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Effect of temperature and humidity regimes on grain mold sporulation and seed quality in sorghum (*Sorghum bicolor* (L.) Moench)

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Abstract
Grain mold, induced by a number of non-specific fungi, causes substantial loss to seed/grain yield and quality in sorghum (*Sorghum bicolor* (L.) Moench). Fungal sporulation and grain mold severity are greatly influenced by temperature and relative humidity (RH) levels. We studied the effects of three incubation temperatures (25, 27 and 28°C) and two sets of RH levels (first set: 85, 90, 95, 98, and 100%, second set: 95, 96, 97, 98, 99 and 100%) on sporulation and grain mold severity in three major mold fungi (*Curvularia lunata*, *Fusarium moniliforme*, and *Bipolaris australiensis*) and on four each of resistant, moderately resistant and susceptible sorghum genotypes for sporulation and mold severity of major fungi. Results indicated that both fungal sporulation and grain mold severity increased on most sorghum genotypes with increasing incubation temperature from 25–28°C and RH levels from 95–98%. A linear relationship was observed among RH levels, grain mold severity and fungal sporulation. The highest sporulation of all the three fungi occurred at 28°C and 98% RH after 5 days of incubation. Among the three fungi, *C. lunata* grew and sporulated faster than *B. australiensis* and *F. moniliforme*, in that order. Among the sorghum genotypes, IS 25017 supported the least sporulation and had the lowest mold severity, followed by IS 8545 and PVK 801. Seed quality parameters, such as seed germination, seedling vigor index, field emergence potential, dehydrogenase and ά-amylase activities declined significantly with increasing temperature and RH levels that supported heavy sporulation and grain colonization.

Keywords: *Grain mold, seed quality, sporulation,* Sorghum bicolor

Introduction

Grain mold is a major obstacle between potential and actual yields in sorghum (*Sorghum bicolor* (L.) Moench) cultivars maturing in high humidity and warm environments in the rainy

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season (Forbes et al. 1992). The estimated yield loss due to grain mold varies from 30 – 100%. Grain mold not only affects the sorghum yield by reducing grain size and weight but also quality, and nutritional value, leading to production of several potential mycotoxins and secondary metabolites due to several mold fungi (Navi et al. 1999). Early infections often result in abortion of caryopsis since the whole seed is damaged, while late infections are generally confined to pericarp.

It is estimated that annual economic losses in Asia and Africa as a result of grain mold are in excess of US$ 130 million. The poverty implications include loss of access to food, exposure to health risks through contaminated food, and income losses through lower prices. Grain mold of sorghum is the greatest constraint for optimum grain yield where anthesis occurs in humid, warm, and rainy seasons (Forbes et al. 1992). Exposure under such conditions allows the floral niche to be exploited in a number of ways prior to grain development. Grain mold has remained a continuous problem for the semi-arid tropics of India, Africa, and the America. Grain mold problem in sorghum is a complex of three phenomena: (1) infection of developing grains by parasitic and saprophytic fungi; (2) grain discoloration and weathering; and (3) loss of seed germinability or sprouting.

A few fungi infect sorghum spikelet tissues during the early stages of grain development. These are (in approximate order of importance) *Fusarium moniliforme* held., *Curvularia lunata* (Wakker) Boedijin, *Fusarium pallidoroseum* (Cooke) Sacc. *F. semitectum* Berg and Rave), and *Phoma sorghina* (Sacc.). *F. moniliforme* and *C. lunata* are distributed worldwide (Williams & Rao 1981; Bandyopadhyay 1986). Several fungi belonging to about 21 genera have been reported so far with sorghum grain by various workers.

Screening of sorghum lines for grain mold resistance under field conditions has successfully been done by spraying panicles with water (Anahosur 1983) or by providing sprinkler irrigation or mist on rain free days. A strong correlation between moldiness and high humidity has been observed and that all photoperiod insensitive cultivars that mature during rainy season under high humidity levels are often diseased, even so-called resistant cultivars rarely escape through it. But mere discoloration does not really mean the loss in total seed quality. In addition, a healthy looking seed may even have internal infection and that may be categorized into normal grain. Mere superficial growth does not mean that the seed is internally infected. Earlier studies on the effect of weather variables on grain mold sporulation have been inconclusive and, hence, an attempt was made to understand the effect of temperature and RH regimes on mold fungal sporulation, and seed quality in sorghum, since the sporulation is an important phase for epidemics. The data obtained by these experiments can be of utmost use to formulate risk assessment models and future breeding strategies to manage the grain mold problem.

Materials and methods

Two sets of experiments were conducted at the International Crop Research Institute for Semi Arid Tropics (ICRISAT), Patancheru, India to understand the effects of temperature and relative humidity regimes on sporulation of sorghum grain mold fungi and the quality of sorghum seed. Unless otherwise specified the first set of experiments were conducted from April – July 2001, and the second set from April – July 2002 under controlled environments.

Effect of RH regimes on infection by major mold fungi upon seed inoculation on the genotype PVK 801

Seed material and grain mold fungi. In this experiment matured grains of PVK 801 were used to understand effects of temperature and RH regimes on infection. Seeds of PVK 801 were

multiplied in the field during the post rainy season 2000 and were presumed to be healthy. The grains were evaluated following dip inoculation technique (Singh & Navi 2001) using major mold fungi isolated at ICRISAT- Patancheru location during the rainy season 2000.

Inoculum preparation. Pure culture of a particular fungus was made by inoculating a small quantity of mycelia from a naturally infected grain in a sterilized Petri dish containing PDA under sterile conditions. After inoculation, the plate was incubated for a few days and used for sub-culturing. This was used to prepare grain culture. For making grain culture, presoaked grains were autoclaved and inoculated with small pieces of agar containing mycelia from pure culture. These were allowed to infect the grains by incubating them. Grain cultures of *Fusarium*, *Curvularia,* and *B. australiensis* were used separately to make spore suspension. In known (measured) quantity of sterile distilled water (SDW), a few grains from the grain culture were added under sterile conditions. In order to remove maximum spores adhering to the grain surface, it was stirred well with the help of a magnetic stirrer or mixer. Grains were then removed with the help of a flame sterilized tea strainer and the filtrate or spore suspension thus obtained was checked for spore concentration by using haemocytometer. If necessary, it was either diluted by adding SDW or concentrated by adding more grains so as to adjust the desired spore concentration, i.e. 1×10^6 spores/ml. This was determined by using the following formula:

$$N1V1 = N2V2$$

where: N1 = measured spore concentration; V1 = initial volume; N2 = required spore concentration, i.e. 1×10^6 spores/ml; V2 = final volume.

