MECHANISM OF CONTAMINATION WITH AFLATOXINS OF COTTON SEEDS AND PRODUCTS OF THEIR PROCESSING

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A possible mechanism of the contamination of cotton seeds and the products of their processing with aflatoxins has been studied. It has been found that the contamination of cotton seeds with aflatoxin takes place mainly on storage in the cotton ginning factory and the oils and fats combine. In the processing of the cotton seeds, part of the aflatoxins passes into the final products and part decomposes. Meal stored in the oil and fats combine undergo secondary contamination with aflatoxins.

It is known that the development of Aspergillus flavus fungi – the producing agents of aflatoxins, which possess hepatoxic and hepatocarcinogenic properties [1, 2] – takes place under certain conditions in various food products [3] and feedstuffs [4] and also in oil crops, including cotton seeds and the products of their processing [5].

TABLE 1. Amounts of Aflatoxins in Samples of Soil

Experiment No.	Soil sample		Amount of aflatoxin, μg/kg			
	month taken (1985)	depth, cm	B_1	C_1	B_2	C_2
1	IV	0–15	0,32	0,32	0,15	0,15
2			0,07	0,07	0,04	0,04
3			0,25	0,25	0,13	0,13
4			0,15	0,15	0,10	0,10
5			0,20	0,20	0,09	0,09
6		15–30	0,20	0,20	0,10	0,10
7			0,10	0,10	0,05	0,05
8			0,15	0,15	0,07	0,07
9			0,05	0,05	0,04	0,04
10			0,02	0,02	0,04	0,04
11	VI	0–15	0,12	0,12	0,06	0,06
12			0,20	0,20	0,10	0,10
13			0,10	0,10	0,10	0,10
14			0,09	0,09	0,09	0,09
15			0,10	0,10	0,08	0,08
16		15–30	0,07	0,07	0,04	0,04
17			—	—	—	—
18			—	—	—	—
19			—	—	—	—
20			—	—	—	—
21	IX	0–15	0,20	0,20	0,10	0,10
22			0,40	0,40	0,20	0,20
23			0,25	0,25	0,18	0,18
24			0,25	0,25	0,12	0,12
25			0,32	0,32	0,10	0,10
26		15–30	—	—	—	—
27			—	—	—	—
28			0,04	0,04	—	—
29			—	—	—	—
30			—	—	—	—
31	XI					
35			Not detected			

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TABLE 2. Amounts of Aflatoxins in the Cotton Plant

Sample No.	Time of taking	Amount of aflatoxin, μg/kg			
		B_1	C_1	B_2	C_2
1	15-Day shoots	0,70	0,70	0,35	0,35
2		0,60	0,60	0,30	0,30
3		0,51	0,51	0,25	0,25
4		0,46	0,46	0,23	0,23
5		0,62	0,62	0,30	0,30
6	Budding and flowering phase	0,50	0,50	0,25	0,25
7		—	—	—	—
8		0,38	0,38	—	—
9		—	—	—	—
10		—	—	—	—
11	Fruit-forming phase	Not detected			
50					

TABLE 3. Amounts of Aflatoxins in Seeds Stored for a Month at a MZhK

Sample No.	Depth of sampling, cm	Amount of aflatoxins, μg/kg			
		B_1	C_1	B_2	C_2
1	20	0,42	0,42	0,21	0,21
2		0,22	0,22	0,11	0,11
3		0,15	0,15	0,08	0,08
4		0,30	0,30	0,15	0,15
5	50	0,35	0,35	0,18	0,18
6		0,10	0,10	0,05	0,05
7		0,18	0,18	0,09	0,09
8		0,20	0,20	0,10	0,10
9	1000	0,21	0,21	0,11	0,11
10		0,09	0,09	0,05	0,05
11		0,09	0,09	0,05	0,05
12		0,10	0,10	0,05	0,05

