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FREQUENCY AND ASSESSMENT OF FUNGI AT DIFFERENT GRAIN FORMATION STAGES AND DISCOLORED SEEDS OF SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH)

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ABSTRACT
Grain infecting fungi are important constraints for the sorghum crop grown during *Kharif* season particularly when grain formation stage is synchronized with high field humidity, as well as when harvesting of matured earhead is delayed. Isolation of grain-infecting fungi from earheads of field grown sorghum revealed the association of *Fusarium moniliforme*, *Fusarium* sp., *Curvularia* sp., *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata*, *Bipolaris* sp., *Macrophomina phaseolina*, *Penicillium* sp. and *Chaetomium* sp. Frequency of the fungi on pinkish discolored, blackish discolored, brown spotted grains and smaller and shriveled grains were ranged from 2.50 to 38.75, 2.00 to 18.00, 2.75 to 18.25 and 2.00 to 14.50 per cent respectively. The loss of grain weight in different categories of grains was in the range of 27.78 to 52.28 per cent. Assessment of fungi at physiological maturity, normal maturity and two weeks after normal maturity stage of earhead revealed association of 6-9 fungi and which was ranged from 2.00 to 7.00, 3.00 to 14.00 and 2.00 to 21.00 per cent respectively

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is a vital life-sustaining food crop for human being as well as for livestock in many parts of world. It is one of the major staple foods for the world's poorest and insecured people. Grain mold is a major problem of early maturing sorghum in regions where flowering and grain filling occur during periods of high relative humidity and warm temperatures (28 to 37ºC). Occurrence of fungi observed in all the stages of grain formation. Many fungi that attack to the sorghum ear head at different stages and caused grain discoloration and these fungi were *Absidia corymbifera* (Cohn) Sacc and Trotter, *Alternaria citri* Ellis and Pierce apud Pierce, *Aspergillus awamori* Nakazawa, *A. candidus* Link ex Link, *A. japonicus* Saito, *A.luchuensis* Inui, *A. panamensis* Raper and Thom, *A. penecilloides* Spegazzini, *A. terricola* Marchal, *A. terreus* Thom, *A. wentii* Wehmer, *Chaetomium globosum* Kunze ex Steud., *C. indicum* Corda, *Cladosporium oxysporum* Berk. and Curt, *Paecilomyces variotii* Bain, *Syncephalastrum racemosum* Cohn ex Schrot, *Trichoderma hamatum* (Bonord) Bain and *Tricothecium roseum* (Pers) Link ex Gray (Rasheed *et al*., 2012). These fungi affect developing grains adversely such as grain discoloration, molding of grain surface, endosperm degradation, reduced grain filling and reduced germination besides softening and mustiness of grains are the common features associated with grain mold infection. Such moldy and discolored grains cause heavy loss in grain weight. Similarly nutritional status of sorghum grains is likely to be hampered (Patil *et al*., 2008).

Degree of damage to the sorghum grains is influenced by various biotic and abiotic factors such as different fungi that attack to the sorghum ear heads at physiological, normal and delayed maturity and leads to discoloration. It is very important to study, at which stage maximum fungal attack was done. So as to reduce the grain discoloration caused by different fungi may be it is beneficial to avoid economic loss of grain weight caused by that fungus. The information available on this aspect in fragmented manner particularly assessment and frequency of fungi at different stages of sorghum crop and different discolored categories of sorghum grains. Keeping this point in a view study undertaken for consideration.

MATERIALS AND METHODS

Fungi damage to sorghum earheads not a single stage they attack to the crop at different stages of grain formation and laterally production of discolored seeds.

Isolation

Isolation of fungi associated with sorghum grains was carried out from randomly taken 400 grains from the composite grain samples by standard blotter method (Bhale *et al*., 2001). Twenty five grains per Petri plates, after surface sterilization by 1% sodium hypochlorite solution for one minute, were placed at equi distance on three layers of properly moistened sterilized blotters, and Petri plates were incubated under 12/12h alternating light and dark period at 25 ± 2ºC. Developing fungal growth on each of the grains after seven days was observed under stereoscopic binocular microscope and

recorded accordingly.

Purification

The fungal growth of different fungi obtained on grains was transferred on PDA Petri plates. Each fungal species isolated was further purified by single spore or hyphal tip method.

