandass *et al.* 2003). Many fungal diseases reported on sorghum are mainly seed transmitted (Richardson 1990) and are built up in the soil. Sorghum anthracnose caused by *Colletotrichum graminicola* (Ces.) Wilson (syn. *C. sublineolum* P. Henn) is one of the most important seed borne diseases in Burkina Faso (Neya and Normand, 1998). Navi *et al.* (2005) reported that the damage resulting to the grains of sorghum infected by fungal pathogens includes reduced kernal development, discolouration

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of grain, colonization and degradation of endosperm, decreased grain density, germination, seedling vigour and possible mycotoxin contamination. Panchal and Dhale (2011) isolated seed-borne fungi of sorghum in Marathwada region by using the blotter and agar plate methods. Gwary *et al.* (2006) studied the survival of *Colletotrichum sublineolum* and other seed-borne fungi in sorghum seeds after 20 months of storage. Khan and Siddiqui (1980) stated that germination of sorghum seeds was reduced from 93% to 4% when seeds having 17% moisture content were stored at 35° C for 90 days after inoculation with nine species of *Aspergillus* and three of *Penicillium*. The seed-borne nature of the pathogen provides primary inoculum during crop growth stage. It is the main source of introduction and spread of pathogen in disease free areas. Therefore, considering the importance of the problem, the present investigation was carried out on various aspects to generate more information on sorghum grain fungi. The object of the present study was to isolate fungi from grains (seeds) of sorghum, an important cereal crop, were stored at different humidities for 18 months and were analysed for their mycoflora with a view to obtain information in seasonal variations in the fungal flora and also their succession in field and during storage at different stages of seed. In addition to this, germination percentage and weight of seeds at different intervals have been determined since fungi are held mainly responsible for deterioration of seeds.

MATERIAL AND METHOD

Freshly harvested grains(seeds) of a widely cultivated Sorghum *(Sorghum vulgare)*, from growers of Meerut (UP) region were stored at different humidities in percentage 65, 75, 85 and 95 for the study. The seed samples were placed in desiccators containing different concentrations of potassium hydroxide as suggested by Solomon (1951). These desiccators were later transferred to incubators which were set at 24 ± 2°C. The moisture contents of the seeds were calculated by method of Anonymous (1947). 10 g of seeds of each sample were taken in weighing dish of known weight, dried in a hot air oven for 5 hours at 110°C and cooled over calcium chloride in a desiccator. The process was repeated till constant weight was registered. The moisture percentage was calculated by the difference in the weight before and after heating the seeds. The mycoflora colonizing seeds at above-mentioned relative humidities was estimated by blotter method of De Tempe (1953) was employed to isolate the externally and internally seed-borne fungi. In this method three circles of thick white blotters were sterilized, moistened with 0.02% solution of 2,4 -dichlorophenoxyacetic acid to suppress seed germination. 400 seeds were plated in Petri dishes of 10 cm diameter. The seeds were sterilized in 0.1% mercuric chloride solution for 3 minutes and washed 3-4 times with sterilized water before plating. The Petri dishes were incubated at 28 ± 2°C. The developing colonies of fungi were recorded on 8th and 12th day. Fungal colonies developed by the above methods were picked up from 3rd day and transferred aseptically to Potato dextrose agar slants for identification and their percentage calculated at intervals of three months using PDA medium and the fungi recorded.

OBSERVATION AND DISCUSSION

To study the effect of relative humidity on seed-borne fungi and their effect on seed deterioration, an experiment was conducted with four levels of relative humidities, viz. 65%, 75%, 85% and 95%. The seeds showed almost similar trends of succession in terms of seed mycoflora. The percentage incidence of fungi increased at all the levels of humidities. The dominant storage fungi isolated in the present study were *Alternaria alternata, Aspergillus flavus, Curvularia lunata, Fusarium semitectum* and *Penicillium citrinum.* The fungi such as *Alternaria alternata, Curvularia lunata* and *Fusarium semitectum* were prevalent on seeds stored at 65% and 75% RH and in control conditions. However, at 85% and 95% RH levels, several field fungi including *Alternaria alternata, Curvularia luanta* and *Myrothehcium verrucaria* which had pathogenic potential on the seeds of *Sorghum vulgare,* gradually declined as the storage time extended, while storage fungi like aspergilli and penicillia attained dominance with increasing storage time. At higher levels of RH, the seeds were moldy. Narnaware *et al*. (2006) reported that *Fusarium* and *Curvularia* are the two most important fungal taxa causing grain discolouration and reduction in viability of seeds. Panasenko (1967) reported that the aspergilli were more prevalent on cereal grains probably due to a lower relative humidity (65-88%) required for their growth, as against high relative humidity (82-100%) required by other storage fungi and some of the osmophilic aspergilli (requiring high sugar or high salt substratum) are encountered on grains stored at RH 65-88%. Dange *et al*. (1985) also noted that ground-

