



## CORRELATION OF AFLATOXIN PRODUCTION ON WHEAT SEED VARIETIES WITH THEIR PROTEIN AND SUGAR CONSTITUENTS

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Infestation of seeds of selected wheat varieties with a highly toxigenic strain of *Aspergillus flavus* under laboratory conditions resulted in varying levels of aflatoxin B<sub>1</sub> production. Biochemical analysis showed that the varieties containing higher levels of protein and sugar showed resistance against the production of aflatoxin B<sub>1</sub> in them, whereas the varieties with lesser amount of protein and sugar were more prone to aflatoxin B<sub>1</sub> production. Significant changes were observed in the sugar and protein levels. Total sugar content enhanced whereas the protein content decreased significantly in the selected varieties. Correlation analysis showed negative correlation between aflatoxin level and protein/sugar content.

**Key words:** Aflatoxin, *Aspergillus flavus*, wheat varieties, sugar and protein levels.

Wheat (*Triticum aestivum* L.) is consumed by millions of people all over the world in different forms. Thus, any form of contamination may prove to be deleterious to health. *Aspergillus flavus* and *A. parasiticus* were reported to contaminate wheat grains with their toxic secondary metabolites known as aflatoxins (Furlong *et al.* 1995 Curtui *et al.* 1998). Among various aflatoxins, aflatoxin B<sub>1</sub> is considered to be very potent hepatotoxin as well as hepatocarcinogen for the animals and human beings.

Warm and humid climatic conditions along with unscientific storage practices prevalent in Bihar provide favourable condition for the growth of *A. flavus* and subsequent production of aflatoxins in food grains including wheat (Sinha and Sinha 1990 Sinha 1991). Aflatoxin production also varies with variations in the biochemical constituents of different seed varieties.

In this investigation, aflatoxin production in different seed varieties has been correlated with the sugar and protein contents of the seed.

## MATERIALS AND METHODS

Seeds of nine wheat varieties were collected from authentic sources viz. Bihar Agricultural College, Sabour, Bihar (UP-262, Lokmanya-T, RR-21, HD-2285, UP-2003, PBW-343, Kundan, and HD-2329) and private dealers of certified seeds (Ankur Kedar research variety).

50 g seeds of each variety were taken in 250ml conical flask, soaked in sterilized distilled water for 2 h and then infested with 1 ml of spore suspension of one highly toxigenic strain of *A. flavus*, isolated earlier from wheat seed. These were then incubated in BOD incubator at 30±2<sup>0</sup>C for 11 days for the growth of *A. flavus* and aflatoxin production. The infested seeds were then kept in an oven at 55±2<sup>0</sup>C for three days. Properly dried seeds were ground for the chemical extraction of aflatoxin as well as for the estimation of protein and sugars (both reducing and non-reducing sugars).

Quantitative estimation of protein was done by the methods of Lowry *et al.* (1951). 100mg seed was crushed in 5ml acetate buffer (pH =

4.8). 0.5ml of this homogenate was mixed with 1ml 15% cold trichloroacetic acid and then centrifuged at 6000 rpm for 30 minutes. The supernatant was decanted and the precipitate was dissolved in 5 ml 0.1N NaOH. The dissolved solution was then diluted ten times. This 5 ml of alkaline reagent was mixed thoroughly and was allowed to stand at room temperature for 10 minutes. 0.5 ml folin ciocalteau reagent (1:1 in distilled water) was then added to it. After 10 minutes the optical density was read at 750 nm with blank prepared by similar process in distilled water. The standard curve of egg albumin was prepared via similar method and correlated.

Total sugar of the wheat seed was estimated according to the method of Dubois *et al.* (1956). Reducing sugar content of the sample was evaluated by the method of Somogyi-Nelson (Plummer 1971). Non reducing sugar was estimated by subtracting the value of reducing sugar from total sugar.

For establishing correlation between different variables, Karl Pearson's coefficient of correlation ( $r$ ) was used.

## RESULTS AND DISCUSSION

None of the wheat seed varieties screened was found to be totally immune to aflatoxin production. However, varying levels of aflatoxin were elaborated in different varieties. Maximum amount of aflatoxin B<sub>1</sub> was produced on the seeds of variety UP-262(300 µg/kg), whereas minimum amount was elaborated on variety Lokmanya-T (71 µg/kg).

Table 1 shows the protein contents of different varieties of wheat seeds under healthy and infested conditions. Total protein content in healthy seeds ranged in between 122.50mg/g (Kundan variety) to 126.12mg/g (Lokmanya-T variety). Significant reduction in protein content was observed during fungal infestation,

**Table 1:** Protein content of seeds of different varieties of wheat under healthy and infested conditions.

Wheat variety	Protein content (mg/g) $\pm$ SE <sub>M</sub>		
	Healthy seeds	Infested seeds	% change
Kundan	122.50 $\pm$ 1.11	102.40 $\pm$ 1.01	16.40
HD-2285	122.60 $\pm$ 1.12	109.52 $\pm$ 1.04	10.66
UP-2003	123.36 $\pm$ 1.24	112.20 $\pm$ 1.06	9.04
PBW-343	124.48 $\pm$ 1.42	103.30 $\pm$ 1.07	17.01
Lokmanya-T	126.12 $\pm$ 1.52	115.90 $\pm$ 1.05	8.10
RR-21	125.45 $\pm$ 1.27	111.30 $\pm$ 1.06	11.27
HD-2329	124.22 $\pm$ 1.32	104.90 $\pm$ 1.09	15.55
Kedar research	123.30 $\pm$ 1.22	109.20 $\pm$ 1.03	11.43
UP-262	123.24 $\pm$ 1.35	107.10 $\pm$ 1.06	13.09

maximum being in Kundan (16.40%).