Evaluation of grains for infection. The grains of PVK 801 were surface sterilized in 1% sodium hypochlorite (Clorox containing 5.25% NaOCl) for 2 – 3 min, washed thoroughly with SDW, and air dried on sterilized filter paper in the microflow. The surface sterilized grains were separately dip-inoculated in the spore suspension of *F. moniliforme*, *C. lunata* and *B. australiensis* at 275 grains fungus^{-1} and another 275 grains were dipped in SDW, as control. After the inoculation, grains were air-dried under aseptic conditions, and were placed in sterilized Petri dish humid chambers at 25 grains plate^{-1}. The plates were incubated in an Incubator (Perceival, USA) at 28°C for 24 h with 12 h light cycle.

Subsequently, the grains from the Petri dish presumed to have initiated the infection process were transferred on sterilized tetrapoid square metallic stands each with 12 wire-mesh chambers (Figure 1A) under aseptic conditions. For each fungus, there were three replications each with 20 grains. The stands were placed in a single plant humidity chambers (SPHC) stabilized at 85, 90, 95, 98, 99 and 100% RHF (Figure 1B). Similarly inoculated (two plate fungus^{-1}) and un-inoculated grains (one plate) were placed outside the chambers but within the growth room for comparison.

Mold score and conidial count. At an interval of 24 h, for seven days each grain was evaluated for overall percent mold score on a 1 – 100 scale where 1% = no grain surface was colonized and 100% ≥ 75% of the grain surface area colonized. Immediately after seven days of incubation in SPHC, 10 grains from each wire mesh chamber and from Petri dishes were sampled in sterilized vial containing 5 ml DSW and a 0.1 ml trypan blue. The stain was added to inhibit conidial germination ahead before counting is completed.

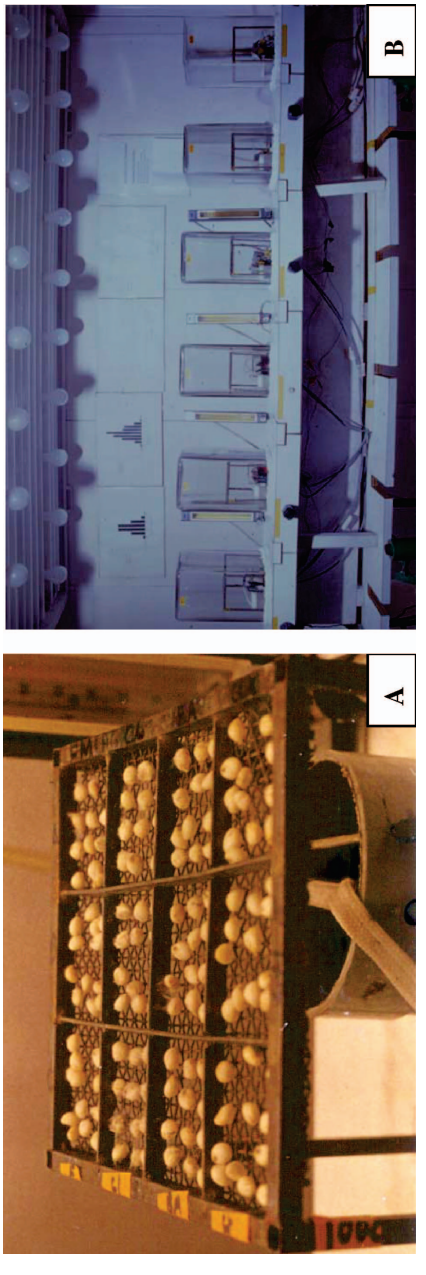

Figure 1. Evaluation of sorghum grains for fungal infection. (A) Placement of grains on sterile tetrapoid stands. (B) Single plant humidity chambers utilized to expose grains to different Relative Humidity levels.

To obtain conidial suspension from the grains, each vial was shaken on a vortex mixture, and the conidial count was measured using haemocytometer. The experiment was repeated twice.

Effect of RH regimes on infection by major mold fungi upon panicle inoculation on the genotype PVK 801

Raising plants and inoculation. In this experiment, following staggered planting, plants of PVK 801 were raised in the greenhouse facility at ICRISAT during the summer 2001. The panicles were inoculated at 50% flowering using fresh conidial suspensions ($1 \times 10^5\,ml^{-1}$) of *F. moniliforme*, *C. lunata* and *B. australiensis*. These were major mold fungi isolated at ICRISAT, Patancheru during the 2000 rainy season. The inoculated panicles were exposed to $>90\%$ relative humidity in the dew chambers for seven days. Subsequently, the panicles were placed in the controlled environment until physiological maturity for three weeks. At maturity, grains that were free from colonization in the panicles were collected in a minigrip bag for sporulation study.

Evaluation of grains for infection. Similar to the experiment mentioned above, the grains were evaluated for fungal infection. In this experiment the grains collected at maturity were surface sterilized; air dried under aseptic conditions and transferred on sterilized tetrapoid square metallic stands. These stands were placed in a single plant humidity chambers stabilized at 85, 90, 95, 98, 99 and 100% RH. There were three replications each with 20 grains per RH regime. As in experiment 1, the grains were evaluated for mold score and for conidial count. The only difference in this experiment was that grains were collected from an inoculated panicle unlike the grains collected from the post rainy season were inoculated in the previous experiment. The experiment was repeated twice.

