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Substrate Suitability of Different Genotypes of Sorghum in Relation to *Aspergillus* Infection and Aflatoxin Production

C. V. RATNAVATHI* AND R. B. SASHIDHAR

Department of Biochemistry, University College of Science, Osmania University, Hyderabad 500 030, India

Grain sorghum is often damaged by rain in the field and severely infected by grain mold, which includes *Aspergillus* infection and aflatoxin production. The objective of the study is to investigate the extent of aflatoxin production with *Aspergillus* infection in vitro in different sorghum genotypes with different pericarps, red, yellow, and white, the physical and chemical characteristics of grain during infection, and the changes in grain polyphenols and phytic acid in comparison to maize and groundnut. The physical characters and biochemical composition of sorghum grain contribute to make it less susceptible to *Aspergillus* infection and aflatoxin contamination compared to maize and groundnut. The lowest amounts of aflatoxin and ergosterol were observed in genotypes with red pericarp, whereas higher amounts of aflatoxin and ergosterol were found in white genotypes followed by maize and groundnut. All of the red genotypes differ in polyphenol composition and aflatoxin produced, showing resistance to mold damage. Another indication of resistance in red genotypes was the delayed peaking of aflatoxin production (9 days after infection). In red sorghum genotypes there was a significant, positive correlation existing between polyphenol content and aflatoxin produced at 3 and 6 days after infection, the r values being 0.589 and 0.513, respectively. The starch content decreased whereas the protein content in all sorghum genotypes increased during infection. Maximum phytic acid was observed in white sorghum genotypes. Phytic acid in yellow genotypes was found to have a significant negative correlation ($r = -0.569$) with aflatoxin produced.

KEYWORDS: Aflatoxin; polyphenol; ergosterol; *Aspergillus*; sorghum; phytic acid; genotype

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the main staples for the world's poorest and most food insecure people (*1*). Sorghum is a truly dual-purpose crop; both grain and stover are highly valued outputs. Grain molds cause significant losses in both grain yield and its nutritional quality. The genus *Aspergillus* is one of the important groups of fungi afflicting sorghum in the field as well as during postharvest periods (*2*). Mycotoxin contamination and deterioration of grain quality are the important consequences affecting the market acceptability of the grain as well as low crop returns to the farmer. Aflatoxins are a group of important mycotoxins that are produced as secondary metabolites in a variety of agricultural commodities under conducive climatic conditions by the fungi *Aspergillus parasiticus* and *Aspergillus flavus* (*2*). The aflatoxins most commonly produced by *A. flavus* are B_1 (AFB_1) and B_2, whereas *A. parasiticus* also produces two additional toxins, namely, G_1 and G_2. Aflatoxin B_1 is the most carcinogenic one. Sorghum is susceptible to *Aspergillus* infestation and aflatoxin production (*3*−*8*); the natural occurrence of aflatoxin B_1 in rain-affected Indian sorghum samples and aflatoxin B_1 contamination in Brazilian samples was reported by Silva et al. (*9*). Dietary exposure to aflatoxin poses a potential health hazard, to both humans and farm animals (*10*−*13*). Among the agricultural commodities, maize, groundnut, wheat, tapioca, sunflower seed, cottonseed, etc. have been recognized as high-risk commodities for aflatoxin contamination (*2*). The moisture content required for growth and aflatoxin production varies with natural substrates. Moisture contents of 18−18.5% in wheat, corn, and sorghum grains, 17−18% in soybeans, and 8−10% in groundnuts were found to be optimum (*14*). The optimum range of temperature for the production of aflatoxin was 28−32 °C, limiting to the substrate and other experimental conditions (*15*). Grain hardness, density, and grain characteristics are reported to be associated with grain mold resistance (*16*). Sashidhar et al. (*17*) reported a systematic study on the mold and mycotoxin contamination in the grain sorghum stored in traditional containers in India. They indicated that percentage contamination was only 8% in one white variety compared with nearly 30% in the red and yellow varieties. However, in red varieties toxin could not be detected due to high tannin contents. Of all the traditional storage forms, "kotlu" storage was most prone to fungal infestation in sorghum. It was reported that even if fungal infestation occurs, the extent of aflatoxin contamination was found to be minimal. However, a careful survey of the literature suggests that grain sorghum is less prone to a high degree of

* Address correspondence to this author at the National Research Centre for Sorghum, Rajendranagar, Hyderabad 500 030, India (telephone 91-40-4015349; fax 91-40-4016378; e-mail cvratnavathi@rediffmail.com).

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aflatoxin contamination (*5*, *18*, *19*). Brown seeds with high tannin contents and the presence of a pigmented testa layer have been associated with fungal resistance (*20*). Genotypes such as E 35-1 and SPV-351, however, are found to be resistant with low levels of tannin, but grain hardness contributes to the resistance in such cases (*21*).

Even though sorghum can be infected by *Aspergillus* and produce aflatoxins, the level of toxin production is less when compared to the aflatoxin production in high-risk agricultural commodities such as maize and groundnut (*2*). The present study was undertaken to investigate the variability in genotypes for aflatoxin and ergosterol production as an index of fungal contamination and also to investigate the role of physical as well as chemical characteristics of sorghum grain, including polyphenols and phytic acid, in conferring resistance to aflatoxin and ergosterol production.

MATERIALS AND METHODS

Samples. A total of 16 sorghum genotypes including 6 red sorghum genotypes, 4 yellow sorghum genotypes, and 6 white sorghum genotypes were collected from the germplasm resource unit, International Crop Research Institute for Semi Arid Tropics, Patancheru, India. These genotypes were multiplied at the farms of the National Research Centre for Sorghum, Rajendranagar and Hyderabad. Groundnut and maize (cv. Madhuri) were obtained from a local market and the Agricultural Research Institute, Amberpet, Hyderabad, respectively.

Mycology. The fungus strain used in this study was *Aspergillus parasiticus* (NRRL 2999), a highly toxigenic strain known to produce copious amounts of AFB_1, AFB_2, AFG_1, and AFG_2 obtained from the U.S. Department of Agriculture (USDA) at Peoria, IL. The cultures were maintained on potato dextrose agar (PDA) slants for 6−8 days at 28 °C in a BOD incubator (Kalorstat, Mumbai, India).

Analytical Standards. Reference standards of aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2) were a gift from WHO, Geneva, Switzerland, under the international check sample program.

Preparation of Samples. Sorghum (50 g) was presoaked in sterile quartz distilled water in a clean Erlenmeyer flask (250 mL) for 1 h. The grains were washed three times with sterile water and surface sterilized with sodium hypochlorite solution (1% v/v). The grains were washed three times with sterile water to remove any residual sodium hypochlorite present in the grains. The sorghum sample was allowed to retain a moisture content of 16−17%, and the grains were transferred to a 250 mL Erlenmeyer flask that had been presterilized in a laboratory autoclave at 15 lb of pressure (121 °C) for 15 min. The transfer was made under aseptic conditions inside the laminar flow chamber. All glassware used was presterilized in an autoclave. Fungal spore inoculum, in a volume of 500 mL, containing 1×10^6 spores prepared in 0.01% (v/v) Tween-20, was added to 10 g of grain sample. The spore count was determined through a hemocytometer (Neubacur counting chamber). Groundnut and maize (cv. Madhuri) were used as high-risk substrates in the study. For groundnut, procedures for sterilization and soaking were similar to those for sorghum, whereas maize grains were soaked overnight in lukewarm water. The moisture contents of maize (16−18%) and groundnut (10−12%) were maintained. The moisture estimations were done according to an AOAC method (*22*). These samples were kept at 28 ± 1 °C in a BOD incubator, and the fungus was allowed to grow for a period of 12 days. At five different time periods of infection, that is, 0, 3, 6, 9, and 12 days, samples were drawn and processed for further analysis. Samples were maintained in duplicate flasks. Two subsamples were withdrawn from each of the duplicate experiments. The samples were dried in a vacuum oven at 20 kg/cm^2 and 40−45 °C for 48 h. After drying, samples were ground in a mechanical grinder (Sumeeth, Mumbai, India) to a particle size of 0.4 mm. The grain samples were initially analyzed for the presence of aflatoxins and ergosterol before inoculation with fungal spores, to avoid any background interference.

Defatted samples were analyzed for biochemical constituents such as starch, protein, phytic acid, and polyphenols, whereas whole samples were used for aflatoxin and ergosterol analysis.

Determination of Aflatoxin. Aflatoxins including AFB_1, AFB_2, AFG_1, AFG_2, and total aflatoxin content were analyzed by the thin-layer chromatography (TLC)−fluorodensitometric method (SLR-TRFF, Biomed Instruments Inc., Indianapolis, IN) as reported by Egan (*23*). The sensitivity of the method is 1 ng/g.