Identification and maintenance of the fungal cultures

Various ear-infecting fungi, developed on sorghum grains, were separately cultured on PDA Petri plates. Each fungal growth was critically observed under stereoscopic microscope as well as research microscope for cultural and morphological characters. Finally, fungal characteristics observed were compared with the characteristics described in various manuals (Watanable, 1993; Booth and Waterston, 1998; Booth, 1998). Cultures were maintained on PDA slants by sub culturing and stored at 5ºC for further study.

Assessment of fungal association with discoloured, shrivelled and smaller grains

Qualitative and quantitative assessment of fungi associated with different categories of grains, sorted out on the basis of grain discoloration and abnormality, was done group-wise from naturally infected earheads of sorghum. Further loss of grain weight was also calculated by taking 1000 grain weight of each category of grain with four repetitions. Assessment was done, from 400 grains from each of the categories, after surface sterilization by 1% sodium hypochlorite solution for 1 minute by standard blotter method (Bhale *et al*., 2001).

Assessment of grain infecting fungi at different stages of grain formation in sorghum crop under field condition

Qualitative and quantitative assessment of grain infecting fungi of sorghum grains was done at physiological maturity, normal maturity and 2 weeks after normal maturity stage from field grown sorghum cultivar GJ 38 by standard blotter method (Bhale *et al*., 2001). For this, five earheads were collected from each of 10 different parts of the field. Thus, total 10 composite samples were taken. After hand threshing, 400 grain samples were drawn randomly from each of 10 composite grain samples for fungal assessment. After surface sterilization of grain by 1% sodium hypochlorite solution for 1 minute, 25 grains per Petri plates were placed on three layers of thoroughly moistened sterilized blotters. After 4 days of incubation at 28ºC with 12h photoperiod, each grain was examined under stereoscopic microscope to record frequency (%) of sorghum grains colonization by various fungi. Various fungi growing on grains were recorded and transferred to PDA Petri plates for further study.

RESULTS AND DISCUSSION

Results on the association of fungi with different groups of discolored and shrivelled grains, collected from field-grown sorghum crop of fields, are presented in Table 1. Overall, 5 to 8 different fungal species were found associated with discolored as well as shrivelled and smaller grains of sorghum.

Table 1: Fungal association with naturally infected grains of sorghum

Fungus	Frequency (%) of fungal association				
	Pinkish discolored grains	Blackish discolored grains	Dark Brown spots on grains	Shrivelled grains	Healthy grains
Fusarium moniliforme	38.75	11.50	18.25	14.50	2.50
Fusarium spp.	21.50	5.00	9.00	5.00	1.00
Curvularia spp.	0.00	18.00	10.00	13.50	0.00
Aspergillus niger	3.00	6.25	2.75	3.75	0.00
Aspergillus flavus	0.00	0.00	0.00	2.00	0.00
Alternaria alternata	0.00	15.00	12.00	6.50	0.00
Bipolaris spp.	0.00	14.50	0.00	6.00	1.25
Macrophomina phaseolina	2.50	6.00	0.00	0.00	0.00
Penicillium spp.	4.00	0.00	0.00	3.00	0.00
Chaetomium sp.	0.00	2.00	3.00	0.00	0.00
Total fungal species	5	8	6	8	3

Table 2: Assessment of grain infecting fungi at different stages of grain formation in sorghum crop under field condition

Fungus	Frequency (%) of fungal association		
	Physiological maturity	Normal maturity	Two weeks after normal maturity
Fusarium moniliforme	7.0	14.0	21.0
Fusarium spp.	4.0	12.0	26.0
Curvularia spp.	7.0	13.0	17.0
Aspergillus niger	0.0	6.0	8.50
Aspergillus flavus	0.0	3.0	6.0
Alternaria alternata	6.0	8.0	12.0
Alternaria sp.	4.0	9.0	14.0
Bipolaris spp.	2.0	3.0	6.0
Penicillium spp.	0.0	0.0	2.0
Total fungal species	6	8	9

Among all fungi, *Fusarium moniliforme*, *Fusarium* spp. and *Aspergillus niger* were associated with each of the four categories of infected grains. Pinkish discolored grains and blackish discolored grains revealed 5 and 8 fungi, whereas grains with dark-brown spots and shrivelled and smaller grains shown 6 and 8 fungi, respectively.