Evaluation of cultivars for grain mold infection and effect of temperature and RH regimes in relation to sporulation and seed quality on selected cultivars

Evaluation of cultivars for infection and fungal frequency. A set of 37 advanced sorghum breeding lines that were exposed to four mold pressures (1 – natural, no artificial rain (shed), 2 – exposed to natural environment, 3 – mist was provided from flowering to hard dough stage, and 4 – mist was provided from hard dough stage to physiological maturity) in a rainout shelter during the rainy season 2001 at ICRISAT constituted this experiment. The grains were harvested from all the four mold pressures to measure fungal frequency. Grains from the third mold pressure were harvested prior to the stage where apparent mold symptoms are observed in the panicles with an assumption that grains are internally colonized but have not shown sporulation. The grains collected were surface sterilized and plated on moist blotter paper and evaluated for fungal frequency and mold severity on a 1 – 9 scale where 1 = no mold and $9 \geq 75\%$ mold. Later severity index was calculated by using the following formula:

$$\text{Mold severity (\% Infection index)} = \frac{Y(1-1) + Y(2-1) + \ldots Y(n-1) \times 100}{N \times (n-1)}$$

where: Y = the number of grains in each reaction category (severity rating); N = the total number of grains in the genotype under test; n = number of days of incubation.

Effect of temperature and humidity regimes. Out of 37 lines, 16 were selected for the sporulation study (IS 8545, IS 3443, IS 18758C-618-2, IS 25017, IS 30469C-140 as resistant ICSV 96101, SEPON 78-1, CSV 4, ICSV 95001, as and Bulk-Y, ICSV 91008, IS 18522, PVK 801 moderately resistant, SPV 351, CSH 9, SPV 104 as susceptible).

A total of three temperatures 25, 27 and 28°C and five relative humidity regimes 85, 90, 95, 98 and 100% were selected. Further to arrival at critical humidity level, another set of relative humidity from 95, 96, 97, 98, 99 to 100% were simulated in SPHC at a constant temperature of 28°C. The temperature in the room and RH regimes in each of the SPHC were programmed immediately before placing tetrapoid square metallic stands. A total of 108 surface sterilized grains (36 grains replication^{-1}) were placed in individual chambers as shown in Figure 1A and B. At an interval of 24 h, individual grains in all the RH regimes were evaluated for number of grains colonized by mold fungi, and their over all mold score on the 1 – 9 scale for seven days. Later severity index was calculated. Seven days after incubation 20 grains from each wire mesh chamber of SPHC were transferred to screw vials containing 5 ml SDW and a drop of trypan blue stain. The vials were shaken on a vortex mixture to obtain spore suspension of fungi colonized grains. The spore concentration of fungi observed was recorded using haemocytometer. The experiment was repeated three times.

Analysis of seed quality. Seeds of sixteen genotypes tested for three temperature and five levels of relative humidity in the SPHC were used for seed quality analysis. The seeds of each genotypes were subjected to various physiological and biochemical tests *viz.,* seed germination, vigour index, field emergence, speed of germination, electrical conductivity of seed leachate, dehydrogenase and α-amylase activity in seeds. These observations on seed quality were recorded twice, once before the incubation and finally at the end of incubation period.

Standard germination test (ISTA, 1996). The germination test was conducted adopting the roll towel method under controlled conditions of temperature and relative humidity (25 ± 3°C and 90 ± 3% RH), in four replications of 100 seeds each. The numbers of normal seedlings were counted at the end of seven days and the percentage germination was calculated.

Vigour index (Abdul-Baki & Anderson 1973). Vigour index was calculated by adopting the following formula and expressed as number: vigour index = germination percentage × whole seedling length (cm).

Seed germination in exhaustion test. One hundred seeds in four replicates were placed on a 'seed placement line' drawn on moist paper towel, folded into a roll and placed in the dark at 10°C for ten days, and then transferred to a germination room maintained at a temperature of 25 ± 3°C and 90 ± 3% relative humidity. After seven days, the seedlings with roots and shoots extended beyond "root line" and "shoot line" drawn respectively at 5 cm below and 3.75 cm above the seed placement line were considered and counted as vigorous seedlings, the results expressed in percentage.

Field emergence. Field emergence potential of seeds was assessed by sowing 400 seeds in four rows in raised seed beds of red sandy loam soil. The seedling was considered emerged when the plumule was just visible on the soil surface. The emergence of the plumule at the end of every 24 h was recorded. The plumule emergence was recorded from fourth day to the tenth day after sowing and expressed as field emergence count in percentage.

Speed of germination (Maguire 1960). The speed of germination was estimated by taking daily counts in germination test using the pleated paper method under controlled conditions of temperature and relative humidity (25 ± 3°C and 90 ± 3% RH), in four replications of 100 seeds each and in field emergence test. In this case, germination is considered to have occurred when the radicle has appeared. The calculation of speed of germination using following indices is given below:

$$\text{Speed of germination} = \Sigma(n/t)$$

where: t = time in days; n = no. of seeds germinated on final day.

Electrical conductivity of seed leachate, dehydrogenase and α-amylase activity activity in seeds. Electrical conductivity of seed leachate (Presley 1958), α-amylase activity (Simpson & Naylor 1962) and dehydrogenase activity (Kittock & Law 1968) in seeds were estimated using standard chemical procedures.

Data analysis

Data were analyzed using Genstat 5 and Microsoft excel package. Analysis of variance was calculated and means were separated by LSD for assessing the significant differences.

Results and discussion

It is well recognized that predisposition of sorghum panicles to wet and humid weather from flowering to grain maturity favors infection by mold fungi and the wet weather and heavy rainfall stimulate the development of molds at all the stages from the emergence of ears to the ripening of grains (Tarr 1962; Gray et al. 1971; Balasubramanian 1977). Siddiqui and Khan (1973) reported that grain maturity and not the flowering stage must coincide with rains for grain and mold development, other factors being normal. Under favorable high moisture conditions mature sorghum grains are invaded by species of *Alternaria, Cladosporium, Phoma* and *Fusarium semitectum*.