Determination of Ergosterol and Other Chemical Constituents. Ergosterol was estimated according to the method of Sashidhar et al. (*24*). Starch was estimated by enzymatic procedure, as reported by Southgate (*25*). Protein content was estimated colorimetrically after Kjeldhal digestion using salicylate (*26*). Fat was estimated by Soxhlet extraction (*22*).

Phytic acid was quantitated according to the method of Wheeler and Ferral (*27*). Polyphenols were quantitated by precipitation of protein in a microassay using tannic acid as a standard (*28*). Physical characteristics of the sorghum genotypes such as hardness index, 1000-grain weight, and endosperm texture were also analyzed. Hardness index was measured as kg/cm^2, the force required to break the grain using a Kiya hardness tester (Kiya Seisakusho Ltd., Japan). Endosperm texture was classified as per the IBPGR manual (*29*).

Statistical Analysis. Data were analyzed by two-way analysis of variance (ANOVA) and correlation (*30*). Computer software, M.Stat statistical package, along with Lotus freelance graphics (ver. 2.1), was used in data analysis. The critical difference (CD) was calculated using the following formula.

$$\mathrm{CD} = \text{standard error (SE)} \times t$$

$$\mathrm{SE} = (2\mathrm{Ms_e}/r)^{1/2}$$

$$\mathrm{Ms_e} = \text{error mean sum of square}$$

RESULTS

Substrate Suitability of Sorghum Genotypes to Fungal Infestation. *Aflatoxin Production.* The variations in total aflatoxin produced and also individual aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2) produced at different stages of fungal infection among 16 genotypes of sorghum over a period of 12 days are presented in **Tables 1−4**. The pie chart of two-way ANOVA of total toxin is shown in **Figure 2.1**.

The two-way ANOVA showed statistically significant differences in red, yellow, and white sorghum genotypes for toxin production as well as statistically significant variation for different periods of infection ($P < 0.01$) (**Figure 2.1**). The level of aflatoxin contamination was in the order red < yellow < white < maize < groundnut. The highest amount of aflatoxin (total) was produced in the genotype CSH 14 day 6 after fungal infection, that is, 46.17 μg/g (**Figure 2.1**; **Tables 1−4**).

Red Sorghum. Total aflatoxin production was lower in red genotypes compared to yellow and white genotypes (**Tables 1−3**). No aflatoxin was detected in red genotypes AON 486 and IS 18528 day 3 after infection. In genotype IS 620 day 3 after infection, the aflatoxin content is 2.03 μg/g. Aflatoxin production peaked among red genotypes 9 days after infection and decreased after that in all red genotypes of sorghum. Aflatoxin production was also different and statistically significant for various time points of infection. In AON 486 and IS 620 aflatoxin produced was least at all stages of infection, that is, 3, 6, 9, and 12 days (0, 5.3, 5.1, and 2.5 μg/g, respectively). In IS 620 and IS 688 aflatoxin produced was less up to 12 days. The range of total aflatoxin content in red genotypes was from 2.0 μg/g (IS 620, 3 days) to 19.4 μg/g (IS 14384, 9 days). The two red genotypes IS 14384 and IS 8014 showed high aflatoxin levels (19.37 μg/g in IS 14384 and 7.82 μg/g in IS 688) on day 9 after infection. The ratio of AFB_1/total toxin ranged from 0.32 (AON 486, 12 days) to 0.62 (AON 486, 6 days). There was a great degree of variation with respect to individual aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2) at different time points (**Table 1**). The variation of AFB_1 was

Table 1. Aflatoxin Content in Grain of Red Sorghum Genotypes

	period of infection									
	3 days					6 days				
genotype	B_1	B_2	G_1	G_2	sum	B_1	B_2	G_1	G_2	sum
IS 14384	1.11 ± 0.11[a]	0.70 ± 0.10	0.51 ± 0.10	0.58 ± 0.02	**2.90 ± 0.33**	2.16 ± 0.20	1.99 ± 0.20	2.50 ± 0.37	0.62 ± 0.19	**7.27 ± 0.96**
IS 688	2.45 ± 0.50[b]	1.06 ± 0.23	0.30 ± 0.02	1.32 ± 0.15	**5.13 ± 0.90**	2.75 ± 0.07	1.14 ± 0.33	1.07 ± 0.25	1.29 ± 0.05	**6.25 ± 0.70**
AON 486	ND[c]	ND	ND	ND	ND	3.30 ± 0.40	1.05 ± 0.01	0.36 ± 0.04	0.63 ± 0.04	**5.34 ± 0.49**
IS 620	1.05 ± 0.09	0.62 ± 0.13	0.25 ± 0.06	0.11 ± 0.01	**2.03 ± 0.29**	2.34 ± 0.47	1.70 ± 0.20	0.72 ± 0.20	0.96 ± 0.18	**5.72 ± 1.05**
IS 18528	ND	ND	ND	ND	ND	2.39 ± 0.53	1.47 ± 0.25	1.53 ± 0.47	1.03 ± 0.06	**6.42 ± 1.31**
IS 8014	1.60 ± 0.35	1.50 ± 0.39	0.70 ± 0.20	0.21 ± 0.02	**4.01 ± 0.96**	2.57 ± 0.60	1.77 ± 0.22	1.55 ± 0.45	1.31 ± 0.08	**7.20 ± 1.35**

	period of infection									
	9 days					12 days				
genotype	B_1	B_2	G_1	G_2	sum	B_1	B_2	G_1	G_2	sum
IS 14384	7.46 ± 2.10	7.37 ± 2.20	2.83 ± 0.28	1.71 ± 0.30	**19.37 ± 4.88**	3.58 ± 0.44	2.60 ± 0.28	1.38 ± 0.23	0.51 ± 0.03	**8.07 ± 0.98**
IS 688	4.47 ± 0.88	1.77 ± 0.27	0.92 ± 0.06	0.66 ± 0.08	**7.82 ± 1.29**	1.46 ± 0.31	0.64 ± 0.18	0.48 ± 0.16	0.35 ± 0.06	**2.93 ± 0.71**
AON 486	1.71 ± 0.24	0.74 ± 0.13	2.06 ± 0.30	0.62 ± 0.19	**5.13 ± 0.86**	0.61 ± 0.26	0.19 ± 0.04	0.41 ± 0.05	1.35 ± 0.07	**2.55 ± 0.42**
IS 620	2.19 ± 0.25	1.10 ± 0.16	1.03 ± 0.04	1.70 ± 0.06	**6.02 ± 0.51**	1.11 ± 0.15	0.80 ± 0.27	0.67 ± 0.21	0.28 ± 0.08	**2.86 ± 0.71**
IS 18528	5.28 ± 0.60	2.41 ± 0.34	1.55 ± 0.30	0.47 ± 0.12	**9.71 ± 1.36**	2.89 ± 0.84	1.14 ± 0.08	1.22 ± 0.08	0.51 ± 0.13	**5.76 ± 1.13**
IS 8014	3.29 ± 0.48	2.53 ± 0.19	1.05 ± 0.09	0.72 ± 0.01	**7.59 ± 0.77**	3.49 ± 0.04	1.15 ± 0.30	2.07 ± 0.09	1.15 ± 0.23	**7.86 ± 0.66**

[a] Values represented are mean ± SD of four replications. [b] Aflatoxin values are expressed as μg/g. [c] ND, not detected.