Table 1. Percentage frequency of fungi recorded on seeds of *Sorghum vulgare* during storage for 12 months by blotter method at various level of humidity (observation based on 400 seeds).

Name of fungi	Control				Relative humidity levels															
					65%				75%				85%				95%			
	Months				Months				Months				Months				Months			
	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12
Alternaria alternata	20.5	24.1	25.2	29.1	23.0	24.5	25.2	30.0	28.0	28.0	30.0	36.0	12.5	10.2	6.2	4.0	10.5	8.0	6.0	7.5
A. brassicicola	-	3.0	-	2.1	-	-	1.0	-	-	3.2	-	-	-	-	-	-	-	-	-	-
Aspergillus Chevalieri	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.0	5.0	4.5
A. clavatus	-	-	-	-	-	-	-	-	-	-	-	-	-	6.0	7.2	-	-	-	10.0	11.5
A. flavus	10.0	15.0	13.2	17.0	11.2	14.0	13.2	15.5	15.5	20.0	26.0	27.0	16.1	22.5	26.7	30.0	13.0	28.0	34.0	38.0
A. fumigates	0.2	2.0	-	-	0.5	-	1.9	3.2	-	-	5.2	5.7	-	-	6.1	7.5	-	-	6.9	8.2
A. nidulans	-	-	-	-	-	-	-	3.1	-	-	0.5	-	-	-	1.9	2.7	-	-	5.1	4.9
A. niger	-	-	8.0	11.0	-	5.1	2.0	4.1	-	3.0	3.5	5.5	-	2.8	6.0	6.2	4.0	3.0	6.0	6.5
A. terreus	-	-	0.5	4.0	-	5.4	6.5	6.9	10.6	12.0	19.5	21.9	8.5	13.0	21.0	30.1	6.2	20.1	29.0	35.0
Candida sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.5	10.2	-	11.0	10.1	10.0
Chaetomium Brasilense	2.0	5.0	7.0	4.0	0.6	-	-	-	1.1	-	-	1.6	-	-	-	-	-	-	-	-
Chaetomium sp.	-	-	4.0	-	2.0	-	-	-	-	-	-	0.5	-	-	-	-	-	-	-	-
Curvularia Iunata	9.0	7.0	11.0	1.0	8.8	8.0	8.5	6.8	3.2	9.1	7.2	6.6	3.2	5.0	2.4	2.1	2.2	5.1	2.8	2.1
Fusarium Moniliforme	2.0	3.0	3.0	4.0	-	3.1	3.6	3.0	-	2.5	4.1	2.8	2.9	3.5	-	-	4.5	5.1	3.1	2.2
F. semitectum	9.5	8.0	9.0	8.5	9	8.9	10.4	8.2	0.6	8.0	6.2	6.1	3.6	4.0	3.4	4.1	0.4	6.1	2.8	2.5
Geotrichum sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	5.8	-	-	3.2	-	3.1	1.3
Mucor mucedo	-	-	2.5	1.2	-	-	-	-	3.2	2.2	-	-	-	-	-	-	-	-	-	-
Myrothecium Verrucaria	3.0	2.7	3.1	2.0	-	-	-	1.3	-	-	3.2	1.4	-	-	-	-	1.2	0.5	-	-
Penicillium citrinum	1.0	5.5	8.1	7.7	3.0	-	6.4	8.0	3.2	3.9	6.8	8.5	4.5	6.0	10.2	14.7	7.3	12.7	12.8	15.5
Penicillium sp.	3.0	4.1	-	4.0	3.6	-	-	4.0	-	-	4.2	-	3.0	6.4	6.0	6.0	6.0	5.9	7.4	7.7
Rhizopus nigricans	2.0	3.2	-	3.1	-	5.4	-	-	3.6	2.2	-	-	-	3.5	-	1.0	4.3	-	-	-
Stachybotrys atra	-	-	-	3.9	-	-	-	-	-	-	-	-	3.1	-	-	-	-	-	-	-
Sterile hyphae	5.4	-	-	-	-	-	3.9	-	4.1	-	-	1.5	-	-	2.5	1.0	3.6	-	2.1	2.6
Thielavia terricola	4.0	-	2.2	-	-	-	2.3	-	-	-	2.5	-	3.6	-	3.3	3.4	-	4.1	5.3	5.2
Trichoderma viride	-	7.8	2.0	-	3.2	2.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Verticillium sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.2	1.8	-	0.3	2.5	1.5
Total organisms	**13**	**13**	**14**	**15**	**10**	**9**	**12**	**12**	**10**	**11**	**13**	**13**	**10**	**12**	**15**	**15**	**13**	**14**	**18**	**18**
Total percentage of incidence	**71.6**	**90.4**	**98.8**	**102.6**	**64.9**	**77.3**	**84.9**	**94.1**	**73.1**	**94.1**	**119.2**	**125.1**	**60.8**	**88.7**	**117.8**	**124.8**	**66.4**	**101.9**	**151.0**	**166.7**