Frequency wise, fungi viz., *Fusarium moniliforme* (38.75%), *Fusarium* spp. (21.50%), *Penicillium* sp. (4.00%), *Aspergillus niger* (3.00%) and *Macrophomina phaseolina* (2.50%) were found associated in descending order on pinkish discolored grains. The highest frequency of association of *Fusarium moniliforme* and *Fusarium* spp. with pinkish discolored grains observed in present study, tallies with the observations of Padghan and Sanap (2008) and Patil *et al*. (2008). Padghan and Sanap (2008) noted that *Fusarium* spp. induced pink

Table 3: Effect of discoloured, shrivelled and smaller grains on grain weight

Grains	1000 grain weight (g)*	% decrease in grain weight over healthy grains
Pinkish discoloured grains	11.80	52.28
Blackish discoloured grains	12.36	50.02
Shrivelled and smaller grains	14.23	42.45
Brown spotted grains	17.86	27.78
Healthy grains	24.73	
S. Em. ±	0.26	
CD 0.05%	0.58	
CV %	2.00	

* Average of four replications

discoloration of sorghum grains.

Fungi viz., *Curvularia* sp. (18.00%), *Alternaria alternata* (15.00%), *Bipolaris* sp. (14.50%), *Fusarium moniliforme* (11.50%), *Aspergillus niger* (6.25%), *Macrophomina phaseolina* (6%), *Fusarium* spp. (5.00%), and *Chaetomium* sp. (2.00%) were associated with blackish discolored grains. Rao and Williams (1977) reported association of *Curvularia* sp. on blackish discolored seeds, whereas Patil *et al*. (2008) found black discoloration of sorghum seeds due to the infection of *Alternaria alternata* and *Curvularia penniseti*. Fungi associated with dark brown spotted grains were *Fusarium moniliforme* (18.25%), *Curvularia* sp. (10.00%), *Fusarium* spp. (9.00%), *Alternaria alternata* (12.00%), *Chaetomium* sp. (3.00%) and *Aspergillus niger* (2.75%). Shrivelled grains of sorghum revealed the association of *Fusarium moniliforme*, *Curvularia* sp., *Alternaria alternata*, *Bipolaris* sp., *Fusarium* spp., *Aspergillus niger*, *Penicillium* sp. and *Aspergillus flavus* showing 14.50, 13.50, 6.50, 5.00, 3.75, 3.00 and 2.00% frequencies respectively. Apparently healthy looking grains did not remain completely free from association of fungi as evident from the association of three fungi viz., *Fusarium moniliforme* (2.50%), *Bipolaris* sp. (1.25%) and *Fusarium* spp. (1.00%).

Thus, by and large, different categories of grains revealed preferential trend of fungal association. Thus, it can be concluded that various fungi induce specific grain abnormality.

Results of the assessment of fungi associated with different stages of grain formation in field-grown GJ 38 variety of sorghum, carried out by standard blotter method, are presented in Table 2. Overall, 6 fungal species were associated with surface sterilized grains at physiological maturity stage, whereas 8 and 9 different fungi were associated with grains at normal maturity and two weeks after normal maturity stage, respectively.

Frequency wise, fungi viz., *Fusarium moniliforme* (7%), *Curvularia* sp. (7%), *Alternaria alternata* (6%), *Alternaria* sp. (4%), *Fusarium* spp. (4%) and *Bipolaris* sp. (2%) were found associated in descending order at physiological maturity stage. At normal maturity stage, 8 different fungi viz., *Fusarium moniliforme* (14%), *Fusarium* spp. (12%), *Curvularia* sp. (13%), *Aspergillus niger* (4%), *Aspergillus flavus* (2%), *Alternaria alternata* (8%), *Alternaria* sp. (9%) and *Bipolaris* sp. (3%) were found associated. *F. moniliforme*, *Fusarium* spp. and *Curvularia* sp. were major ones in terms of their frequencies.

The frequency of fungi 2 weeks after normal maturity revealed presence of *Fusarium moniliforme* (21%), *Fusarium* spp. (26 %), *Curvularia* sp. (17%), *Alternaria alternata* (12%), *Alternaria* sp. (14 %), *Aspergillus niger* (6%), *Aspergillus flavus* (4%), *Bipolaris* sp. (6%) and *Penicillium* sp. (2%). Thus, it was interesting to note that fungal infection increased with increasing trend of frequency of fungal association from physiological maturity to two weeks after normal maturity. These findings confirm the observations of Kumar (1991). He reported less deterioration of sorghum grains due to grain molds at normal harvesting stage than delayed harvesting. Sawant (2000) also reported that sorghum grain mold increased as the harvesting of sorghum was delayed. Recent study conducted by Somwanshi and Kurundkar (2008) revealed the higher intensity of *Fusarium* and *Curvularia* species with sorghum grains in delayed harvesting than harvesting at physiological maturity. These observations tally the findings of present investigation. Once fungal infection of grains takes place in the standing crop, it is likely that infection gets aggravated with passage of time due to congenial field humidity resulting into more grain deterioration when harvesting is delayed.

