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Aflatoxin contents of stored and artificially inoculated cereals and nuts

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Abstract

Aflatoxin contents of cereals and nuts, collected from local markets of NWFP, were determined by thin layer chromatography (TLC). The seeds of these crops were also inoculated with *Aspergillus flavus* and the aflatoxin content and its relation with the proximate composition of seeds was studied. The effect of storage for different durations of time (2–3 and 12–18 months) on the aflatoxin content of seeds was also assessed. Aflatoxin content of cereals (wheat, maize and rice) ranged from 14 to 45 μg/kg, and that of nuts (almond, walnut and peanut) varied from 5 to 17 μg/kg. The aflatoxin content was within the safe limit (50 μg/kg) recommended by FAO. The aflatoxin content of inoculated seeds was significantly ($p < 0.05$) increased over control (un-inoculated seeds). This was positively related ($r = 0.65$) to moisture content of the seeds. However, negative correlation ($r = -0.50$) existed between aflatoxin and ash contents of the seeds. Protein, fat and total carbohydrate (NFE) contents were not much affected by inoculation. Prolonged storage for 18 months (1.5 years) significantly ($p < 0.05$) increased aflatoxin contents of seeds compared to short storage periods (2–3 months). It was concluded that aflatoxin content of food should be monitored to ensure food safety. Prolonged storage of cereal and nuts in warm humid condition should be avoided to minimize the risk of aflatoxin contamination.
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Keywords: Aflatoxin; Cereals; Nuts; Storage; Nutritional quality

1. Introduction

Aflatoxins are a group of related bisfuranocoumarin compounds produced by fungi, *Aspergillus flavus* and *Aspergillus parasiticus*. The term aflatoxin is derived from *Aspergillus* (A-) flavus (-fla-) and toxin. It has been reported that, out of the known strains of *Aspergillus parasiticus*, only about one-half produce toxins. There are fourteen known aflatoxins but most of these are metabolites formed endogenously in animals. The well-known ones among these are aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 ($AFLG_1$) and aflatoxin G_2 ($AFLG_2$). (Structure 1). Aflatoxins B_1 and B_2 are so designated because of their strong blue fluorescence under ultraviolet light and aflatoxins G_1 and G_2 show greenish yellow fluorescence (Wogan & Busby, 1980).

Aflatoxicosis (ill effect of aflatoxin) causes acute liver damage, liver cirrhosis, induction of tumors, impaired central nervous system, skin disorders and hormonal defects (Pitt, 1989).

Malabsorption syndrome and decreased bone strength have been associated with aflatoxin consumption (Nelson, Jonson, Kirby, & Beasley, 1982; Osborne, Huff, Hamilton, & Burmeister, 1982). The overall toxicity of aflatoxin in an animal appears to be determined by the rate of formation of the reactive intermediate, its binding to the largest macromolecules (DNA, RNA) and the rate of detoxification and other competing reactions (Sermand, Karchesy, & Deinzer, 1999).

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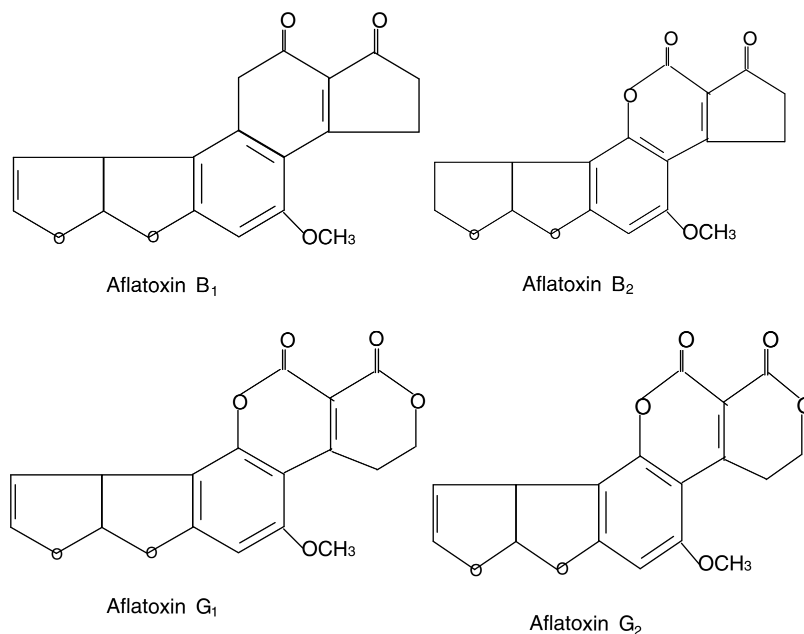

Structure 1. Structure of Aflatoxins (Wogan & Busby, 1980).