Table 2. Aflatoxin Content in Grain of Yellow Sorghum Genotypes

	period of infection									
	3 days					6 days				
genotype	B_1	B_2	G_1	G_2	sum	B_1	B_2	G_1	G_2	sum
LPJ	2.35 ± 0.20[a]	3.13 ± 0.44	1.42 ± 0.37	1.40 ± 0.35	**8.30 ± 1.36**	13.65 ± 0.12	3.21 ± 0.37	1.68 ± 0.36	3.02 ± 0.23	**21.56 ± 1.08**
IS 17777	3.34 ± 0.54	1.34 ± 0.54	2.23 ± 0.16	4.30 ± 0.64	**11.21 ± 1.88**	9.31 ± 1.29	8.42 ± 0.12	2.52 ± 0.37	2.17 ± 0.30	**22.42 ± 2.08**
IS 17780	3.25 ± 0.21	0.97 ± 0.03	0.24 ± 0.05	1.26 ± 0.16	**5.72 ± 0.45**	7.11 ± 0.30	7.05 ± 0.20	1.69 ± 0.11	1.30 ± 0.14	**17.15 ± 0.75**
IS 17779	1.64 ± 0.39	0.41 ± 0.04	0.42 ± 0.01	0.09 ± 0.01	**2.56 ± 0.45**	4.08 ± 0.18	1.14 ± 0.16	2.99 ± 0.21	0.97 ± 0.03	**9.18 ± 0.58**

	period of infection									
	9 days					12 days				
genotype	B_1	B_2	G_1	G_2	sum	B_1	B_2	G_1	G_2	sum
LPJ	4.08 ± 0.18	6.87 ± 0.47	3.31 ± 0.27	1.54 ± 0.41	**15.80 ± 1.33**	3.02 ± 0.60	0.75 ± 0.27	2.17 ± 0.04	8.13 ± 2.60	**14.07 ± 3.51**
IS 17777	9.48 ± 0.53	3.27 ± 0.41	2.35 ± 0.35	1.35 ± 0.21	**16.45 ± 1.50**	4.92 ± 0.08	2.44 ± 0.17	1.48 ± 0.01	2.98 ± 0.18	**11.82 ± 0.44**
IS 17780	7.99 ± 0.21	9.53 ± 0.11	3.14 ± 0.08	3.17 ± 0.07	**23.83 ± 0.47**	2.06 ± 0.44	0.88 ± 0.14	2.54 ± 0.59	5.50 ± 0.42	**10.98 ± 1.59**
IS 17779	7.26 ± 0.44	4.43 ± 0.60	1.43 ± 0.40	1.26 ± 0.40	**14.38 ± 1.84**	3.73 ± 0.22	0.84 ± 0.18	3.02 ± 0.24	1.52 ± 0.37	**8.81 ± 1.01**

[a] Values represented are mean ± SD of four replications. [b] Aflatoxin values are expressed as μg/g.

statistically significant among red sorghum genotypes, ranging from 1.05 to 7.46 μg/g. At the peak period of production, that is, day 9 after infection, AON 486 showed 1.71 μg/g of AFB_1, which was the lowest content among the red genotypes. The other red genotype, IS 620, resistant to aflatoxin, showed 2.19 μg/g of AFB_1. In IS 14384 significantly higher AFB_1 was produced followed by IS 18528 (5.28 μg/g). However, the amount of AFB_1 at days 3, 6, and 12 after infection was not significantly different. The other three aflatoxins were significantly higher in the red genotype IS 14384 at day 9 after infection. The variation among the other toxins was not significant at all time points in all red sorghum genotypes.

Yellow Sorghum. The aflatoxin production in yellow genotypes at different periods of infection is presented in **Table 1**. Of the four yellow sorghums tested, IS 17779 showed less aflatoxin production (14.4 μg/g). Peak production of aflatoxin was observed on day 6 after infection in all genotypes except IS 17779, in which it was found to peak at day 9 after infection.

Statistically significant variation for total aflatoxin content as well as for various time points of infection was observed in yellow sorghum genotypes. At day 9 after infection in genotype IS17780, the total aflatoxin produced was 23.83 μg/g, which was found to be highest among yellow genotypes. In LPJ and IS 17777 toxin production peaked at day 6 after infection. The range of toxin in yellow genotypes was from 2.53 μg/g (IS 17779, 3 days) to 23.9 μg/g (IS 17780, 6 days). In yellow sorghum genotypes, the AFB_1 content was peaking at day 6 after infection, and it was found to be maximum in LPJ, 13.65 μg/g, a local yellow genotype (**Table 2**). The range of AFB_1 contents at peak production, that is, day 6 after infection, was 4.08 μg/g in IS 17779 and 13.65 μg/g in LPJ. The AFB_1 content in LPJ genotype was statistically significant and higher than AFB_1 in other yellow genotypes day 6 after infection. No significant difference of AFB_1 was observed among the yellow genotypes days 3 and 12 after infection. AFB_2 was significantly higher in IS 17777 and IS 17780 days 6 and 9 after infection (**Table 2**). The highest level of AFG_2 was observed in LPJ and IS 17780 at day 12 after infection. There was no significant variation of the AFG_1 and AFG_2 toxins among the other yellow genotypes.

White Sorghum. Aflatoxin production in white genotypes is depicted in **Table 3**. Total aflatoxin production in all sorghum genotypes, maize, and groundnut at different periods of infection differed significantly.

Table 3. Aflatoxin Content in Grain of White Sorghum Genotypes

	period of infection									
	3 days					6 days				
genotype	B_1	B_2	G_1	G_2	sum	B_1	B_2	G_1	G_2	sum
CSH 9	2.14 ± 0.03	1.47 ± 0.40	3.08 ± 0.19	1.53 ± 0.66	**8.22 ± 1.28**	14.8 ± 0.28	6.10 ± 0.28	2.88 ± 0.13	4.69 ± 0.33	**28.47 ± 1.02**
CSH 14	3.72 ± 0.17	1.03 ± 0.10	2.71 ± 0.30	1.69 ± 0.24	**9.15 ± 0.81**	25.1 ± 3.00	6.55 ± 0.45	9.42 ± 0.42	5.10 ± 0.19	**46.17 ± 4.06**
SPV 86	5.13 ± 1.60	2.76 ± 0.35	1.01 ± 0.16	1.33 ± 0.17	**10.23 ± 2.28**	5.67 ± 0.21	1.32 ± 0.05	2.42 ± 0.30	1.05 ± 0.19	**10.46 ± 0.75**
SPV 462	1.31 ± 0.20	1.14 ± 0.20	1.22 ± 0.05	0.58 ± 0.05	**4.25 ± 0.50**	9.97 ± 1.00	6.02 ± 0.40	3.08 ± 0.15	3.00 ± 0.13	**22.07 ± 1.68**
IS 25017	1.55 ± 0.21	1.50 ± 0.42	2.33 ± 0.30	1.54 ± 0.16	**6.92 ± 1.09**	9.80 ± 0.15	2.35 ± 0.10	1.21 ± 0.18	2.69 ± 0.24	**16.05 ± 0.67**
GM 13	6.00 ± 1.40	ND	0.47 ± 0.08	ND	**6.47 ± 1.48**	2.01 ± 0.23	0.84 ± 0.12	1.07 ± 0.12	1.25 ± 0.21	**5.17 ± 0.68**

	period of infection									
	9 days					12 days				
genotype	B_1	B_2	G_1	G_2	sum	B_1	B_2	G_1	G_2	sum
CSH 9	7.40 ± 0.85	7.00 ± 0.28	3.07 ± 0.13	3.54 ± 0.42	**21.01 ± 1.68**	5.46 ± 0.38	2.93 ± 0.10	2.00 ± 0.10	2.09 ± 0.16	**12.48 ± 0.74**
CSH 14	5.11 ± 0.03	1.59 ± 0.13	3.84 ± 0.19	1.36 ± 0.06	**11.90 ± 0.41**	3.70 ± 0.10	2.15 ± 0.21	2.00 ± 0.21	0.59 ± 0.10	**8.44 ± 0.62**
SPV 86	3.51 ± 0.30	0.93 ± 0.06	2.96 ± 0.12	1.15 ± 0.04	**8.55 ± 0.52**	3.23 ± 0.11	2.34 ± 0.21	1.52 ± 0.10	1.29 ± 0.05	**8.38 ± 0.47**
SPV 462	5.30 ± 0.54	2.26 ± 0.20	3.00 ± 0.28	2.00 ± 0.04	**12.56 ± 1.06**	2.11 ± 0.10	0.98 ± 0.22	1.65 ± 0.30	4.30 ± 0.10	**9.04 ± 0.72**
IS 25017	3.61 ± 0.45	1.39 ± 0.10	3.25 ± 0.40	1.20 ± 0.11	**9.45 ± 1.06**	0.63 ± 0.18	2.10 ± 0.21	0.44 ± 0.13	1.82 ± 0.30	**4.99 ± 0.82**
GM 13	3.45 ± 0.18	1.32 ± 0.09	2.62 ± 0.14	2.29 ± 0.20	**9.68 ± 0.61**	2.23 ± 0.21	1.49 ± 0.11	1.04 ± 0.00	1.40 ± 0.15	**6.16 ± 0.47**

[a] Values represented are mean ± SD of four replications. [b] Aflatoxin values are expressed as μg/g. [c] ND, not detected.