TABLE 4. Amounts of Aflatoxins in Seeds Stored for 3 Months in a MZhK

Sample No.	Depth of sampling, cm	Open method				Closed method			
		Amount of aflatoxins, μg/kg							
		B_1	C_1	B_2	C_2	B_1	C_1	B_2	C_2
1	20	0,85	0,85	0,42	0,42	2,50	1,20	1,25	0,60
2		—	0,15	—	0,07	1,90	1,90	0,95	0,95
3		1,23	0,09	0,61	0,05	1,13	1,85	—	—
4		0,57	0,57	0,50	0,28	3,00	1,50	0,56	0,93
5		3,00	3,00	1,50	1,50	2,55	1,28	0,72	—
6		0,78	0,38	0,19	0,19	0,81	—	—	—
7	50	0,19	—	—	—	0,50	0,50	0,25	0,25
8		0,73	0,55	0,35	0,27	0,19	0,19	0,38	0,09
9		0,21	0,21	0,22	0,11	2,40	2,40	1,20	1,20
10		1,10	—	—	—	1,90	1,35	1,40	1,40
11		1,35	0,77	0,48	0,38	0,70	0,90	0,50	0,50
12	1000	0,15	0,15	0.07	0.07	—	—	—	—
13		0,15	0,15	0.07	0.07	0.20	—	—	—
14		0,30	0,30	0.15	0.30	0.20	—	—	—
15		0,15	0,15	0.07	0.07	0.15	—	—	—
16		0,14	0,14	0.07	0.07	—	—	—	—

We have previously [6] reported the results of the determination of the amount of aflatoxins in cottonseed meal and some samples of protein obtained from it. In the present paper we give the results of a study of a possible mechanism of the contamination of cottonseed meal with the products of the vital activity of Aspergillus flavus fungi.

Cotton seeds before sowing, samples of soil, 15- and 50-day cotton-plant shoots (epigeal part), the seeds of a ripe crop, and samples of crushed seeds, meal, and refined oil were investigated. Samples were taken from control sections of the experimental fields of the Institute of the Chemistry of Plant Substances in the Kommunizm kholkhoz [State farm] and in the Tashkent oils and fats combine (MZhK) in 1985.

TABLE 5. Amounts of Aflatoxins in the Processing Products of Cotton Seeds

Amount of aflatoxins, μg/kg																	
in the crushed seeds				in the seed husks				in the meal from the extraction shop				in the meal from the loading station				in the refined oil			
B_1	C_1	B_2	C_2	B_1	C_1	B_2	C_2	B_1	C_1	B_2	C_2	B_1	C_1	B_2	C_2	B_1	C_1	B_2	C_2
2,08	2,08	1,04	1,04	0,62	0,40	0,31	0,31	0,91	0,90	0,85	0,85	1,80	1,80	1,73	0,95	0,04	0,04	0,02	0,02
0,69	0,69	0,35	—	0,30	0,30	0,35	0,35	0,28	0,24	0,10	0,10	0,50	0,50	0,30	0,30	0,04	0,04	0,02	0,02
1,90	1,90	0,45	0,45	1,00	1,00	0,72	0,53	0,81	0,80	0,40	0,40	1,74	1,38	0,36	0,36	0,04	0,04	0,02	0,02
1,95	0,95	0,50	0,50	0,92	0,45	0,92	0,20	0,69	0,78	0,50	0,50	0,90	1,50	1,00	1,00	0,04	0,04	0,02	0,02
3,15	3,15	2,07	2,07	0,80	0,40	0,50	0,50	2,00	2,17	1,54	1,54	5,02	5,02	3,00	3,00	0,04	0,04	0,02	0,02

In none of the samples investigated did the level of aflatoxins exceed the maximum acceptable concentration (MAC) laid down, which agrees with results obtained by Kyuz [7, 8]. The MAC for aflatoxin B_1 (the most toxic variety [9]) corresponds to 5 μg/kg for all food products [10].

No aflatoxins were detected in seeds taken for analysis before sowing, which can apparently be explained by their treatment with fungicidal preparations.

It is also interesting to note that samples of soil taken in November contained no aflatoxins, although they were detected in the preceding months (Table 1). This is possibly connected with the irrigation period [11].

Table 2 gives the results of a study of 50 samples of the epigeal part of the cotton plant.

With the growth of the plant, the concentration of aflatoxins in it fell. While in the epigeal part of 15-day shoots the concentrations of aflatoxins B_1, B_2, C_1, and C_2 were comparatively high, no aflatoxins were detected in the mature plant (leaves, stems, unopened bolls, fibers, seeds). The variation in the amounts of aflatoxin at the end of the vegetation of the cotton plant is connected with the nature of the vital activity of the Aspergillus fungi [11], i.e., conditions not favorable for the development of these fungi inhibit their vital activity.