Aflatoxin can be detoxified by treating the products with NH_4OH and H_2O_2 at the rate of 1% on a dry matter basis (Gontijo, Teixeira, & Gomes, 1988). Aflatoxin may be prevented by packing the dried products in polythene or propylene bags (Siriacha, Kawashima, Saito, Tonboon, & Buangsuwon, 1990). Antoxigenic strains may be used for the prevention of Aflatoxin production (Brown, Cotty, & Cleveland, 1991).

This paper deals with the aflatoxin contents of stored and inoculated cereal and nuts used as human food. The effect of contamination was also assessed in order to ensure nutritional quality and food safety.

2. Materials and methods

2.1. General

Two sets of experiments were conducted at the Department of Agricultural Chemistry and Department of Animal Nutrition of the North West Frontier Province (NWFP) Agricultural University, Peshawar, Pakistan during the session 2000–2002. In one set of experiments, the aflatoxin content of cereals and nuts was determined, and the effect of aflatoxin production on the proximate composition of cereal and nuts was evaluated. In the second set of experiments, the aflatoxin content of cereals and nuts stored for different periods of time was determined. Cereals (rice, wheat and maize) and nuts (almonds, walnuts and peanuts) were collected from different local markets of NWFP. Samples stored for different durations of time were also obtained from local shops. About 1.0 kg of each sample was obtained from at least four shops. They were mixed to make a composite sample. This was reduced to small lab size and used of analysis.

Aflatoxin from each sample was extracted by an aqueous acetone procedure and the level was detected by the Jones (1972) method. This method was also used by other workers with some modifications for the determination of aflatoxin in milk, milk products and animal tissue (Brown, Neshim, Stack, & Ware, 1973; Pones, Cucullu, Franz, Lee, & Goldblatt, 1973; Stubblefield & Van Egmond, 1989).

2.2. Extraction of aflatoxin

Sample analysis was carried out by taking a known quantity (25 g) of the powdered sample in a 250 ml flask and treating with 19 ml distilled water and 100 ml acetone. This mixture was shaken for 50 min at 200 rpm on a shaker. It was then filtered through Whatman paper (No. 1). To the filtrate, 1.5 g cupric carbonate was added. Another solution of 85 ml of 0.2 N NaOH and 15 ml of 6.67% $FeCl_3$ was prepared to which 2.5 g celite powder was added. This solution was mixed with filtrate containing cupric carbonate and then the mixture was shaken for 20 min at 200 rpm. After shaking for 20 min the mixture was filtered through Whatman No. 1 filter paper. To the filtrate, 75 ml of 0.03% H_2SO_4 and 10 ml of chloroform were added and the mixture was transferred to a separatory funnel. After 30 min, the lower layer was separated and treated with KOH + KCl (1:1) solution, again in a separatory funnel. The lower layer was collected in a crucible. The solvent was allowed to evaporate and the oven-dried residue was dissolved in 5 ml chloroform.

2.3. Preparation of aflatoxin standards

Stock solution of aflatoxin (from Merck) was prepared in chloroform. For this purpose, 1000 μg of the aflatoxin was accurately weighed by a sensitive electronic balance and dissolved in 1 ml of chloroform. This stock solution was diluted to 100 μg/l by dissolving 0.01 ml in 100 ml of chloroform. This was used to check the fluorescence density of sample spots.

2.4. Sample assay

The dissolved residue was then spotted onto a silica gel plate of about 0.5 mm thickness as 1, 2, 3, 4, 5, 10, 15, 20 μl drops. The standard solution of aflatoxin was also spotted onto the same plate as drops of 1, 2, 4, and 6 μl. The plate was developed in chloroform–acetone (1:9). After development, the plate was air-dried and observed under UV light. The fluorescence intensities of aflatoxin spots of sample were compared with those of standard spots. The sample spot, which matches one of the standard spots, was selected. For this purpose, the plate was moved away from the lamp to attenuate UV light so that particular pairs of spots could be compared at extinction. If the spot containing the least amount of the sample was too intense to match the standard, then the sample was again diluted and rechromatographed. Standards were also used to compare the colour and rf value of unknown sample streak on the plate. The amount of aflatoxin was estimated using the following formula.

$$\text{Aflatoxin } B_1 \ (\mu\text{g/kg}) = \frac{C \times V_{\text{std}} \times TV}{V_{\text{spl}} \times W},$$

where C is the concentration of B_1 (standard), μg/kg, V_{spl} is the μl of sample extract spotted to give equal fluorescence intensity like standard B_1, V_{std} is the volume of standard spot, TV is the total volume of sample extract in μl, and W is the wt of sample in grammes.