In the present study, two sets of experiments were conducted, i.e. effect of RH regimes on infection by major mold fungi upon seed and panicle inoculation and genotypic interaction with temperature and RH in relation to sporulation of mold fungi at ICRISAT, Patancheru, India to understand effects of temperatures and relative humidity regimes on sporulation of sorghum grain mold fungi and the quality of sorghum seed.

Influence of RH on sporulation of Fusarium moniliforme, Curvularia lunata *and* Bipolaris australiensis *on the genotype PVK 801upon seed inoculation and panicle inoculation*

During the first two to three days of exposure to RH regimes, grains showed cottony mycelial growth on the surface, followed by dark colored growth from the fourth day onwards indicating sporulating phase of a particular fungus. This pattern increased with an increase in RH regime from 85–100% but over a period of time, it attained a constant score. Initially it showed a lag phase, the duration of which depended on RH, i.e. higher RH levels showed shorter lag period. All fungi followed a sigmoid pattern based on their mold score, even grains placed in Petri dishes showed the same pattern but they had shorter lag phase and they attained plateau earlier than the grains placed in SPHC. Grains

inoculated with *Curvularia lunata* showed first visible growth followed by grains inoculated with *Bipolaris australiensis* and *Fusarium moniliforme*. The mold severity of all the three fungi reached its peak at 98 and 100% RH within 24 h of incubation (Table I). Spore concentration exponentially increased with increase in RH levels, i.e. from 85 – 100% for grains inoculated with *Fusarium moniliforme* and *Curvularia lunata* while for *Bipolaris australiensis*, it showed normal distribution pattern with maximum spore concentration at 95% RH (Table II). Hence 98% RH and 28°C are the threshold humidity and temperature regimes for sporulation of these fungi.

Evaluation of cultivars for mold infection and genotypic interaction with temperature and RH in relation to sporulation

Out of 37 cultivars evaluated for mold infection, 10 classified as resistant, 18 classified as moderately resistant and nine as susceptible based on the severity index (Table III). From this a total of 16 lines were selected for sporulation study. Among 16 genotypes tested, IS 25017

Table I. Mold score (1 – 100%) at 24 h interval in single plant humidity chamber after inoculation with three fungi at 28°C*.

		Mold score hours after incubation in SPHC						
RH (%)	Fungi	24 h	48 h	72 h	96 h	120 h	144 h	168 h
85	FM	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	CL	0.0	0.0	0.0	0.5	0.5	1.0	2.0
	BA	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	DSW	0.0	0.0	0.0	0.0	0.0	0.0	0.0
90	FM	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	CL	0.0	0.0	0.0	1.2	3.0	3.0	3.0
	BA	0.0	0.0	0.0	0.0	1.3	1.3	1.3
	DSW	0.0	0.0	0.0	0.0	0.0	0.0	0.0
95	FM	0.0	4.0	4.0	5.0	5.0	5.0	6.0
	CL	3.0	3.0	3.0	3.0	4.0	5.0	5.0
	BA	2.0	2.0	3.0	3.0	5.0	5.0	8.0
	DSW	0.0	0.0	0.0	0.0	0.0	0.0	0.0
98	FM	2.7	2.7	2.7	3.0	8.0	8.0	8.7
	CL	10.0	10.7	12.7	15.0	23.3	23.3	36.7
	BA	10.0	10.0	10.0	13.3	16.7	16.7	16.7
	DSW	0.0	0.0	0.0	0.0	0.0	0.0	0.0
100	FM	10.0	10.7	12.7	13.3	28.3	73.3	80.0
	CL	11.7	15.0	20.0	21.7	28.3	73.3	83.3
	BA	10.0	10.7	15.0	18.3	21.7	70.0	80.0
	DSW	0.0	0.0	0.0	0.0	1.0	1.0	2.0

RH, relative humidity; *FM, *Fusarium moniliforme*; CL, *Curvularia lunata*; BA, *Bipolaris australiensis*; DSW, distilled sterile water. CD ($p = 0.01$).

Temperature	= 0.31
Relative humidity	= 0.26
Fungi	= 0.23
Temperature × relative humidity	= 0.68
Relative humidity × fungi	= 0.52
Temperature × fungi	= 0.61
Temperature × relative humidity × fungi	= 1.37

Table II. Spore concentration ($\times 10^4$) of *Fusarium moniliforme, Curvularia lunata and Bipolaris australiensis* at different RH regimes at 28°C after seven days incubation in SPHC.

RH	FM	Cl	Ba	Others*
85	$0.3^{d\dagger}$	0.7^{c}	0.4^{d}	0.0^{c}
90	2.1^{d}	0.9^{c}	1.1^{b}	0.0^{c}
95	31.9^{c}	0.9^{c}	1.7^{a}	2.4^{a}
98	58.4^{b}	1.8^{b}	0.9^{c}	0.3^{b}
100	369.2^{a}	8.4^{a}	1.1^{b}	0.0^{c}

*Other than the fungi inoculated were not counted for spore concentration, however, some of the immature conidia of the fungi inoculated were observed. FM, *Fusarium moniliforme*; CL, *Curvularia lunata*; BA, *Bipolaris australiensis*.
†Means followed by the same letter within a column are not significantly different at $p = 0.01$ by LSD test.

Table III. Severity index of 37 cultivars evaluated for infection and fungal frequency at ICRISAT.

