

EFFECT OF SUBSTRATE IN PRODUCING AFLATOXIN

The fungi that produce toxic substances were suspected ever since some mushrooms were found to be poisonous. Investigations of Blount (1961) on the cause of Turkey 'X' disease led to the discovery that strains of the fungus, *Aspergillus flavus* present in groundnut meal used as feed material were responsible for producing the toxic factor which was named as aflatoxin.

Production of aflatoxin in culture medium by isolates of *A. flavus* has been reported by Diener and Davis (1966), and Bilgrami (1985). Production of aflatoxin B₁ by *A. flavus* has been reported in groundnut oil cake by Balasubramonian (1985), in coconut, groundnut, and sesamum oil cakes by Niza (1981) while the production of aflatoxin B₁ and B₂ in groundnut oil cake was reported by Neelakantan *et al.* (1981).

In the present investigation, a study was undertaken to find the effect of substrate in producing aflatoxin. The isolates of *Aspergillus flavus* (20 nos.) and *A. niger* (19 nos.) obtained from oil cakes collected from different regions during different periods of the year were tested for their comparative efficiency to produce aflatoxins (B₁, B₂ and G₂) in culture medium and their respective host materials (Table 1).

Aflatoxin production in culture medium was estimated by the method of Diener and Davis (1966) using SMKY liquid medium. The toxin production in oil cakes was estimated following the

procedure of Pons *et al.* (1971). The quantitative estimation of aflatoxins was carried out spectrophotometrically (Anon., 1983).

Of the 20 isolates of *Aspergillus flavus*, 14 were found to produce aflatoxin. Out of the 14 toxigenic isolates, eight produced B₁, B₂ and G₂, two produced B₁ and B₂ and four produced B₁ alone. Out of the 19 isolates of *A. niger*, eight produced B₁ alone (Table 2). Sinha *et al.*, (1988) noted that all strains of *A. flavus* did not possess the capacity to elaborate aflatoxins. Production of aflatoxin by *A. niger* has been reported by Bilgrami (1985).

Of the *Aspergillus flavus* isolates from coconut oil cake, the isolates 7 (CO Ik), 5 (CO IIIb) and 2 (CO IIIa) produced maximum quantity of B₁, B₂ and G₂ respectively, both in the host material as well as in the culture medium. *Aspergillus flavus* isolates from groundnut oil cake, 10 (G IIb), 9 (G IIa) and 11 (G IIc) produced maximum quantity of B₁, B₂ and G₂ respectively in culture medium as well as in the substrate. In sesamum oil cake isolates, 20 (S IIIc) produced maximum quantity of B₁ whereas 16 (S IIb) produced both B₂ and G₂ in the substrate as well as in the culture medium (Table 3).

Of the *Aspergillus niger* isolates, 1 (CO IIIa) from coconut, 9 (G IIb) from groundnut and 12 (S IIa) from sesamum oil cake produced maximum quantity of aflatoxin B₁ in the substrate as well as in culture medium (Table 4).

Table 1. Isolates of fungi tested for aflatoxin production

<i>Asperillus flavus</i>		<i>Aspergillus niger</i>	
Isolate No.	Code	Isolate No.	Code
1	CO IIa	1	CO IIIa
2	CO IIIb	2	CO Ib
3	CO Ib	3	CO IIb
4	CO IIb	4	CO IIIb
5	CO IIIb	5	CO Ic
6	CO Ic	6	CO IIc
7	CO IIc	7	CO IIIc
8	CO IIIc	8	G IIa
9	G IIa	9	G IIb
10	G IIb	10	G IIc
11	G IIc	11	S Ia
12	S Ia	12	S IIa
13	S IIa	13	S IIIa
14	S IIIa	14	S Ib
15	S Ib	15	S IIb
16	S IIb	16	S IIIb
17	S IIIb	17	S Ic
18	S Ic	18	S IIc
19	S IIc	19	S IIIc
20	S IIIc		

I = Southern region; CO = Coconut; II = Central region; G = Groundnut; III = Northern region; S = Sesamum; a = February-March; b = June-July; c = October-November

Table 2. Production of aflatoxins by species of *Aspergillus* isolated from oil cakes