Table 2. Percentage moisture content, germination and infection of *Sorghum vulgare* seeds at different relative humidities at room temperature.

Relative humidity	Months				
	3	6	9	12	
65 %	11.6	11.2	11.3	13.3	
75 %	12.6	12.6	12.2	12.7	
85 %	13.8	13.9	13.3	14.3	**Moisture**
95 %	14.9	15.0	15.0	15.1	**content %**
65 %	92.5	79.0	32.0	16.3	
75 %	92.2	77.5	31.8	6.0	**Germination %**
85 %	86.3	75.0	30.5	2.4	
95 %	81.0	73.0	5.0	2.0	
65 %	64.9	77.3	84.9	94.1	
75 %	73.1	94.1	119.2	125.1	**Incidence %**
85 %	60.8	88.7	117.8	124.8	
95 %	66.4	101.9	151.0	166.7	

nut seeds stored at higher RH (85 and 95%), suffered greater invasion by *Aspergillus flavus* and *A. niger*. Some fungi, viz. *Candida* sp., *Geotrichum* sp. and *Verticillium* sp. which were not obtained on the seeds of *Sorghum vulgare* at low humidity levels and control sets in the present study were obtained at higher levels (85% and 95%) of RH(Table 1). Owolade *et al*. (2001) isolated mycofungi associated with maize seed discolouration and abnormalities. Several workers (Ellis *et al*. 1974) have suggested that higher temperature and wet conditions either alone or occurring together late in the growing season probably favour seed colonization by fungi. Spilker *et al*. (1981) observed that high humidity-high temperature resulted in soybean seeds with severe *Phomopsis* infection (49%) and the poorest germination (32%) but low humidity-low temperature and low humidity-high temperature resulted in the fewest *Phomopsis*-infected seeds. Burroughs and Saucer (1971) reported that *Alternaria, Cladosporium* and *Fusarium* were dominant genera found in sorghum seeds immediately after harvest but they declined gradually during storage period and were dominated by *Aspergillus niger, A. terreus, Circinella* sp., *Rhizopus* sp. and *Penicillium* sp. Similarly Ghosh *et al*. (1981) studied seed mycoflora of different varieties of wheat and found that field fungi were overwhelmingly predominant constituting more than 90% of the total number of species at harvest. The number of field fungi was found to decrease significantly, with prolonged storage in all cases. The percentage of storage fungi (species of *Aspergillus* and *Penicillium*), on the other hand, which were present only occasionally at harvest, showed continuous increase during the storage period(Table 1). Aliyu and Kutama (2007) isolated and identified fungal flora associated with groundnut in diffferent storage facilities. Gwary *et al.* (2006) studied the survival of *Colletotrichum sublineolum* and other seed-borne fungi in sorghum seeds after 20 months of storage. Storage fungi, viz. *Aspergillus, Chaetomium, Nigrospora, Penicillium* and *Rhizopus* have been reported to have negative effect on the viability of seeds (Malaker *et al.*, 2008). Javaid *et al.* (2010) investigated the mycoflora associated with different varieties of shisham and found that seeds of all the 12 varieties of shisham are equally susceptible to fungal attack during storage. Abdulsalaam and Shenge (2011) conducted an experiment to determine the type of seed-borne fungal pathogens associated with farmer-saved sorghum seeds. They identified seven fungal genera growing on the seed samples. These were *Helminthosporium* sp., *Aspergillus* sp., *Fusarium* sp., *Rhizoctonia* sp., *Penicillium* sp., *Sclerotium* sp., and *Curvularia* sp. Butt *et al.* (2011) studied the storage grains of five varieties of rice to investigate the occurrence of seed-borne mycoflora using blotter paper method. Four fungal species namely *Fusarium moniliforme, Alternaria* sp., *Helminthosporium* sp. and *Curvularia* sp. were isolated from different test rice varieties. Nandi *et al*. (1982), while

