Detection of Seedborne Grain Mold Fungi in Sorghum and their Control with Fungicidal Seed Treatment

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Introduction

Grain mold, caused by a complex of fungi, is a serious problem of sorghum (*Sorghum bicolor*) that affects its grain yield, quality and market value. The annual economic loss in Asia and Africa as a result of grain mold is more than US$130 million (Chandrashekar et al. 2000). Early-maturing, short-statured, high-yielding hybrids that flower and mature during wet weather are particularly vulnerable to attack by mold fungi. Among the fungi involved in the mold complex, species of *Fusarium, Curvularia* and *Alternaria* are more abundant than others (Girish et al. 2004). The risk of introducing new species or strains of a pathogen could be minimized if we have sound knowledge of their seedborne nature and the treatment to reduce the possibility of seed transmitted inoculum. Efforts to produce sorghum genotypes with tolerance to grain mold by conventional breeding have been only partially successful (Thakur et al. 2003). This study was undertaken to investigate the seedborne nature of predominant mold fungi and to identify a fungicide as seed treatment to minimize the seed infection of these fungi.

Materials and Methods

Seedborne nature of mold fungi. Seed of mold resistant (IS 8545 and PVK 801) and susceptible (Bulk Y, SPV 104 and CSH 9) genotypes from the crop grown during the rainy season in 2002 was used. Twenty-five seeds from each of five genotypes were surface sterilized in 2% Clorox® for 2 min and thoroughly washed with sterile distilled water (SDW), and soaked in 4% potassium hydroxide (KOH) for 15 min and then transferred into SDW. Each seed was dissected to separate its seed coat, endosperm and embryo. These were plated separately on potato carrot agar (PCA) medium, and incubated at 22±2°C with 12 h near-ultraviolet light (NUV) for 5 days. Data were recorded on the number of seed components infected by individual fungi in each genotype. The experiment was repeated once.

Seed treatment with fungicide. Three treatments, Bavistin (carbendazim) (2.5 g kg^{-1}) seed as dry seed dressing (SD), thiram (3 g kg^{-1} seed) as SD and soaking seed in Bavistin solution (2%) for 4 h as seed steeping (ST), were used (Munghate and Raut 1982). From each genotype 300 seeds were used with 100 seeds in each of three replications. Seed were surface sterilized in 2% Clorox® for 2 min, washed with SDW and air-dried before treating with fungicides. Seed without fungicidal treatment served as control. The seed were evaluated for fungal colonization using the standard blotter method at 22±2°C with 12 h NUV for 7 days (ISTA 1992). Overall mold colonization and seed germination were recorded and subjected to analysis of variance to determine significant differences among the treatments. The experiment was repeated once.

Table 1. Infection by *Curvularia lunata* and *Fusarium verticillioides* on seed components of five sorghum genotypes plated on potato carrot agar medium.

	Infection[1] (%)					
	C. lunata			*F. verticillioides*		
Genotype[2]	Seed coat	Endosperm	Embryo	Seed coat	Endosperm	Embryo
Bulk Y (S)	20	32	38	74	56	34
CSH 9 (S)	84	78	62	36	24	12
SPV 104 (S)	54	50	30	18	18	14
PVK 801 (R)	30	30	6	20	18	0
IS 8545 (R)	0	8	2	38	34	4
Mean	38	40	28	37	30	13
SEm±	5.2	6.0	5.1	10.6	4.3	2.8

1. Data are means of two experiments. In each experiment, 25 seeds per genotype were tested.
2. S = Susceptible; R = Resistant.

Table 2. Effect of fungicide treatment on grain colonization by mold fungi in five sorghum genotypes.

Treatment[2]	Grain colonization (%) by mold fungi[1]				
	Bulk Y (S)	CSH 9 (S)	SPV 104 (S)	IS 8545 (R)	PVK 801 (R)
Thiram SD	20 (74)[3]	15 (85)	0 (100)	3 (96)	17 (80)
Bavistin SD	54 (30)	96 (4)	63 (29)	54 (27)	53 (37)
Bavistin ST	64 (18)	100 (0)	55 (38)	50 (32)	77 (8)
Control	78	100	89	74	84
SEm±	9.6	0.7	9.5	13.5	6.5
Mean reduction	43	30	52	42	56

1. Data are means of two experiments with three replications in each experiment; 100 seeds per replication were tested. S = Susceptible; R = Resistant.
2. SD = Seed dressing; ST = Seed steeping.
3. Figures in parentheses indicate reduction percentage compared to control.

Results and Discussion

Fungal infection in seed components. Infection of seed components (seed coat, endosperm and embryo) occurred only by *Fusarium verticillioides* and *Curvularia lunata* and not by *Alternaria alternata.* The incidence of infection varied significantly among the component tissues and among the sorghum genotypes (Table 1). Infection of tissues was significantly lowered in resistant (IS 8545 and PVK 801) than in susceptible genotypes (Bulk Y, SPV 104 and CSH 9). In the susceptible genotypes, the component tissue infection ranged from 20 to 84% by *C. lunata* and 12 to 74% by *F. verticillioides*. In an earlier study (Girish et al. 2004), we found six fungi (*A. alternata, Bipolaris sorghicola, C. lunata, F. verticillioides, Exserohilum rostratum* and *Phoma sorghina*) commonly associated with sorghum grain mold complex and these caused seed rot and reduced seed germination to a considerable extent. In this investigation, of these six fungi only *C. lunata* and *F. verticillioides* were found to be seedborne and highly infectious as infection was detected in seed coat, endosperm and embryo in most sorghum genotypes.