Sl. No.	Fungus	No. of isolates	No. of toxigenic isolates	% of toxigenic isolates	Aflatoxins produced
1	<i>Aspergillus flavus</i>	20	14	70.00	B ₁ , B ₂ and G ₂ B ₁ and B ₂ B ₁
			8	57.14	
			2	14.29	
			4	28.57	
2	<i>Aspergillus niger</i>	19	8	42.10	B ₁

Table 3. Production of aflatoxins by isolates of *Aspergillus flavus*

Sl. No.	Isolate		Quantity of aflatoxin (ppb)					
	No.	Code	B ₁		B ₂		B ₃	
			C	O	C	O	C	O
1	2	CO IIIa	413	660	302	315	151	272
2	4	CO IIb	214	900	200	385	100	110
3	5	CO IIIb	231	757	1040	1092	85	98
4	6	CO Ic	242	1044	-	-	-	-
5	7	CO IIc	1210	1517	-	-	-	-
6	8	CO IIIc	434	1085	215	398	-	-
7	9	G IIa	185	255	467	1011	63	115
8	10	G IIb	960	1210	344	310	51	96
9	11	G IIc	410	1011	235	510	145	211
10	14	S IIIa	195	896	-	-	-	-
11	15	S Ib	208	285	408	422	43	76
12	16	S IIb	307	622	940	577	68	110
13	17	S IHb	185	495	213	278	-	-
14	20	S IIIc	502	948	-	-	-	-

Table 4. Production of aflatoxin by isolates of *A. niger*

Sl. No.	Isolate		Quantity of aflatoxin B ₁ (ppb)	
	No.	Code	Culture	Oil cake
1	1	CO IIIa	210	419
1	2	CO Ib	170	95
3	4	CO IIIb	101	210
4	6	CO IIc	122	335
5	9	G IIb	110	238
6	12	S Ha	222	246
7	13	S IIIa	93	138
8	17	S Ic	112	198

In general, host materials were found to be preferred substrates for the production of aflatoxin.

Among the *Aspergillus flavus* and *A. niger* isolates from different oil cakes, the isolates from coconut oil cake produced maximum quantity of all the three aflatoxins. Goldblatt (1969) stated that high carbohydrate substrate supported luxuriant growth of *A. flavus* with consequent production of large quantities of aflatoxin. In our studies also it was revealed that coconut oil cake contained higher carbohydrate than groundnut and

sesamum oil cake.

The present investigation further revealed that the isolates which produced maximum quantity of aflatoxin in culture medium could also produce similarly in their host material, thereby indicating their inherent ability for aflatoxin production.

The senior author is thankful to the Kerala Agricultural University for providing the facilities and the ICAR for awarding the senior fellowship.

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REFERENCES

- Anonymous, 1983. *A Manual of Laboratory Techniques*. (Raghuramulu, N. et al., Eds.), National Institute of Nutrition, Indian Council of Medical Research, Jamai-Osmania, Hyderabad, India, p. 235
- Balasubramonian, T. 1985. Incidence of aflatoxin B₁ in animal feeds. *Indian Vet. J.* 62 : 982-988
- Bilgrami, K.S. 1985. Mycotoxins in dry fruits and spices. Narendra Publishing House, New Delhi, p. 80
- Blount, W.P. 1961. Turkey 'X' disease. *Turkeys* 9 : 52-57
- Diener, U.L. and Davis, N.D. 1966. Aflatoxin production by isolates of *Aspergillus flavus*. *Phytopathology* 56 : 1290-1393
- Neelakantan, S., Balasubramonian, T., Jasmine, G.I. and Balasraswathi, R. 1981. Presence of aflatoxins in foods and feeds available in Tamil Nadu Region. *Madras agric. J.* 68 : 189-95
- Niza, T.J.R. 1981. Microbial deterioration of copra. M.Sc.(Ag.) thesis, Kerala Agricultural University, Trichur, p. 103
- Pons, W.A.J., Cocullu, A.F. and Lee, L.S. 1971. Determination of aflatoxin in mixed feeds. *Proc. 3rd Int. Congr. Food Sci. Technol.* p.705-709
- Sinha, A.K., Bilgrami, K.S., Ranjan, K.S. and Prasad, T. 1988. Incidence of aflatoxins in mustard crop in Bihar. *Indian Phytopath.* 41 : 434-437