Table 4. Aflatoxin Content in Grain of Maize and Groundnut

	period of infection									
	3 days					6 days				
genotype	B_1	B_2	G_1	G_2	sum	B_1	B_2	G_1	G_2	sum
maize (Madhuri)	2.35 ± 0.08	0.78 ± 0.19	1.70 ± 0.14	0.89 ± 0.05	**5.72 ± 0.46**	7.05 ± 1.19	2.85 ± 0.35	6.78 ± 0.25	2.75 ± 0.21	**19.73 ± 2.00**
groundnut (commercial)	3.72 ± 0.11	1.95 ± 0.21	3.15 ± 0.08	1.40 ± 0.28	**10.22 ± 0.68**	11.70 ± 0.71	5.10 ± 1.20	10.30 ± 0.71	4.44 ± 0.65	**31.54 ± 3.27**

	period of infection									
	9 days					12 days				
genotype	B_1	B_2	G_1	G_2	sum	B_1	B_2	G_1	G_2	sum
maize (Madhuri)	5.5 ± 0.42	1.5 ± 0.42	4.6 ± 0.42	1.15 ± 0.07	**12.75 ± 1.33**	3.40 ± 0.28	1.00 ± 0.02	3.00 ± 0.14	0.89 ± 0.16	**8.29 ± 0.60**
groundnut (commercial)	14.2 ± 0.35	4.1 ± 0.98	12.9 ± 1.10	4.75 ± 1.20	**35.95 ± 3.63**	4.84 ± 1.20	1.97 ± 0.18	4.35 ± 0.64	1.70 ± 0.14	**12.86 ± 2.16**

[a] Values represented are mean ± SD of four replications. [b] Aflatoxin values are expressed as μg/g.

Among the six genotypes of white sorghum, four lines, CSH 9, CSH 14, SPV 86, and SPV 462, are released through All India Coordinated Sorghum Improvement Project. IS 25017 and GM 13 were germplasm accessions having grain mold resistance. White genotypes showed higher toxin production at day 6 of infection than yellow genotypes (**Tables 2** and **3**). The variability for total toxin production in varieties was statistically significant. The individual toxins AFB_1, AFB_2, AFG_1, and AFG_2 were also significantly different (**Table 3**). Aflatoxin production was highest at day 6 after fungal infection in white sorghums except in GM 13, that is 28.5 and 46 μg/g (**Table 3**). The temporal trend in aflatoxin production in SPV 86, SPV 462, and GM 13 was comparable to that of red sorghum genotypes: In GM 13 maximum aflatoxin production was observed at day 9 after infection (9.67 μg/g). GM 13 and SPV 86 produced fewer aflatoxins at all stages of infection. At day 3 after fungal infection, no AFB_2 and AFG_2 toxins could be detected in GM 13.

Maize and Groundnut. Aflatoxins in maize and groundnut at all stages of infection differed significantly. Contents of total and individual aflatoxins are given in **Table 4**. The amount was also high in maize and groundnut compared to red sorghum genotypes (maize, 19.42 μg/g; groundnut, 31.5 μg/g). However, aflatoxin production in two white genotypes (CSH 9 and CSH 14) was found to be higher as compared to maize and groundnut. In both groundnut and maize the aflatoxin production peaked at days 6 and 9 after infection, respectively.

Ergosterol Production. Ergosterol contents were different and statistically significant in all genotypes at all stages of fungal growth ($P < 0.01$). The variation in ergosterol contents of different genotypes of sorghum, maize, and groundnut are given in **Figure 1**. The pie chart of ANOVA of ergosterol is represented in **Figure 2.2**. It shows that variation exists between genotypes as well as period of infection, which was different and statistically significant ($P < 0.01$). The ranking order for the ergosterol content was different from that of total toxin (yellow > white > red > maize > groundnut). Red genotypes were found to have low amounts of ergosterol (**Figure 1.1**). Varieties are significantly different for the ergosterol content. As the fungal growth increases, ergosterol also increased significantly in all of the genotypes up to day 9 after fungal infection, and it decreased or showed no change on day 12, after fungal infection. The range of ergosterol in red genotypes observed was from 17.0 μg/g (IS 620, 3 days) to 228 μg/g (AON 486, 12 days). The lowest content of ergosterol (87.5 at day 12) was observed in IS 14384 among the red genotypes at all stages of infection. LPJ, a local cultivar, showed a low content of ergosterol (132 μg/g). The range of ergosterol observed in yellow sorghums was 18.0−248 μg/g (**Figure 1.2**). In white sorghum SPV 86, a Rabi-based cultivar, showed a low amount

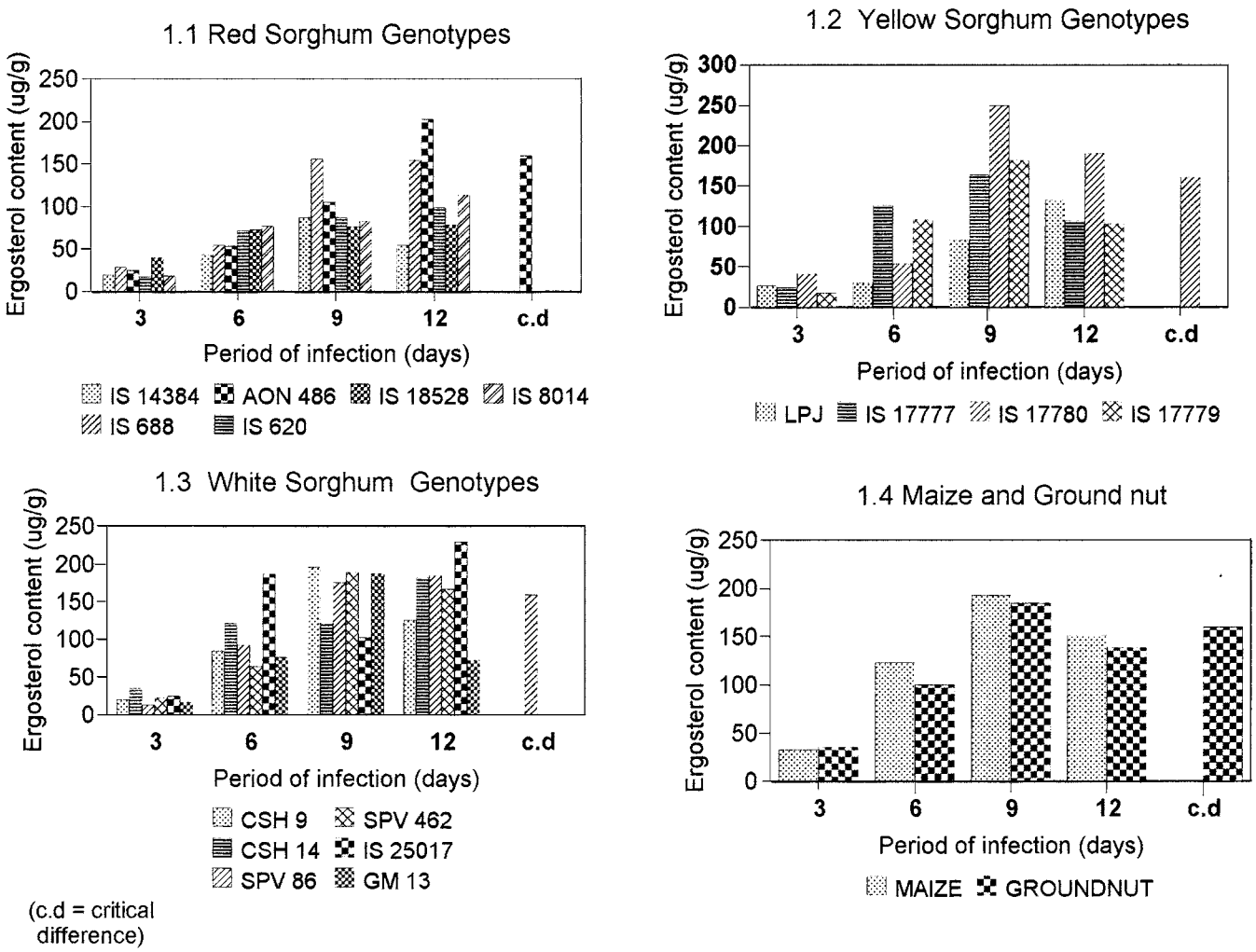

Figure 1. Ergosterol content in sorghum.

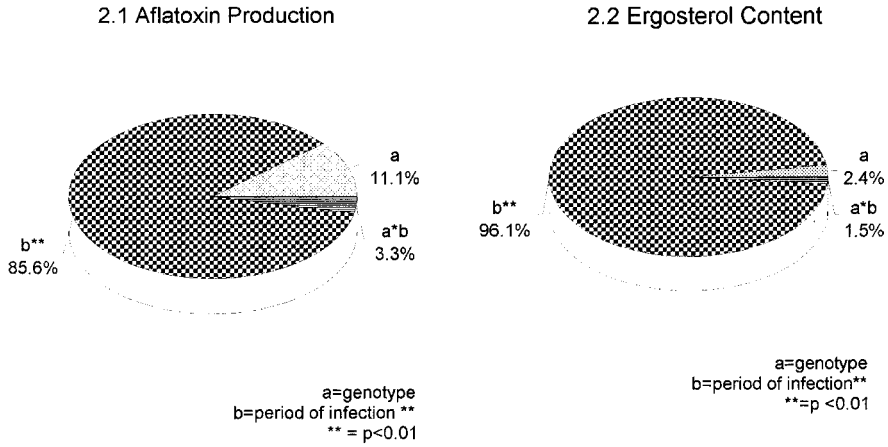

Figure 2. Pie charts of ANOVA.