S. no	Category	Cultivars
1	Highly resistant (severity index: < 1 – 2)	IS 8545 (0.2)*, IS 3443 (0.6), IS 18758C-618-2 (0.7), SPV 819 (0.9), IS 14384 (1.2), IS 9478 (1.7), IS 30469C-140 (1.8), SPV 472 (1.9) and CSH 16 (2.0)
2	Moderately resistant (severity index: 2.1 – 4)	SPV 386 (2.1), SPV 876 (2.1), SPV 881 (2.1), SURENO (2.3), ICSV 96101 (2.3), SEPON 78-1 (2.4), IS18758 (2.5), CSV 15 (2.5), PSV 16 (2.8), CSV 4 (2.8), ICSV 95001 (2.9), SEPON 79-26 (3.1), BULK Y (3.2), ICSV 239 (3.3), ICSV 91008 (3.6), IS 18522 (3.7), SWARNA (4.0) and PVK 801
3	Susceptible (severity index: > 4)	SPV 351 (4.1), IS 14332 (4.4), SEPON 79-2 (4.6), ICSV 8802 (5.1), SPV 475 (5.5), IS 18467 (5.6), CSH 15R (5.8), ICSV 89102 (5.1), CSH 9 (6.0) and SPV 104 (7.2)

*Severity index values in parentheses.

showed least or no sporulation followed by IS 8545 and PVK 801. However, CSH 9 and Bulk Y have shown high sporulation across temperatures and RH regimes. The linear regression values indicated highly significant interaction of genotypes with temperature and RH (Figure 2). The mean interaction of three genotypes, three temperatures and six RH levels; and 16 genotypes across six RH levels at 28°C is given in Tables IV and V.

The most predominant sporulating fungi observed in the order of their predominance were *Alternaria* spp., *Curvularia* spp., *Fusarium* spp., *Colletotrichum* spp. and *Drechslera* spp. (Figure 3). Sporulation increased significantly with increasing humidity and temperatures. The spore count was highest at 100% RH and 28°C. However, the most optimal temperature and RH for sporulation of mold fungi were 28°C and 98% respectively.

Effect of temperature and RH regimes on selected cultivars with reference to mold sporulation

The results indicated that the incidence and severity of mold increased with increased temperatures from 25 – 28°C. Sporulation was highest at 28°C and could be seen after three days of incubation. However, sporulation was visible after 4 days at 25 and 27°C. The linear regression values in relation to mold development were highly significant at all the RH

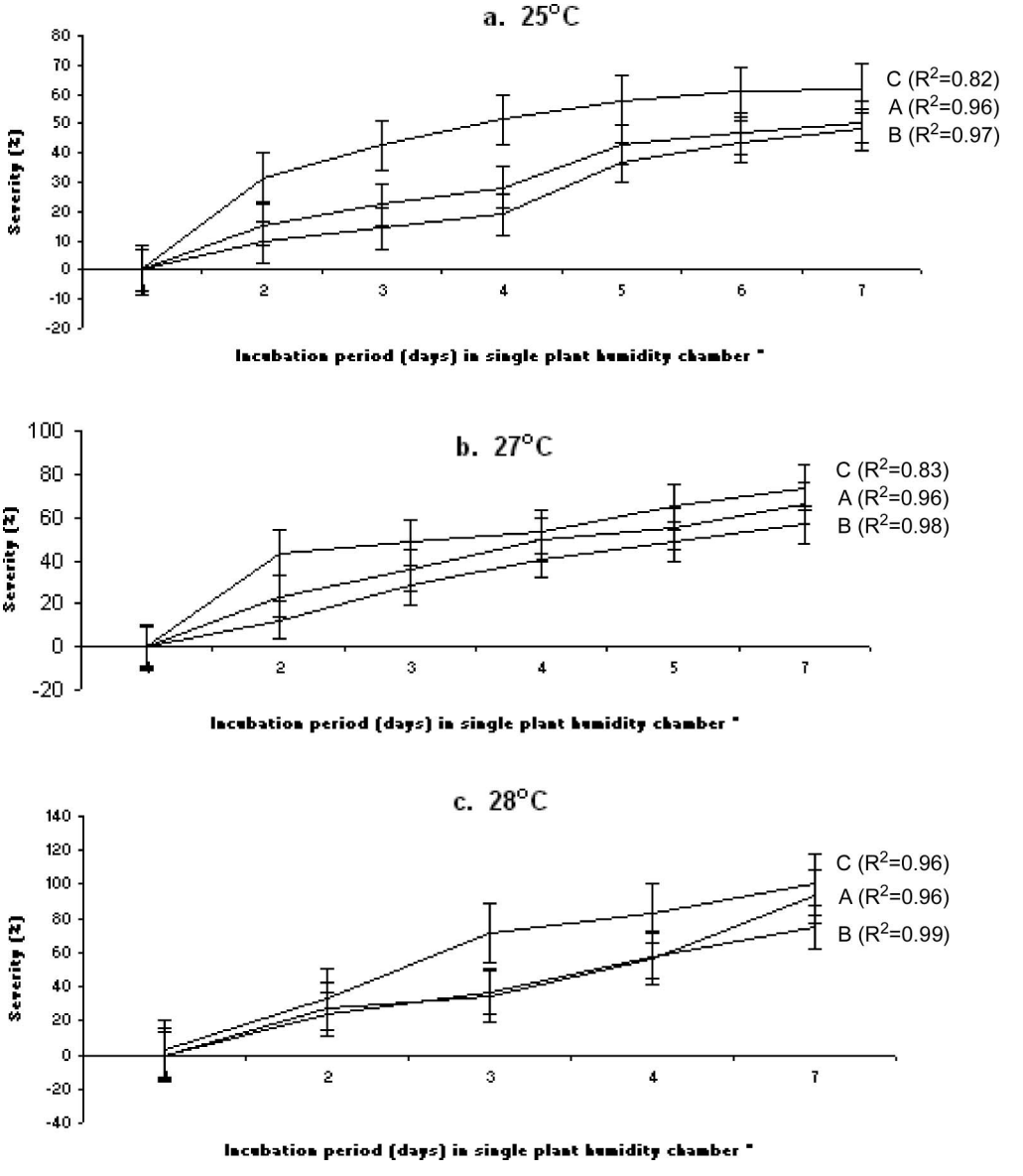

Figure 2 (a,b,c). Mean mold severity of three representative genotypes at different temperatures (°C) across RH regimes. A = IS 25017, B = PVK 801, C = CSH 9. Vertical bars indicate standard error. *Mold severity for a maximum of seven days recorded on respective days as indicated on X-axis.

regimes and temperature levels (Figure 4). Mold severity *vis-à-vis* grain colonization increased proportionately with increasing RH levels from 85 – 100% until the fifth day, but attained constant score thereafter. Initially the grain colonization by various fungi showed an extended lag phase at lower RH levels (85 – 90%), but at higher RH (95 – 100%) shorter lag period indicating a sigmoid pattern. The most congenial RH level for sporulation of mold fungi was 98% which is in fact in the same threshold cluster as that of 99 and 100% relative humidity (Figure 5).