Effect of fungicides on grain mold colonization. Seed treatment with thiram SD significantly reduced grain mold colonization in all the five sorghum genotypes (Table 2). Compared with thiram SD, Bavistin SD or Bavistin ST was less effective in reducing grain colonization. Among the methods of fungicide application, SD was more effective in controlling the mold colonization than ST.

Effect of fungicides on grain infection by seedborne fungi. Seed treatment with thiram significantly reduced

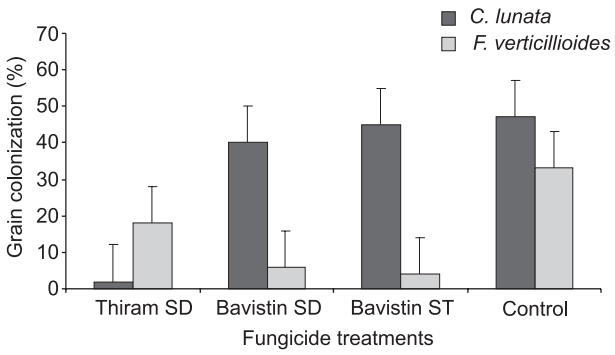

Figure 1. Effect of fungicide treatments on grain mold colonizaion by *Curvularia lunata* and *Fusarium verticillioides* across five sorghum genotypes. (Note: SD = Seed dressing; ST = Seed steeping.)

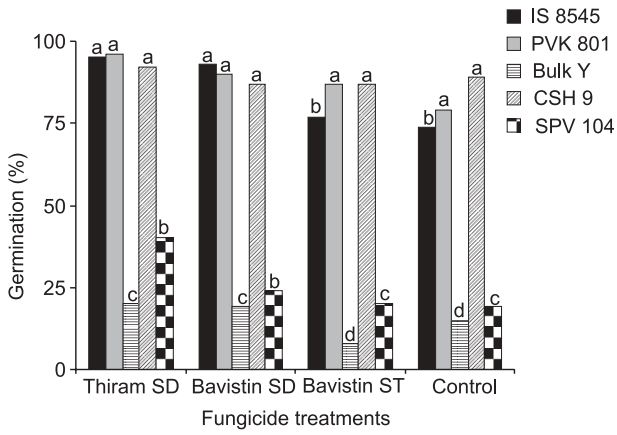

Figure 2. Effect of fungicide treatments of moldy grain on germination of five sorghum genotypes. (Note: The values of bars with common letters in each treatment group are not significantly different at $P<0.05$. SD = Seed dressing; ST = Seed steeping.)

infection by *C. lunata,* while Bavistin treatments significantly reduced infection by *F. verticillioides* across sorghum genotypes (Fig. 1). With reduction in grain mold infection (by 30–56%), there was a subsequent increase in seed germination by 4–47% over the control across sorghum genotypes (Fig. 2). In this study, out of the two SD fungicides, thiram proved superior to Bavistin in eliminating infection by *A. alternata* and *C. lunata* and Bavistin was superior to thiram in eliminating infection by *F. verticillioides*. Thus, it is suggested that seed treatment with a mixture of thiram and Bavistin (1:1) could be routinely used to eliminate the seedborne infection by *C. lunata* and *F. verticillioide*s during sorghum germplasm exchange.

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Effect of Two Mold Causing Fungi on Physical and Nutritional Properties of *Kharif* Sorghum Grains

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Introduction

Sorghum (*Sorghum bicolor*) was the second largest grain crop in India until the Green Revolution and presently ranks third in area sown and fifth in production among the food grains in the country. The principal grain mold fungi in India are *Fusarium* spp and *Curvularia lunata* and both cause severe yield losses. In severely affected grains nutritional quality can be seriously impacted (Williams and Rao 1980). Bhatnagar (1971) reported marked reduction in the size and weight of sorghum grains artificially infected with *C. lunata*. Considering the gravity of grain quality deterioration in terms of physical and nutritional properties, the present investigations were undertaken to study the impact of grain mold on physical and nutritional properties of sorghum grains.

Materials and Methods

Field experiments were carried out to obtain grains with different mold intensities during the *kharif* (rainy) season in 2002. A split plot design was used with eight sorghum lines (CSH 9, CSH 14, CSH 16, CSH 17, CSH 18, CSV 13, CSV 15 and PVK 801) as main treatments and three harvest situations as sub-treatments with three replications. Sorghum lines were inoculated with *Fusarium moniliforme* and *C. lunata* in the field. The observations for each treatment and sub-treatments were taken in three replications under laboratory conditions.

A correlation of fungi associated with grains to the pathological, physical and nutritional parameters was recorded. A simple statistical correlation technique was used to study the relationship between fungal infection and physico-chemical properties of sorghum grains.

Grain quality parameters related to fungal infection were threshed grain mold rating (TGMR), germination and physical properties such as test weight, grain volume and density, floaters and grain hardness. Nutritional parameters measured included moisture, crude protein, crude fat, crude fiber, soluble sugars, starch, ash and appropriate calorific value.