2.5. Inoculation and chemical analysis

Potato dextrose agar medium was used for the growth of the fungus and inoculation of sample with *Aspergillus flavus*. Moisture, crude protein, crude fat and ash contents in each sample were estimated by the standard method of AOAC (1990). Nitrogen-free extract, representing total carbohydrates, was calculated by difference.

2.6. Statistical analysis

The data relating to aflatoxin content and storage effect were statistically analyzed with a CRD 2 × 3 factorial design (Yates, 1993). LSD (Waller & Duncan, 1969) was computed to compare treatment means. Cor-

Table 1
Aflatoxin content μg/kg of cereals and nuts stored for short and long periods of time

	Storage period	
	2–3 months	12–18 months
Cereals		
Rice (paddy)	17.7	23.3
Wheat	18.4	22.3
Maize	45.7	50.2
Mean	27.1b	31.9a
SE±		3.2
Nuts		
Peanut	19.7	30.0
Almond	7.5	11.9
Walnut	17.7	26.4
Mean	14.9b	22.7a
SE±		1.9

*SE± = Standard error (12 df). Means in horizontal row within each category of crops (showing storage effect) followed by different letters are significantly ($p = 0.05$), different.

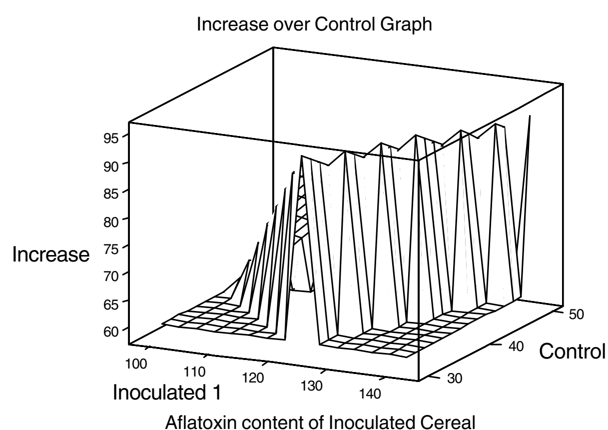

Fig. 1. The aflatoxin content of control (un-inoculated), inoculated cereal, and its increase (%) over control.

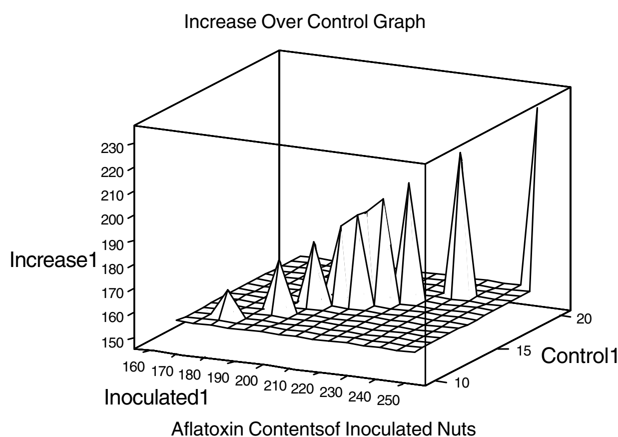

Fig. 2. The aflatoxin content of control (un-inoculated), inoculated nuts, and its increase (%) over control.

Table 2
Proximate composition of artificially inoculated cereals and nuts

Proximate composition	Cereals					Nuts				
	Popcorn	Fresh maize	Kashmir maize	Means	*SE±	Walnut	Almond	Peanut	Means	*SE±
Moisture (%)										
Controlled[a]	7.3	47.7	6.5	20.5a	17.8	2.3	2.7	2.3b	2.4	4.1
Inoculated[b]	47.2	58.5	40.5	48.6a		50.4	41.3	39.3a	43.3	
% Increase over control	39.9	10.8	34.0	28.1		48.1	38.6	37.0	40.9	
Crude Protein (%)										
Controlled	11.7	9.7	8.5	10.0a	1.2	19.3	22.3	29.2	23.6a	1.4
Inoculated	11.1	8.9	7.2	9.1a		18.4	20.4	28.5	22.4a	
% Decrease over control	5.1	8.2	15.3	9.0		4.6	8.5	2.3	5.0	
Crude Fat (%)										
Controlled	17.3	8.7	7.5	11.2a	3.8	71.2	60.3	57.3	63.0a	7.5
Inoculated	6.4	5.1	4.5	5.3a		64.5	52.7	51.1	56.0a	
% Decrease over control	170.0	70.5	66.6	111.3		10.6	14.5	12.3	7.0	
Ash (%)										
Controlled	0.9	1.3	1.1	1.1a	0.31	2.0	3.2	2.5	2.5a	0.7
Inoculated	0.8	1.5	0.9	1.0a		1.8	3.1	1.4	2.1b	
% Decrease over control	11.1	15.4	22.0	9.09		10.0	3.1	44.0	16.0	
NFE (%)										
Controlled	73.2	33.6	70.4	59.1a	21.0	12.5	17.4	17.1	14.8a	13.0
Inoculated	61.3	30.8	68.2	53.2a		5.1	12.4	14.6	14.8a	
% Decrease over control	16.9	8.3	3.1	9.9		58.9	28.7	14.6	33.0	