Table IV. ANOVA for three genotypes, three temperatures and six relative humidity levels.

Source of variation	Degrees of freedom	Mean square	F value	Probability 1%	Probability 5%
Replication stratum	3	10.82	0.569	5.42	3.29
Replication, RH stratum					
RH	5	39204.76	2061.24	4.56	2.90
Residual	15	19.02			
Replication, RH, Temperature stratum					
Temperature	1	3071.01	84.90	8.28	4.41
RH, Temperature	5	1396.42	38.61	4.25	2.77
Residual	18	36.17			
Replication, RH, Temperature, Entry stratum					
Entry	2	2382.47	100.91	4.92	3.13
RH, Entry	10	743.09	31.47	2.59	1.97
Temperature, Entry	2	253.30	10.73	4.92	3.13
RH, Temperature, Entry	10	233.09	9.87	2.54	1.97
Residual	72	23.61			
Total	143				

Table V. ANOVA for 16 genotypes, six relative humidity levels at 28°C.

Source of variation	Degrees of freedom	Mean square	F value	Probability 1%	Probability 5%
Replication stratum	2	236	2.341	7.56	4.10
Replication, RH, Stratum					
RH	5	21970.76	217.92	5.64	3.33
Residual	10	100.82			
Replication, RH, Entry stratum					
Entry	15	1621.20	37.01	2.22	1.71
RH, Entry	75	564.58	12.89	1.69	1.45
Residual	180	43.81			
Total	287				

Influence of RH and temperature on seed quality

The recently improved early-maturing cultivars have higher harvest index and give stable and high yields under favorable environments, but when they flower, fill grains and often mature in wet weather, it results in increased susceptibility to parasitic and saprophytic fungi that destroy the grain and food quality, leading to loss of seed viability and sprouting on the panicle. Certain grain mold pathogens have repeatedly been associated with losses in seed mass, grain density (Ibrahim et al. 1985), and germination (Maiti et al. 1985). Other types of damage that arise from grain mold relate to storage quality (Hodges et al. 1999), food and feed processing quality, and market value. In spite of general agreement that grain mold is important, there have been few attempts to quantify losses resulting from the disease (Williams & McDonald 1983).

Our results from 16 genotypes at 28°C and 98% RH in the SPHC indicated that seed germinability in lab and in exhaustion tests, speed of germination, seedling vigor index, field

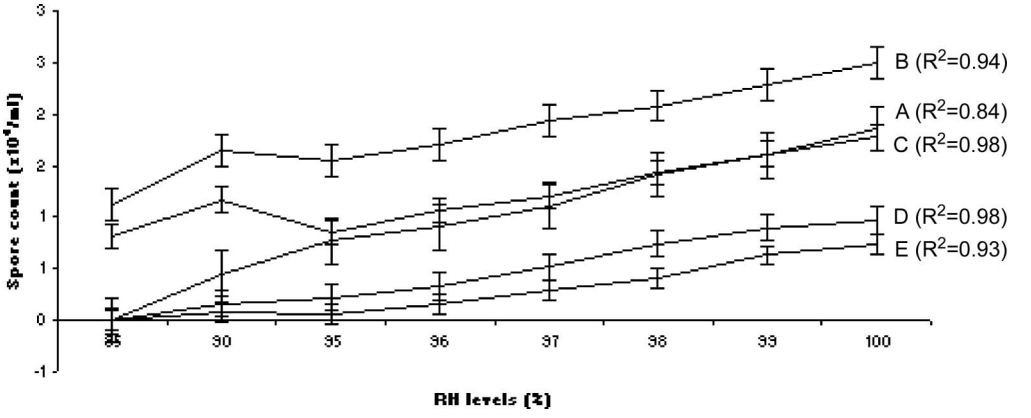

Figure 3. Effect of RH levels on grain mold sporulation across genotypes and temperatures. A = *Fusarium spp*, B = *Alternaria spp*, C = *Curvularia spp*, D = *Colletotrichum spp*, E = *Drechslera spp*. Vertical bars indicate standard error.