Thus, aflatoxins pass from the soil into the plant and then with the growth of the plant their amount falls and ripe cotton seeds contain none of them.

Cotton seeds after harvesting are stored for an average of 3 months at the collecting station and the cotton-ginning factory, and are then transferred to MZhK. We analyzed seeds stored in the MZhK by the open method – in bales – and found that aflatoxins had accumulated in small amounts mainly in the upper layers (Table 3).

With an increase in the time of storage to 3 months there was a vigorous development of the Aspergillus flavus fungi and, correspondingly, of their metabolites, aflatoxins (Table 4), the level of contamination of the seeds stored by the open and closed methods being practically the same, which, in all probability, is explained by the use of active ventilation on storage.

We simultaneously investigated the products of the processing of cotton seeds (Table 5).

The amount of aflatoxins in the crushed seeds ranged from 0.69 to 2.08 μg/kg for B_1 and C_1 and from 0.35 to 1.04 μg/kg for B_2 and C_2, while in the meal from the extraction shops the amount of aflatoxins had fallen under the action of the heat treatment and the gasoline extraction of the initial product. However, in samples kept in a store and intended for the working up of the meal, the amount of aflatoxins had increased, i.e., there was secondary contamination with aflatoxins. Some of the aflatoxins passed in the micellae, and in the final product – the refined oil – they were found at levels of 0.04 μg/kg for B_1 and C_1 and 0.02 μg/kg for B_2 and C_2, which are considerably below the MAC.

Thus, freshly gathered seeds contain no aflatoxins but subsequently, on processing in the cotton-ginning factories, they undergo invasion by the Aspergillus fungi the development of which depends on the conditions and nature of storage. The aflatoxins detected in the cotton seeds and meal after preliminary storage are the result of secondary contamination.

EXPERIMENTAL

The amounts of aflatoxins B_1, C_1, B_2, and C_2 in the soil were determined by the method of [10] with some modifications. A sample (100 g) of soil that had been ground and passed through a sieve with apertures having a diameter of 1 mm was extracted with an 80% solution of acetone by shaking for 30 min, and the mixture was filtered. To 50 ml of the filtrate was added 25 ml of a 15% solution of lead acetate, and the resulting precipitate was filtered off. Of this filtrate, 80 ml was transferred to a separatory funnel, and 40 ml of hexane was added, after which the aqueous acetone layer was separated off. The aflatoxins were extracted from the aqueous acetone solution successively with 40 ml of chloroform and with chloroform–acetone (3:1). The combined extracts were dried with sodium sulfate and evaporated in a rotary evaporator in vacuum to dryness at a temperature not exceeding 60°C. The residue was dissolved in 0.1 ml of chloroform and the solution was analyzed with the aid of two-dimensional TLC: first direction, ether; second direction, chloroform–acetone (9:1).

The detection in UV light of spots corresponding in their chromatographic mobilities and the color of their fluorescence to the spots of aflatoxin standards showed the presence of aflatoxins in the samples investigated. Confirmatory tests were carried out by spraying the TLC plates with a solution of nitric acid in water (1:2). The aflatoxins were determined quantitatively by matching corresponding dilutions, which made it possible to trap on the plate spots with vanishingly low fluorescence. According to the literature [12], such spots contain 0.004 μg/kg of B_1 and 0.0002 μg/kg of C_2, which enables the amount of aflatoxin in the sample to be calculated.

The amounts of aflatoxins B_1, C_1, B_2, and C_2 in cotton-plant shoots were determined as described above with additional purification, consisting in three treatments of the aqueous acetone extract with hexane to eliminate pigments.

The amounts of aflatoxins B_1, C_1, B_2, and C_2 in cotton seeds and in the meal obtained from them were determined after additional purification of the aqueous acetone extract from lipids on a chromatographic column containing silica gel.

Determination of the Amounts of Aflatoxins B_1, C_1, B_2, and C_2 in the Cottonseed Oil. The aflatoxins were extracted by a published method [13]. The extract of aflatoxins was analyzed by the method described above with additional chromatographic purification using hexane.

EXPERIMENTAL

The investigations have shown that the contamination of the cotton seeds with aflatoxin takes place mainly on storage in the cotton-ginning factory and the oils and fats combine (MZhK).

In the processing of cotton seeds, part of the aflatoxins passes into the final products and part decomposes. Meal stored in the MZhK undergoes secondary contamination with aflatoxins.

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