(13−85 μg/g) of ergosterol, whereas IS 25017, a germplasm line, had a high amount of ergosterol (230 μg/g) (**Figure 1.3**). In IS 25017, the range of ergosterol observed was 13.3−230 μg/g. In maize and groundnut ergosterol contents were highest at day 9 after fungal infection, that is, 193 and 185 μg/g, respectively (**Figure 1.4**).

Physical and Chemical Characteristics of Deteriorated Sorghum Grain. *Physical Characteristics.* The physical characteristics of grain in sorghum genotypes are presented in **Table 5**. They represent 1000-grain weight, color of the grain, endosperm character, and hardness index. Grain size of red genotypes was small, whereas yellow and white sorghum grains are of medium size. The 1000-grain weight ranged from 15.8 to 42 g. The grain weight in red genotypes again varied from 15.8 g (IS 8014) to 22 g (IS 18528) (**Table 5**). In yellow sorghums, it varied from 29.8 g (LPJ) to 32.0 g in IS 17779. In white sorghums the grain weight varied from 16.6 g (GM 13) to 42.0 g (SPV 86, a post rainy season cultivar). Among the red genotypes IS 688, AON 486, and IS 8014 were found to have a hard corneous endosperm. IS 14384 and IS 620 had a chalky or floury endosperm. The hardness index of red genotypes varied from 7.6 to 9.5 kg/cm^2, the highest being for

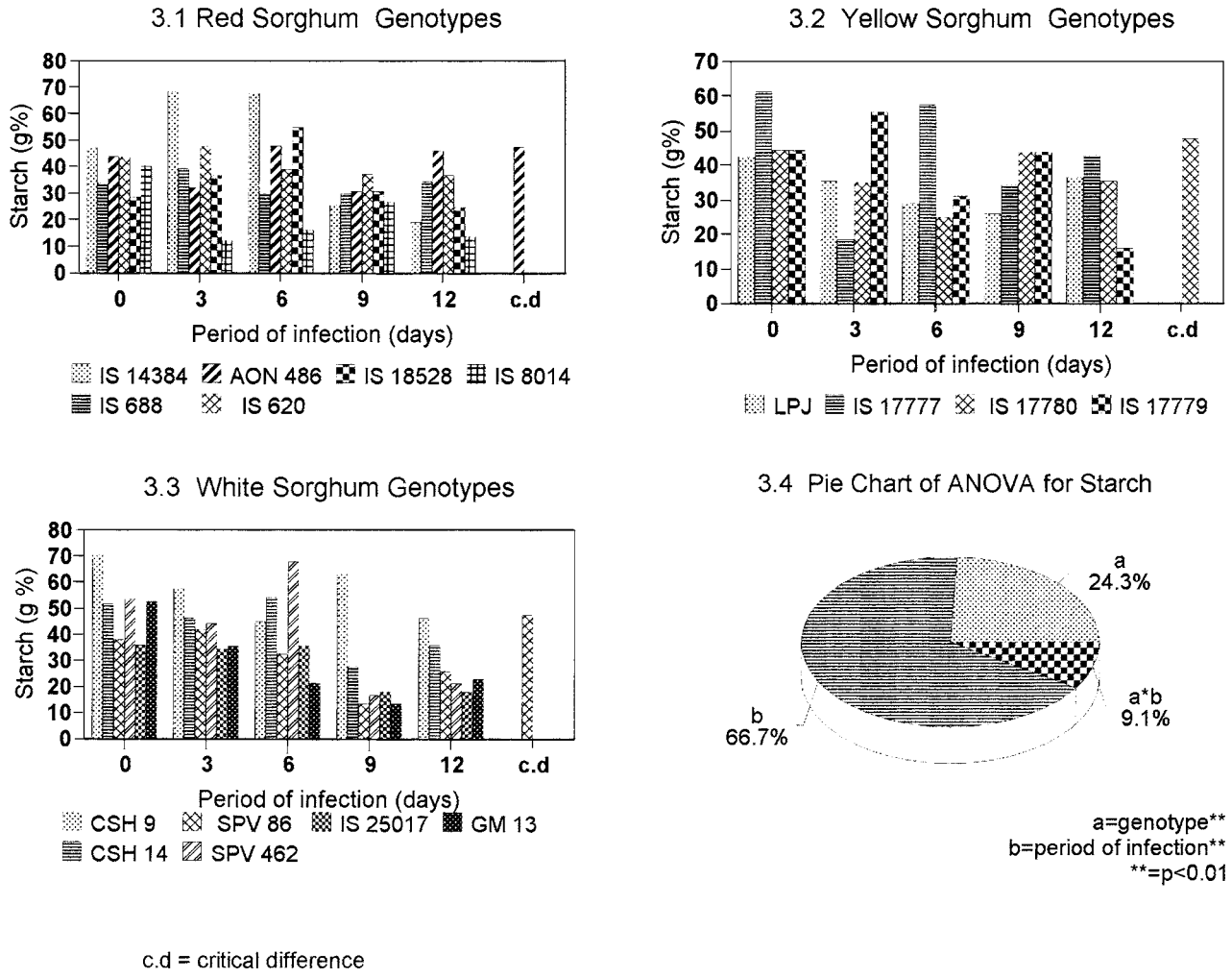

Figure 3. Percent starch content in sorghum.

IS 8014 (**Table 5**). All yellow and white genotypes had corneous endosperm. The hardness index varied from 9.0 to 10.1 kg/cm^2. The hardness index in white sorghums ranged from 8.98 to 12.5 kg/cm^2. IS 25017 was found to have the highest hardness index.

Chemical Characteristics. (a) Starch. The pie chart of analysis of variance in starch is depicted in **Figure 3.4**. A significant difference in starch content was found to exist among the varieties. It also differs significantly with the period of fungal infection. The starch content in red genotypes was low as compared to yellow and white genotypes. Maximum amount of starch was observed in CSH 9 (70%).

Among the red genotypes IS 18528 was found to have the lowest amount of starch (28.7%), and the highest starch content was observed in IS 14384 (47.5%) (**Figure 3.1**). In yellow sorghum IS 17777 was found to have a high percentage of starch (61%). The other three yellow genotypes were found to have lower contents of starch (44%) (**Figure 3.2**). The percent starch content in white sorghums varied from 36% (IS 25017) to 70% (CSH 9, **Figure 3.3**) on day 6. In general, starch content decreases during the course of infection. Furthermore, the correlation between starch content and aflatoxin produced was not statistically significant (**Table 6**).

(b) Protein. The protein content in all sorghum genotypes tended to increase as the fungal infection increased. Varieties differ significantly for the protein content. Aflatoxin production was significantly and positively correlated to the grain protein content in red sorghum genotypes ($r = 0.413$). Red sorghum genotypes contain slightly higher amounts of protein compared to yellow and white sorghums (**Figure 4.1**). The overall protein content ranged from 6.88 to 29.7%. As the protein increased, the toxin production also increased. However, there was no

Table 5. Physical Characters of the Grain in Sorghum Genotypes

genotype	hardness index[a] (kg/cm^2)	1000 grain[a] wt (g)	endosperm nature
red			
IS 14384	8.80 ± 0.45	18.9 ± 2.0	floury
IS 688	9.00 ± 0.53	16.3 ± 1.5	corneous
AON 486	9.10 ± 0.91	19.3 ± 1.8	corneous
IS 620	7.60 ± 1.00	16.8 ± 2.0	floury (soft)
IS 18528	8.60 ± 0.30	22.0 ± 0.5	corneous
IS 8014	9.50 ± 0.50	15.8 ± 0.5	corneous
yellow			
LPJ	10.10 ± 0.62	29.8 ± 1.0	corneous
IS 17777	9.00 ± 0.16	31.0 ± 1.0	corneous
IS 17780	9.80 ± 0.24	30.0 ± 1.5	corneous
IS 17779	9.35 ± 0.20	32.0 ± 2.0	corneous
white			
CSH 9	9.70 ± 0.30	29.2 ± 1.5	corneous
CSH 14	9.20 ± 0.20	28.5 ± 1.8	corneous
SPV 86	10.70 ± 0.90	42.0 ± 2.5	corneous
SPV 462	8.98 ± 0.53	28.7 ± 1.5	corneous
IS 25017	12.50 ± 0.22	22.9 ± 2.0	corneous
GM 13	9.65 ± 0.30	16.6 ± 1.0	corneous

[a] Values represented are mean ± SD of four replications.