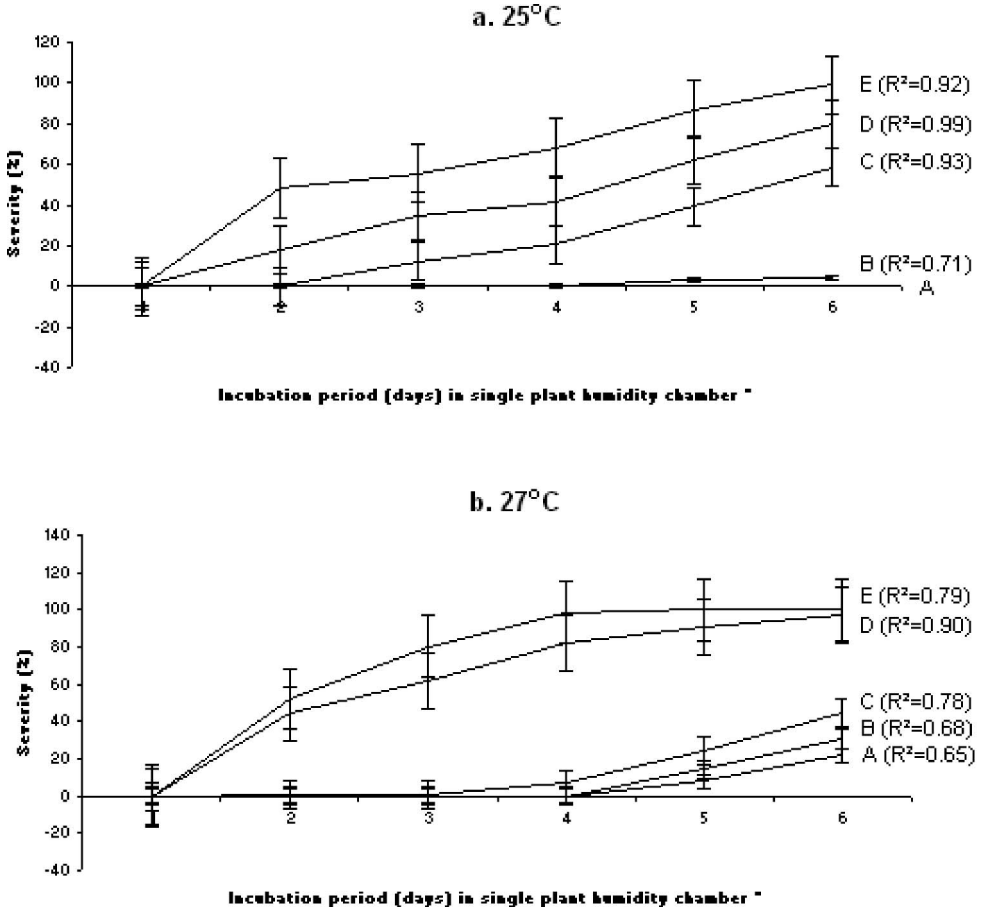

Figure 4 (a,b). Effect of RH levels on mean mold severity across genotypes at different temperatures (°C). A = 85% RH, B = 90% RH, C = 95% RH, D = 98% RH, E = 100% RH. Vertical bars indicate standard error. *Mold severity for a maximum of seven days recorded on respective days as indicated on X-axis.

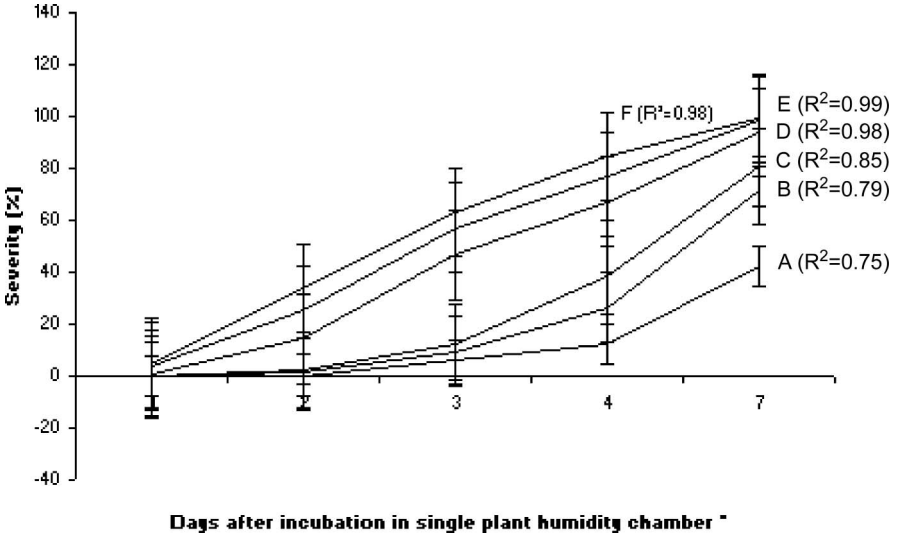

Figure 5. Effect of RH levels on mean mold severity across genotypes at 28°C. A = 95% RH, B = 96% RH, C = 97% RH, D = 98% RH, E = 99% RH, F = 100% RH. Vertical bars indicate standard error. *Mold severity for a maximum of seven days recorded on respective days as indicated on X-axis.

emergence potential, dehydrogenase and ά-amylase activity declined significantly at the end of the incubation period, the stage at which there was maximum sporulation (Table VI). This may be mainly due to infection to the embryo and exhaustion of all the endospermic components by the sporulating fungi. Hence the decline in performance potential of the seeds was evident. The membrane integrity measured in terms of solute leakage (electrical conductivity) increased with the incubation period, indicating higher loss of solutes into the seed steep water due to loss of membrane integrity and seed ageing phenomenon as indicated by loss of degree of aliveness of the seed and germinating ability as reflected in declined dehydrogenase and ά-amylase activity in seeds. Hence seed deterioration due to mold is clearly evident.

Infection by *F. moniliforme* and *C. lunata* has been reported to interfere with carbohydrate translocation to developing kernels, and thus causing reduction in size and weight of seed (Bhatnagar 1971; Gray et al. 1971; Mathur et al. 1975; Castor & Frederiksen 1977). Significant grain weight losses (40–70%) due to infection by several grain mold fungi have been reported (Gray et al. 1971; Sundaram et al. 1972; Glueck & Rooney 1976; Castor & Frederiksen 1981; Singh & Agrawal 1989). Forbes et al. (1989) compared severity with loss in grain weight, the standard deviation of grain weight, grain density, electrolyte leachate, percentage germination and visual appraisal of moldy, off colored or smaller grain. Martinez et al. (1994) reported decrease in test weight and percentage of seed germination and molding was higher in the white cultivars than in red or brown ones.

In conclusion, it is evident that threshold temperature and humidity regimes for mold sporulation in sorghum is 28°C and 98% RH, leading to significant losses in seed, grain and food quality. Hence, future research on sorghum grain mold should emphasize on etiology and the role of host maturity, resistance mechanism and epidemiology in relation to changing weather variables and to search for sustainable seed traits that may confer resistance to grain molds.

Table VI. Seed germinability, seed germination under stress and changes in biochemical components in sorghum as influenced by sporulation at 28°C and 98% RH.