Table 2 depicts the levels of total sugar, reducing sugar and non-reducing sugar in seeds of wheat varieties. In healthy seeds, total sugar was estimated to be maximum (18.82 mg/g) in the variety Lokmanya-T and minimum (16.75 mg/g) in variety UP-262. Reducing sugar was also estimated maximum in Lokmanya-T (5.45 mg/g) whereas minimum level was estimated in variety UP-262 (4.12 mg/g). Non-reducing sugar was recorded maximum (13.37 mg/g) in Lokmanya-T and minimum (11.94 mg/g) in UP-262. In all the cases, sugar contents were found to increase on fungal infestation

Analysis of protein contents of seeds of selected wheat varieties shows that higher levels of protein in seeds somehow inhibit the production of aflatoxin B<sub>1</sub>. Thus, the varieties containing higher amount of proteins were found to resist the production of aflatoxin B<sub>1</sub>. Significant reduction in protein content was observed in case of those varieties that facilitated higher amount of aflatoxin B<sub>1</sub>. Correlation coefficient ( $r$ ) was calculated to be **-0.58**, which shows moderate negative correlation between the amount of protein and levels of aflatoxin production.

Analysis of sugar contents shows that higher levels of sugar (both reducing and non-reducing) have an inhibitory effect on the production of aflatoxin B<sub>1</sub>. Correlation coefficient between sugar levels and aflatoxin B<sub>1</sub> production was

**Table 2:** Levels of total sugar, reducing sugar and non-reducing sugar in the seeds of different varieties of wheat under healthy and infested conditions.

Wheat variety	Total sugar content (mg/g) $\pm$ SE <sub>M</sub>			Reducing sugar (mg/g) $\pm$ SE <sub>M</sub>			Non-reducing sugar (mg/g) $\pm$ SE <sub>M</sub>		
	Healthy seeds	Infested seeds	% change	Healthy seeds	Infested seeds	% change	Healthy seeds	Infested seeds	% change
Kundan	16.98 $\pm$ 0.12	24.54 $\pm$ 0.19	44.52	4.22 $\pm$ 0.21	6.19 $\pm$ 0.01	46.68	12.76 $\pm$ 0.12	18.35 $\pm$ 0.24	43.80
HD-2285	16.96 $\pm$ 0.14	25.24 $\pm$ 0.17	48.82	4.21 $\pm$ 0.28	6.16 $\pm$ 0.02	46.31	12.75 $\pm$ 0.13	19.08 $\pm$ 0.25	49.64
UP-2003	17.35 $\pm$ 0.17	26.21 $\pm$ 0.18	51.06	4.12 $\pm$ 0.23	6.17 $\pm$ 0.07	49.75	13.23 $\pm$ 0.17	20.04 $\pm$ 0.23	51.47
PBW-343	16.85 $\pm$ 0.11	24.45 $\pm$ 0.16	45.10	4.13 $\pm$ 0.27	6.22 $\pm$ 0.06	50.60	12.72 $\pm$ 0.21	18.23 $\pm$ 0.21	43.31
Lokmanya-T	18.82 $\pm$ 0.13	25.56 $\pm$ 0.18	35.81	5.45 $\pm$ 0.25	7.14 $\pm$ 0.03	31.00	13.37 $\pm$ 0.20	18.42 $\pm$ 0.27	37.77
RR-21	18.26 $\pm$ 0.11	26.31 $\pm$ 0.14	44.08	5.12 $\pm$ 0.22	7.11 $\pm$ 0.02	38.86	13.14 $\pm$ 0.13	19.20 $\pm$ 0.23	46.11
HD-2329	17.24 $\pm$ 0.17	25.41 $\pm$ 0.13	47.38	4.88 $\pm$ 0.23	6.88 $\pm$ 0.04	40.98	12.36 $\pm$ 0.18	18.53 $\pm$ 0.28	49.91
Kedar research	17.25 $\pm$ 0.19	24.65 $\pm$ 0.10	42.89	4.98 $\pm$ 0.27	6.91 $\pm$ 0.09	38.75	12.27 $\pm$ 0.25	17.74 $\pm$ 0.23	44.58
UP-262	16.75 $\pm$ 0.12	23.96 $\pm$ 0.18	43.04	4.81 $\pm$ 0.21	6.82 $\pm$ 0.07	41.78	11.94 $\pm$ 0.14	17.14 $\pm$ 0.18	43.55

calculated to be **-0.98**, which shows almost perfect negative correlation.

Above-mentioned conclusions are in conformity with the earlier reports of Sinha (1991) in wheat variety HP-1209 and Maharjan (2000) in wheat variety Rojo-64. These workers reported lesser production of aflatoxin in varieties having higher protein levels.

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