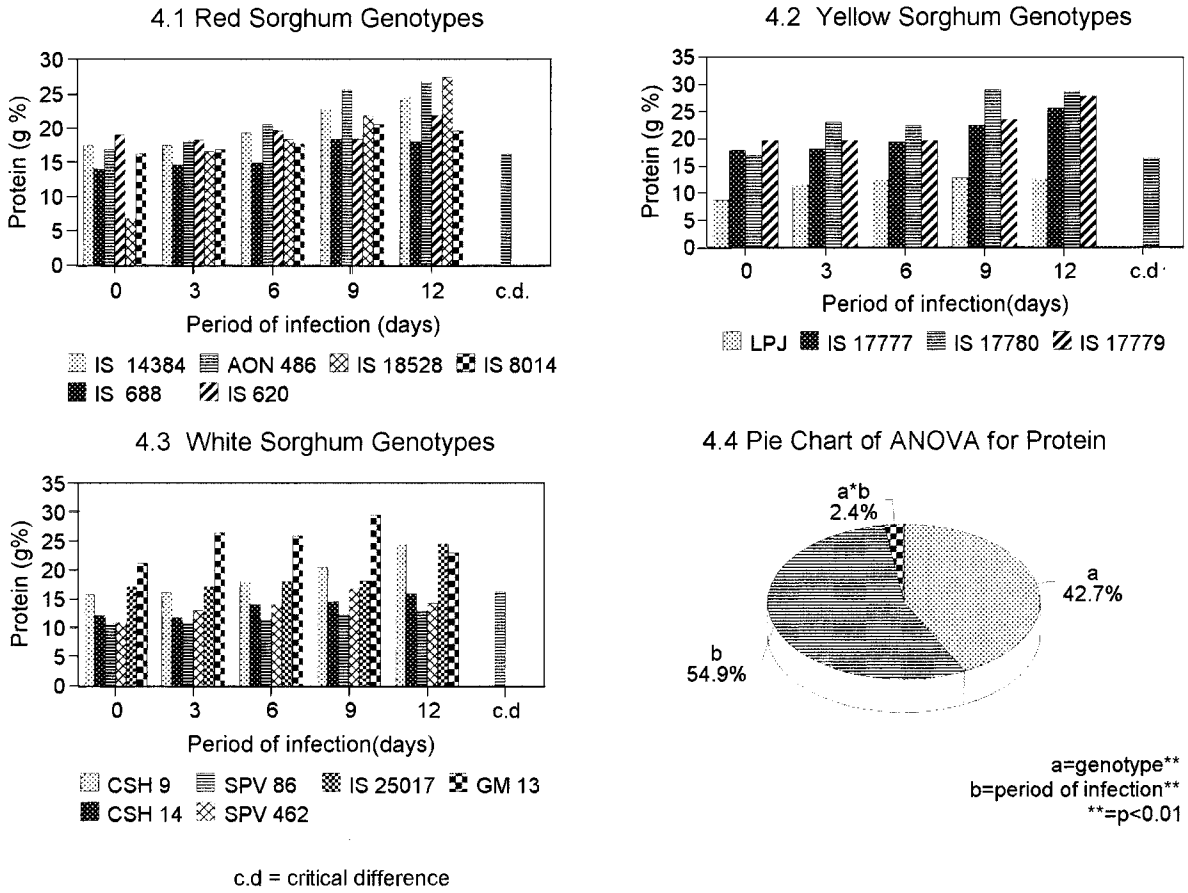

Figure 4. Percent protein content in sorghum.

Table 6. Correlation Coefficients of Total Aflatoxin

sample	parameter	total aflatoxin correlation value
1	polyphenol (red sorghum)	
	day 3 of infection	0.589**[a]
	day 6 of infection	0.513**
2	polyphenols (white sorghum) all time points	0.505*
3	protein (red sorghum)	0.413*
4	starch	–0.008[b]
5	fat (white sorghum)	–0.526**
6	phytic acid (yellow sorghum)	–0.569**

[a] Level of significance: *, $P < 0.05$; **, $P < 0.01$. [b] (–) negative correlation.

significant correlation between protein and aflatoxin contents in yellow and white sorghums (**Table 6**).

In red genotypes, protein content ranged from 14.1% (IS 688, 0 day) to 26.9% (AON 486, 12 days), and in yellow sorghums it varied from 8.75% (LPJ, 0 days) to 29.1% (IS 17780, 9 days) (**Figure 4.2**), whereas in white cultivars protein content ranged from 10.9% (SPV 86) to 21.3% (GM 13) (**Figure 4.3**). The pie chart of two-way ANOVA of proteins is presented in **Figure 4.4**. All of the genotypes were significantly different in their protein contents ($P < 0.05$). Protein content during various periods of infection was also significantly different.

(c) Fat. Among the 16 genotypes of sorghum percent fat content varied from 0.6 to 4.5. In red genotypes percent fat content ranged from 0.9 (IS 14384) to 4.5 (IS 8014) (**Figure 5.1**−**5.3**). The highest fat content was observed in IS 8014 and the lowest in IS 688 (12 days, 0.7%) (**Figure 5.1**). In yellow genotypes it ranged from 1.0% (LPJ, 3 days) to 4.0% (17780, 0 days) (**Figure 5.2**). In general, a decrease in fat content was observed over the period of infection. In white genotypes fat content was in the range from 2.6% (CSH 14, 0 days) to 4.0% (GM 13) (**Figure 5.3**). The pie chart of two-way ANOVA of fat is shown in **Figure 5.4**. However, the decrease in fat content during the period of fungal infection was statistically significant (**Figure 5.4**) ($P < 0.01$). The correlation coefficient of percent fat to aflatoxin content in white sorghum was −0.526 ($P < 0.01$). However, there is no correlation in red and yellow genotypes between fat and aflatoxin elaborated (**Table 6**).

(d) Polyphenols. The content of polyphenols is shown in **Figure 6.1**−**6.3**. The pie chart of two-way ANOVA of polyphenols is shown in **Figure 6.4**. The 16 sorghum genotypes differed significantly in their polyphenol contents. Red sorghums had a high content of polyphenols as compared to yellow and white genotypes. Polyphenol content was increased in response to infection. Polyphenols in red sorghums varied from 1.58% (AON 486) to 8.64% (IS 8014) (**Figure 6.1**). The amount of polyphenols in yellow sorghums was marginally higher than that of white sorghums, ranging from 0.23 to 6.46 μg/g (**Figure 6.2**). In white sorghums, polyphenols were not detected in SPV 462, whereas GM 13 had a low level of polyphenols. At all stages of infection no polyphenols were detected in these genotypes. In white sorghums, polyphenol content increased as the period of infection and toxin production increased (**Figure 6.3**). There was a positive, significant correlation between polyphenol content and aflatoxin production in white sorghums ($r = 0.505$) (**Table 6**). In red sorghum genotypes there was a significant, positive correlation between polyphenol and aflatoxin contents at days 3 and 6 after infection, the r values being 0.589 and 0.513, respectively (**Table 6**). Correlation in yellow sorghums, however, was not significant.