Genotype (Severity Index)	Seed germination (%)		Speed of germination		Vigour index [seed germination (%) × whole seedling length (cm)]		Field emergence (%)		Seed germination in exhaustion test (%)		Electrical conductivity of seed leachate (μmhos/cm)		Dehydrogenase activity in seeds (OD)		ά-amylase activity in seeds (mm)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
IS 8545 (0.2)	89.2^{ab}*	76.1^{ab}	37.3^{a}	28.1^{a}	2980^{a}	2623^{a}	85.0^{abc}	73.3^{ab}	81.0^{a}	72.5^{ab}	127^{g}	138^{hi}	0.47^{a}	0.42^{a}	7.0^{ab}	5.9^{a}
IS 3443 (0.6)	90.3^{ab}	72.0^{bcde}	36.9^{a}	26.7^{b}	2873^{ab}	2398^{bc}	87.5^{a}	72.2^{ab}	80.2^{a}	70.3^{ab}	126^{g}	134^{i}	0.47^{a}	0.41^{a}	7.1^{a}	5.9^{a}
IS 18758C-618-2 (0.7)	87.1^{abc}	74.7^{abcd}	37.9^{a}	25.3^{c}	2817^{b}	2469^{b}	82.4^{bcde}	72.5^{ab}	80.6^{a}	73.4^{a}	127^{g}	139^{ghi}	0.47^{a}	0.42^{a}	7.0^{ab}	5.8^{ab}
IS 25017 (1.0)	91.0^{a}	75.3^{abc}	36.6^{a}	26.0^{bc}	2889^{ab}	2467^{b}	86.6^{ab}	72.0^{ab}	81.0^{a}	72.6^{ab}	132^{fg}	142^{ghi}	0.45^{ab}	0.42^{a}	6.7^{b}	5.5^{bc}
IS 30469C-140 (1.8)	85.7^{bc}	78.4^{a}	34.7^{b}	25.7^{bc}	2765^{b}	2399^{bc}	83.3^{abcd}	70.6^{ab}	78.9^{a}	72.2^{ab}	136^{ef}	146^{fgh}	0.44^{b}	0.38^{b}	6.2^{c}	5.5^{bc}
ICSV 96101 (2.3)	83.4^{cd}	71.8^{cde}	32.5^{cd}	23.6^{d}	2498^{cd}	2187^{e}	79.1^{def}	73.6^{a}	74.5^{bc}	71.8^{ab}	139^{def}	148^{efg}	0.44^{b}	0.37^{bc}	6.1^{c}	5.3^{c}
SEPON 78-1 (2.4)	85.2^{bcd}	70.6^{de}	31.6^{c}	21.4^{e}	2345^{def}	2116^{ef}	78.2^{efg}	72.9^{ab}	74.3^{bc}	70.6^{ab}	138^{def}	152^{def}	0.40^{c}	0.38^{b}	6.0^{cd}	5.3^{c}
PVK 801 (2.5)	89.6^{ab}	69.7^{ef}	32.9^{bc}	24.8^{cd}	2869^{ab}	2331^{cd}	81.0^{cdef}	72.5^{ab}	78.4^{ab}	73.2^{a}	140^{cdef}	139^{ghi}	0.4^{c}	0.35^{c}	6.1^{c}	5.4^{c}
CSV 4 (2.8)	82.3^{cd}	69.6^{ef}	27.6^{de}	21.2^{e}	2318^{ef}	2118^{ef}	79.6^{cdef}	71.0^{ab}	71.6^{cd}	69.0^{b}	142^{bcde}	156^{de}	0.38^{c}	0.35^{c}	5.7^{d}	5.0^{d}
ICSV 95001 (2.9)	85.7^{bc}	71.5^{cde}	28.2^{d}	20.9^{e}	2539^{c}	2211^{de}	85.1^{ab}	70.2^{ab}	69.0^{de}	62.3^{c}	142^{bcde}	158^{cd}	0.39^{c}	0.36^{bc}	5.7^{d}	5.0^{d}
BULK Y (3.2)	80.4^{d}	61.6^{h}	26.9^{de}	18.4^{f}	2319^{ef}	1997^{fg}	76.5^{fgh}	73.4^{ab}	63.6^{f}	60.5^{cd}	142^{bcde}	159^{cd}	0.34^{de}	0.3^{d}	5.3^{e}	4.1^{f}
ICSV 91008 (3.6)	87.3^{abc}	66.3^{fg}	27.1^{de}	19.0^{f}	2445^{cde}	1876^{gh}	87.2^{ab}	69.3^{b}	65.2^{ef}	61.2^{cd}	144^{bcde}	158^{cd}	0.35^{d}	0.31^{d}	5.1^{ef}	4.5^{e}
IS 18522 (3.7)	87.1^{abc}	68.8^{ef}	26.0^{ef}	17.8^{f}	2196^{f}	1921^{gh}	87.0^{ab}	62.5^{c}	64.3^{f}	59.8^{cd}	145^{abcd}	158^{cd}	0.34^{de}	0.31^{d}	5.1^{ef}	4.5^{e}
SPV 351 (4.1)	83.3^{cd}	65.7^{fgh}	26.5^{def}	16.3^{g}	2216^{f}	2005^{fg}	74.2^{gh}	60.6^{c}	62.8^{fg}	57.6^{d}	148^{abc}	166^{bc}	0.32^{ef}	0.3^{d}	4.8^{f}	3.9^{f}
CSH 9 (6.0)	82.7^{cd}	63.9^{gh}	24.9^{fg}	15.0^{gh}	2456^{cde}	1878^{gh}	71.8^{h}	61.3^{c}	59.2^{g}	53.6^{e}	149^{ab}	169^{ab}	0.32^{ef}	0.29^{d}	4.2^{g}	3.0^{g}
SPV 104 (7.2)	83.2^{cd}	56.4^{i}	23.3^{g}	14.1^{h}	2198^{f}	1796^{h}	66.6^{i}	59.7^{c}	53.1^{h}	47.8^{f}	153^{a}	178^{a}	0.3^{f}	0.29^{d}	3.9^{g}	2.7^{h}
CD ($p=0.01$)	6.92	5.62	2.50	1.77	206.33	176.41	6.52	5.60	5.78	5.32	11.25	12.33	0.03	0.03	0.47	0.40

Initial: at the time of incubation; final: end of incubation period. *Means followed by the same letter within a column are not significantly different at $p=0.01$ by LSD test.

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