studying the seed mycoflora of oil seeds (sesame, mustard and linseed) also noted that in all cases, both seed moisture and fungal infections were higher at 90% RH and 20°C than in other treatments. A gradual decrease in infection by field fungi with concomitant increase by storage fungi, accompanied by a reduction in seed germination occurred as storage progressed. Similar observations were made by Vijayalakshmi and Rao (1985) on sunflower seeds. The decrease of field fungal infection which was predominant in the early period of storage in the present study could be partly due to the absence of the required levels of RH necessary for their growth and development as pointed by Richardson (1970) and partly by the overgrowth of storage fungi which increased gradually to reach a peak at the end of the experimental period. At 95% RH, invasion by storage fungi was much more than other fungi(Table 1).

The amount of water in grains affects both grade and storability. For this reason, moisture content limits are included in the specifications for the various grades of different grains in the official grain standard of the country. In the present study, germinability was better retained in seed with lower moisture than in those with higher moisture content. After 9 months, the seeds kept at room temperature at different levels of RH lost their germinability. Some germination was recorded in seeds kept at RH 85%. After 12 months of storage, germination was lost in all cases particularly at 85-95% RH. In the present study, a relationship between moisture content and relative humidity was observed(Table 2). When relative humidity is more, the moisture content of the seed increases rapidly and possibly due to the absorption of moisture by seeds (Snow, 1945). The storage of seeds at low RH apparently led to the death of some mycelia that were not resistant to comparatively drier conditions. On the other hand, seeds stored at a high RH in laboratory incubators had an increased moisture content that favoured a more profuse overgrowth of storage fungi which increased gradually to reach a peak at the end of the experimental period in the present study. As a result, the seeds germinability declined (Christensen and Kaufmann, 1969; Richardson, 1970; Harrington, 1973; Jorgensen, 1974; Ghosh *et al*., 1981). Kotgire (2009) reported ten different fungi, viz., *Fusarium moniliforme, Fusarium* sp., *Curvularia* sp., *Aspergillus niger, A. flavus, Alternaria alternata, Bipolaris* sp., *Macrophomina phaseolina, Penicillium* sp. and *Chaetomium* sp. and mixture of ten fungi exhibited significant adverse effects on seed germination as well as shoot and root length.

The drastic reduction in seed germination at higher humidity levels may be attributed to the invasion of embryonic tissues by *Aspergillus* spp. as suggested by Singh *et al*. (1981).They stated that the viability of seeds got reduced during storage which might be due to the storage fungi under normal conditions of temperature and moisture. Mondal *et al.* (1981) during studies on deterioration of oil seeds in storage found a gradual decrease in field fungi with simultaneous increase in storage fungi accompanied by a reduction in seed germinability as storage proceeded. Poor post harvest management can lead to rapid deterioration in grain quality, severely decreasing the germinability and nutritional value of stored grains. Mould growth in grains may cause deleterious changes in addition to the formation of mycotoxins. Many spoilage fungi cause loss of germination in seed grains, discolouration and darkening of the grains, reduction in protein content, musty odours and changes in fatty acid profiles and other constituents of the grains. Mould development may also reported to be encouraged the mite and insect infestation (Wicklow, 1995). The higher rate of infection was, however, mainly due to storage fungi. The percentage of storage fungi increased with increase in RH, evidently due to high RH that generally favoured growth and germination of most storage fungi in the present study. These findings are in accordance with those of Qasem and Christensen (1958) in maize, Papavizas and Christensen (1958) and Ghosh *et al*. (1981) in wheat seeds and Nandi *et al*. (1982) in oil seeds. According to Christensen (1973), in general, storage fungi grow at moisture contents in equilibrium with relative humidity of 65-70 to 85-90 per cent.

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