[a] Un-inoculated with *Aspergillus flavus*.
[b] Inoculated with *Aspergillus flavus*.
* SE± Standard error (4 df). Means in columns of within each crop followed by same letter are not significantly different ($p = 0.05$).

relation analysis was conducted for determining the relationship of aflatoxin with the proximate composition of the samples. This analysis was completed by mstatc Ver. 2 package.

3. Results and discussion

Samples collected after 2–3 and 12–18 months of storage from different locations were analyzed for aflatoxin contents. The results presented in Table 1 indicate that the aflatoxin contents of both cereals and nuts were significantly ($p < 0.05$) affected by storage period. They were increased from 27.1 to 31.9 μg/kg in cereals and 14.9 to 22.7 μg/kg in nuts by 18 months of storage. Among the cereals, rice (paddy) was much affected by storage period. The aflatoxin content of rice increased from 17.7 to 23.3 μg/kg. Interaction of storage period with the types of cereals was non-significant ($p > 0.05$). Likewise, the nuts data revealed that the minimum variation in aflatoxin occurred in almonds that changed from 7.5 to 11.9 μg/kg and the maximum in peanut, which increased from 19.7 to 30.0 μg/kg. The mean storage effect was also significant ($p > 0.05$) in the case of nuts. The effect of storage period on the kind of nuts was variable.

The cereals and nuts were inoculated with *Aspergillus flavus* and their aflatoxin contents were determined. The graphical representation of the (Figs. 1 and 2) revealed that inoculation significantly ($p < 0.05$) increased the aflatoxin content of both cereals and nuts. Popcorn was less affected then other maize cultivars (fresh maize and Kashmir maize). Among the nuts, the minimum increase in aflatoxin content was found in peanuts and the maximum in walnuts. This might be due the presence of more moisture in inoculated walnuts than in peanuts. Moreover, variation in the amount of substances resistant to mold in various types of cereals and nuts cannot be ignored (Lebron, Buckmaster, & Cheeke, 1989; Yoshizawa, Sakamoto, Ayano, & Microcha, 1995). These results are in fair to agreement with those of Que-King, TongRong, Wei, and Chen (1997).

The inoculation affected the proximate composition of both cereals and nuts. The fat content (Table 2) decreased significantly ($p < 0.05$) in both cereals and nuts inoculated with *Aspergillus flavus*. The minimum decline among the cereals occurred in Kashmir maize (66.6%) and the maximum decrease in popcorn (170%). Among the nuts, walnut (10.6%) was less affected then almond (14.5%). Like fat, the ash content was also decreased significantly ($p < 0.05$), both in cereals and nuts, due to inoculation with *A. flavus*. This effect was more pronounced in peanuts (44% decrease) than in other cereals and nuts. These results are in fair agreement with Bojle, Shannon, and Shatwell (1998).

4. Conclusions

From the preceding discussion it can be concluded that the aflatoxin contents in the tested samples of cereals and nuts are dependent on storage condition and

duration. The higher the humidity the faster is the Aspergillus growth and the higher the toxins production; besides this the anaerobic condition inhibits the growth of Aspergillus and thus decreases the risk of toxin production. Although the amounts in tested samples were within the safe limit (50 μg/kg), as recommended by FAO (1979) it could exceed the limit in food products stored for longer duration, particularly at room temperature (30–37 °C) in tropical regions, aerobic and humid conditions.

The presence of aflatoxin in cereals and nuts collected from different locations of NWFP suggested that food commodities must be handled with care and prolonged storage must be avoided. If prolonged storage is required for certain foodstuffs, then MAP (modified atmosphere packaging) or vacuum-packaging should be practised at low temperature; also, consumption of infected food should be discouraged. Aflatoxin contamination of different stored and processed foods should be monitored to ensure food safety. Governments must fix a legislative limit for aflatoxin in various foods and their products. Further work should be carried on mycotoxins in various foods/feeds to check health problems for humans and animals.

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