Results on effect of different types of discolored as well as smaller and shriveled grains on grain weight, presented in Table 3, revealed that all types of discolored as well as smaller and shriveled grains showed significant loss of grain weight as compared to healthy grains. Overall, 27.78 to 52.28 % loss of grain weight was noted over healthy grains. Pinkish discolored grains revealed significantly the lowest grain weight (11.80g) which was at par with blackish discolored grains (12.36g). Pinkish discolored grains revealed 52.28% loss of grain weight followed by blackish discolored grains (50.02%), shrivelled and smaller grains (42.25%) and brown spotted grains (27.78%).

It is quite possible that fungal infection to the developing grains leads to poor nourishment resulting into loss of grain weight besides other deteriorating effects on seed health. Umechuruba (1986) noted that *Fusarium moniliforme*, *Fusarium* sp., *Aspergillus flavus* and *A. niger*, responsible for ear rot, reduced grain size and induced physiological alteration in grains in maize. Castor (1977) also reports reduced yield and test grain weight in sorghum due to grain mold caused by *Fusarium* spp.

REFERENCES

Anonymous 2009a. Economic intelligence service, Center for Monitering Indian Economy Pvt. Ltd., Mumbai, pp. 37-38.

Anonymous 2009b. Monthly review of the Indian Economy. Monitering Indian Economy. *Pvt. Ltd., Ahmedabad.* p. 25.

Bhale, M. S., Khare, D., Rawt, N. D. and Singh, D. 2001. Seed borne diseases objectionable in seed production and their management. *Scientific Publishers, Jodhpur (India).* pp. 10-16.

Booth, C. 1998. *Fusarium oxysporum*. *CMI descriptions of fungi and bacteria*. **22**: sheet 221.

Booth, C. and Waterston, J. M. 1998. *Fusarium oxysporum* f. sp. *vasinfectum*. *CMI descriptions of fungi and bacteria*. **3**: sheet 28.

Castor, L. 1977. Seed molding of grain sorghum. Development of high yielding, disease and insect resistant sorghum cultivars. Annul Progress Report. TAES-USAID Contact ta-c 1092. *Texas Agricultural*

Experiment station Texas, USA.

Kumar, L. S., Prakash, S. H., Shetty, H. S. and Mallieshi, N. G. 1991. Influence of seed mycoflora and harvesting conditions on milling, popping and malting qualities of sorghum. *J. Sci.Food Agri*. **55(4):** 617-625.

Padghan, P. R. and Sanap, J. H. 2008. Efficacy of fungicidal treatment on incidence of grain mold in sorghum. *International J.Plant Protection*. **1(2):** 54-57.

Patil, P. J., Padule, D. N., Suryawanshi, J. S. and Pinjari, S. S. 2008. Fungi associated with mouldy seeds of sorghum cv.CSH-9 IN Western Maharashtra. *International J. Plant Protection*. **1(2):** 84-87.

Rao, K. N. and Williams, R. J. 1977. The ICRISAT Sorghum Pathology Program. International sorghum workshop 6-13, Mar. 1977. *ICRISAT, Hyderabad, India.*

Rasheed, S., Dawar, S., Ghaffar, A. and Shaukat, S. S. 2012. Seed-borne Mycoflora of Sorghum. *Pak. J. Bot.* **36(1):** 199-202.

Sawant, L. V. 2000. Effect of grain mold fungi on physiological and nutritional qualities of grains in sorghum M. Sc. (Ag.). *Thesis M.A.U., Parbhani (M.S.).*

Somwanshi, S. D. and Kurundkar, B. P. 2008. Prevalence of Fusarium and Curvularia Species in mold affected grains of sorghum genotypes. *J. Mycol. Pl. Pathol*. **38(1):** 148-150.

Umechuruba, G. I. 1986. Effect of thioral on seed-borne fungi associated with maize varieties grown in eastern Nigeria. *Trop. Pest. Manage.* **32**: 27-30.

Watanable, T. 1993. Pictorial atlas of soil and seed fungi, morphologies of cultured fungi and key to species. *Lewis publisher, Washington, D. C.* pp. 168-327.