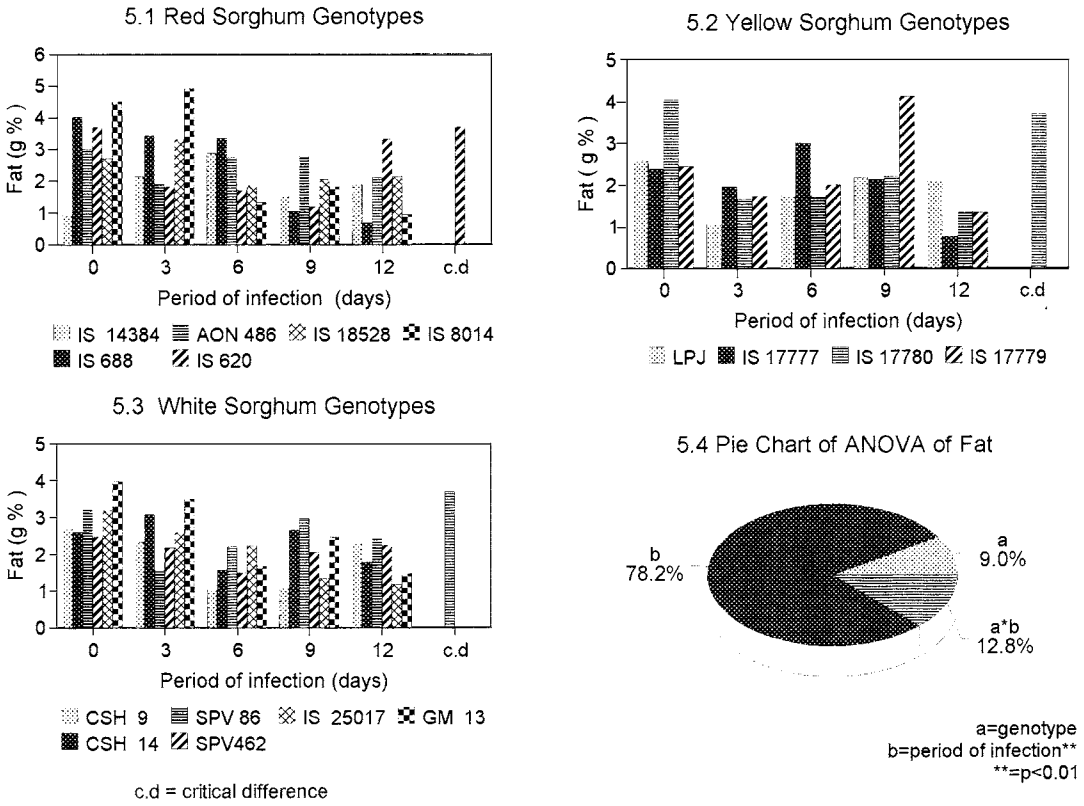

Figure 5. Percent fat content in sorghum.

(e) Phytic Acid. The phytic acid in different genotypes of sorghum is shown in **Figure 7.1**−**7.3**. The pie chart of two-way ANOVA of phytic acid is shown in **Figure 7.4**. Genotypes were not significantly different in their phytic acid contents. The decrease of phytic acid during infection was also not significant. Phytic acid content in red sorghum genotypes was slightly lower compared to yellow and white genotypes (**Figure 7.1**). A negative significant correlation was found between phytic acid and toxin production in yellow sorghums, the *r* value being −0.569 (**Table 6**). The correlation in red and white sorghums was also negative, but it was not significant.

DISCUSSION

Resistance to fungal infection in sorghum is dependent on grain characteristics such as grain hardness, colored testa, or pericarp (*21*, *31*). The rain-damaged grains weigh only 50−70% of the weight of the normal grain. The physical characters of grain such as hardness of kernel and corneous endosperm impart a physical barrier to fungal entry. An earlier study by Kumari and Chandrasekhar (*32*) showed a positive correlation between grain hardness and prolamins or alcohol soluble proteins. The nature of the endosperm in two genotypes (IS 14384 and IS 620) was floury type, whereas the rest of the genotypes were corneous (**Table 5**). The high resistance of IS 620 was possibly caused by the presence of high levels of polyphenols, whereas IS 14384 had a floury endosperm with a low level of polyphenols. However, the nature of the polyphenols present may be different from that of IS 620. Among the white sorghum genotypes GM 13 was shown to have maximum resistance for aflatoxin production. GM 13 is a low-fat genotype having average starch and high protein contents. The corneous endosperm of GM 13 combined with low fat might have been the basis for the lowered aflatoxin production. IS 17779, a yellow genotype, showed a lower aflatoxin production as compared to the other three yellow genotypes, although the endosperm nature was corneous in all of them. A wide variation was observed for aflatoxin production and ergosterol production among the 16 sorghum genotypes. Miguel and Andres (*19*) also reported variability in aflatoxin production in 16 varieties of sorghum. This indicated variation in substrate suitability for aflatoxin production. Aflatoxin contamination in some sorghum varieties under laboratory conditions in the present study was less compared to that of other susceptible agricultural commodities, maize and groundnut.

A survey of sorghum samples collected in India from fields, stores, and markets were tested, and *Aspergillus* was one of the predominant fungi identified (*7*, *24*). The natural incidence of aflatoxin contamination in sorghum is lower compared to other high-risk agricultural commodities such as groundnut, maize, and soybeans (*2*). Aflatoxin contamination levels in sorghum samples drawn from threshing floors were 7.25−75 ng/g (*7*). The occurrence of AFB_1 in improperly stored maize samples was 6.25−15.6 μg/g (*33*). The incidence of AFB_1 in the normal and rain-affected samples of sorghum ranged from 0.18 to 30.34 mg/kg and from 2.0 to 830.0 mg/kg, respectively (*8*, *9*). McMillan et al. (*5*) collected preharvest grain samples from several sorghum fields in Georgia and Mississippi that contained 90 ng of aflatoxin. Australian reports of aflatoxin contamination of postharvest sorghum indicate a level of aflatoxin of ∼9.6 mg/g (*34*). Aflatoxin production was lowest in red genotypes in the initial stages of infection, that is, 3 days after infection (**Table 1**). In addition to this it is also observed that the production of aflatoxin is slightly delayed in the red genotypes and hence peak aflatoxin production is observed at day 9 after infection. This could be due to the presence of polyphenols in red genotypes. Several reports indicated that red genotypes contain colored pericarp with polyphenols, flavan 4-ols, and pigments, which may offer some resistance to fungal

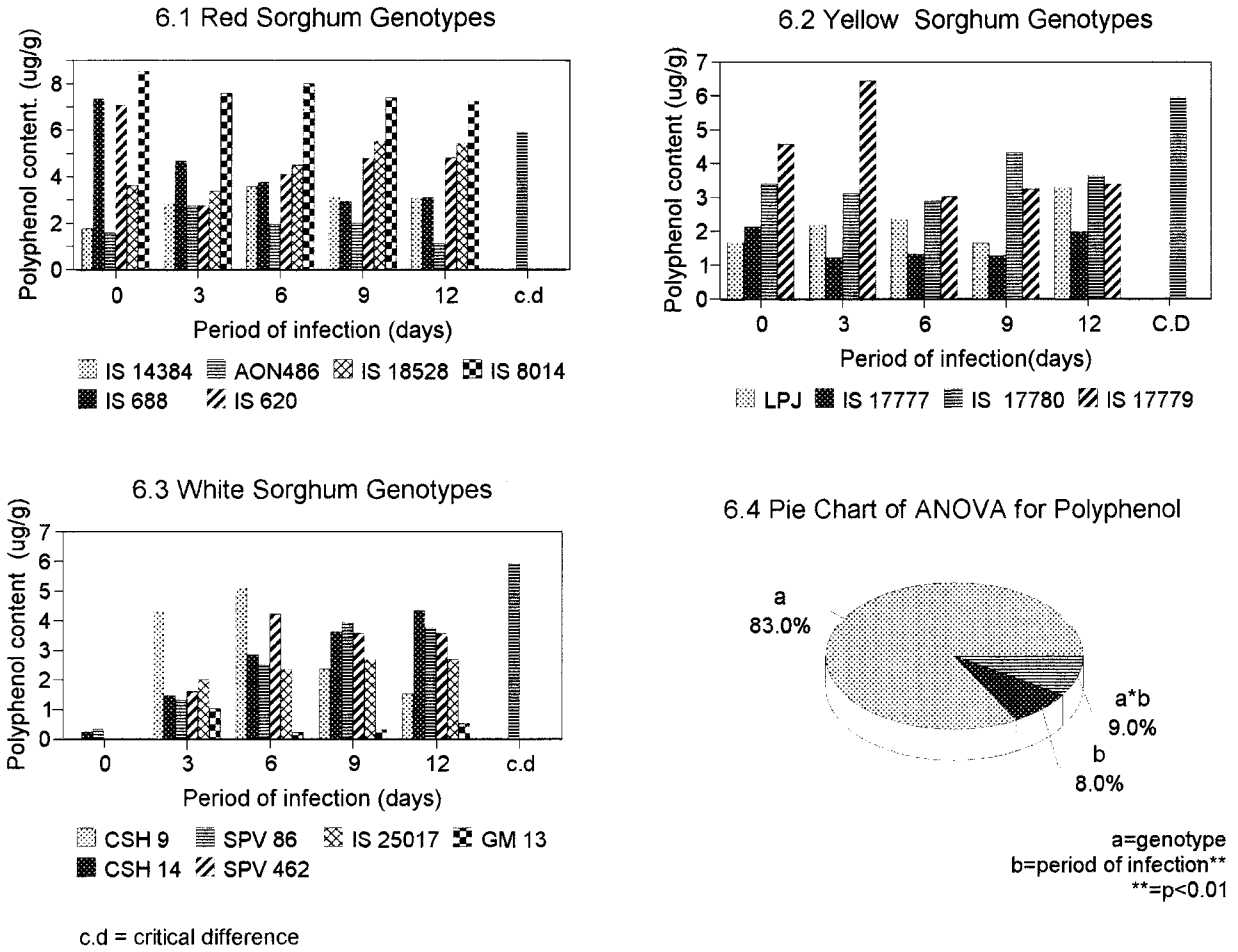

Figure 6. Polyphenol content in sorghum.

infection. In the present study, it would appear that red genotypes offer some resistance to fungal attack and lower aflatoxin.

Yellow sorghum genotypes showed less resistance to aflatoxin production compared to that of red genotypes. Peak toxin production in yellow and white sorghum genotypes was on day 6 after infection, thus indicating a lower degree of resistance compared to red genotypes. The yellow genotype IS 17779 was an exception; its aflatoxin production peaks at day 9 after infection. At the initial stage of infection toxin production was similar to that of red genotypes. Aflatoxin production in sorghum was compared to that of maize and groundnut in order to study suitability of the substrate for the growth of the fungus *A. parasiticus*. Groundnut, a rich source of protein and fat, was found to be a good substrate followed by maize and sorghum, respectively. The total toxin content in groundnut was 4−5-fold higher as compared to that of red genotypes and 2-fold higher as compared to that of white sorghums. Reddy et al. (*35*) reported that groundnut having maximum amount of lipids supported the highest production of AFB_1. In maize, toxin production was comparable to that of yellow sorghums. Within the sorghum genotypes variation in substrate suitability exists, with respect to fungal infestation and aflatoxin production. Sashidhar et al. (*18*) studied the mycotoxin contamination in grain sorghum stored in traditional containers in India. It was reported that, even if fungal infestation in sorghum occurs, the extent of aflatoxin contamination was minimal. However, in red varieties toxin was not detected, possibly due to high polyphenol contents. They also reported that sorghum stored in the "kotlu" traditional storage structure was more susceptible to fungal infestation. The ergosterol content was less in red sorghum compared to yellow and white genotypes at all growth stages of the fungus studied. Ergosterol in yellow genotypes was much higher than that of white sorghums, and maximum content was observed at day 9 after fungal infection. The amount of ergosterol in maize and groundnut was on par with white sorghum genotypes (**Figure 1.4**).

Fat content in cereals is relatively low, and in sorghum it ranges from 2.52 to 5.1% (*36*). Red sorghums were found to have low fat contents, whereas white sorghums had higher fat contents. Percent fat was negatively correlated to total aflatoxin elaborated in white genotypes. The amount of fat decreased during the course of infection while the toxin production was increased. Somani and Pandrangi (*37*) made similar observations in the grains affected by field fungi, *Curvularia lunata* and *Fusarium moniliforme*, that fat content decreased during the course of infection. Furthermore, it was also reported that fat content in moistened ground grains of sorghum decreased markedly during storage (*38*). Red cultivars being low-fat genotypes had low aflatoxin production. The only red genotype with high fat and polyphenol contents was IS 8014. Apart from its high polyphenol content, it showed a higher level of aflatoxin production within the red genotypes, probably due to the presence of high fat.

The fungus infecting the grain would draw most of its nourishment from the grain reserves such as starch, protein, and fat. Padule and Salunkhe (*39*) earlier reported the decrease in carbohydrate content during fungal infestation of sorghum grain. The correlation between starch percent and aflatoxin contamination was negative but not significant ($r = -0.008$). Hence, it

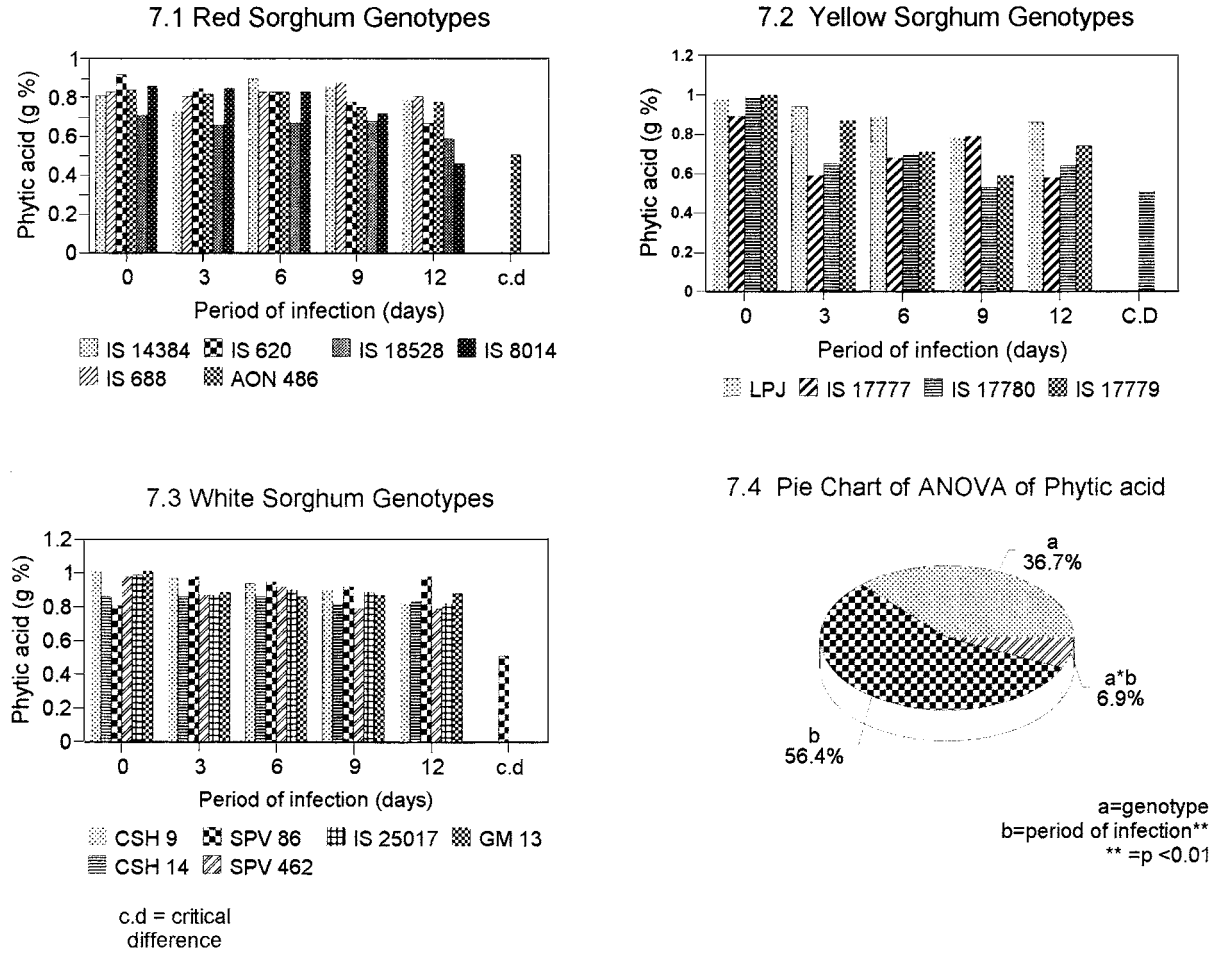

Figure 7. Percent phytic acid in sorghum.

was concluded that there is no specific association between starch and toxin production. However, starch content decreased during the period of infection. Somani and Pandrangi (*37*) also reported the decrease in starch content in deteriorated sorghum grain. However, this study was limited to a single sorghum hybrid. The protein content of the deteriorated grain showed an increase in total protein during the course of infection. However this increase was not statistically significant.

In the present study, polyphenol content increased in response to fungal infection (**Figure 6.1−6.4**), indicating polyphenols to be a possible factor conferring resistance to fungal attack. The increase in polyphenol content with fungal infection was suggested to be a response of the plant immune system (*40*). Resistant cultivars respond to fungal infection via an increased level of phenolic compounds and pigmentation of spikelet tissues (*41*). Doherty et al. (*42*) reported a significant increase in free phenolic compounds and polyphenols in caryopses during development. Snyder and Nicholson (*43*) reported production of phytoalexins in sorghum, which were identified as 3-deoxyanthocyanidins. These compounds inhibited the growth of phytopathogenic field fungi.

No genotypic variation was found for phytic acid. No significant changes were observed in all genotypes for phytic acid content during the course of fungal infection. Phytic acid content was high in yellow sorghum, and it was negatively correlated with total aflatoxin contents. Thus, phytic acid may contribute as an additional factor in conferring resistance to mycotoxin production in yellow genotypes.

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