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FORMATION OF AFLATOXINS BY SOME LOCAL AFLATOXIGENIC ISOLATES OF *ASPERGILLUS FLAVUS*

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Six local aflatoxigenic isolates of Aspergillus flavus isolated from smoked herrings and raisins were tested for the quantitative formation of aflatoxins in two synthetic liquid medium at different incubation times. All the isolates produced higher amounts of aflatoxins in rice powder-corn steep (RC) medium in comparison with the amounts produced in Czapek-yeast extract liquid (CY) medium. The maximum yields of aflatoxins were obtained at the 6th day of incubation at 28°C and did not coincide with the maximum fungal growth. The amounts of aflatoxins produced in CY by using shake cultures were higher than those produced in the same medium but by static cultures. A.flavus isolate No.170 (from raisins) proved to be the highest aflatoxins producer followed by A. flavus No. 51 which was isolated from smoked fish.

Keywords : *Aspergillus flavus; Smoked herrings; Raisins; Rice-corn steep liquor; Czapek-yeast extract*

Introduction

Smoked fish products and dried fruits are susceptible to be contaminated by different species of moulds including *Aspergillus flavus* group. Some species of these moulds are major cause of spoilage and others are able to produce specific mycotoxins in these food products.

Of these mycotoxins, aflatoxins which are toxic metabolites of the fungi *Aspergillus flavus* Link: Fr. and *A. parasiticus* Spear, are potent carcinogens possessing serious health hazards to humans and domestic animals (Diener et al., 1987). Increased attention has been paid during the last and present decade to aflatoxins as environmental chemicals that find their way into feed and foodstuff. The amount and type of aflatoxins produced depend on many factors, most important of which are media used, substrate (the composition of the food), mould strains, time of incubation and environmental conditions (Bullerman et al., 1984; El-Bazza et al., 1991; Pitt, 1993).

The main aim of the present work was to investigate the amount and type of aflatoxins produced by 6 local aflatoxigenic isolates of *A. flavus* (isolated from smoked herrings and raisins) when grown on two synthetic liquid media in relation to different incubation periods at 28°C.

Materials and Methods

Organisms

Six local aflatoxigenic isolates of *A. flavus* (*A. flavus* No. 1, 51, 108 from smoked herrings and *A. flavus* No. 170, 184 and 195 from raisins) which were isolated from samples purchased at random from different shops, supermarkets and groceries at various parts of Cairo were used in these studies.

Media used

1- Czapek-yeast extract (CY) (g/l) : Yeast extract 5; sucrose 30; sodium nitrate 3; magnesium sulphate 0.5; potassium chloride 0.5; ferrous sulphate 0.01; dipotassium hydrogen phosphate 1.0 and chloramphenicol 100 mg (Pitt and Hocking, 1985).
2- Rice-corn steep liquor (RC): 5% rice powder + 4% corn steep liquor (Bullerman, 1974).
3-Potato-dextrose agar (PDA): Potato extract 4 g; dextrose 20 g and agar 15 g (Oxoid, 1982).

Cultivation

The cultures were grown in 250 ml erlenmeyer flasks, each containing 50 ml. For RC medium, 2.5 g powdered rice were introduced into each flask, followed by 50 ml 4% corn steep liquor. Sterilization was carried out for 15 minutes at 1.5 psi. Shaking cultures were not used for RC medium because of the difficulty of separating mycelial pellets from rice powder in the medium.

Transfers were made from subcultures on PDA slants to PDA plates, the plates were incubated for 7 days at 28°C. Two discs, each of 1 cm diameter were cut

from the 7- day -old culture plates and used for inoculating each flask. After incubation for the required time at 28°C, the liquid was filtered off and the pH was measured. For each treatment, the mycelial dry weight was determined.

Mycelial dry weight was determined after Saito et al. (1971), followed by filtration through anhydrous sodium sulphate. Extraction of aflatoxins from culture filtrates was carried out according to the techniques described by Bullerman (1974). The extracts were concentrated under vacuum. Aflatoxins were separated by thin layer chromatography on silica gel

Table 1. Formation of aflatoxins by 3 isolates of *A. flavus* from smoked herrings using tow nutrient media at different incubation periods

Isolate No.	Culture medium	Culture condition	Incubation period (days)	Final pH	Dry wt. (g/50ml)	Aflatoxins (µg/50ml)						Total aflatoxins
						Culture filtrate			Mycelium			
						B	G	Total	B	G	Total	
1	RC	Static	3	3.5	1.15	10.1	3.7	13.8	21.5	16.1	37.6	51.4
			6	3.7	1.20	22.9	7.4	30.3	17.5	14.3	31.8	62.1
			12	4.2	2.50	17.3	11.1	28.4	17.1	14.3	31.4	59.8
	Cy	Static	3	5.2	0.81	4.5	2.3	6.8	2.4	2.4	4.8	11.6
			6	4.4	1.22	5.7	4.3	10.0	4.6	4.6	9.2	19.9
			12	3.4	1.55	2.9	2.6	5.5	2.3	2.3	4.6	10.4
		Shake	3	5.0	0.85	7.9	4.9	12.8	5.1	3.9	9.0	21.8
			6	4.6	1.32	10.7	4.8	15.5	7.9	5.4	13.3	28.8
			12	4.5	1.58	4.3	3.6	7.9	3.5	2.8	6.3	14.2
51	RC	Static	3	3.2	0.99	12.8	8.1	20.9	28.6	7.1	35.7	56.6
			6	3.5	1.67	22.1	10.7	38.8	38.2	10.1	48.3	81.1
			12	5.0	3.04	12.9	4.0	17.9	25.3	6.5	31.8	49.7
	Cy	Static	3	4.5	0.55	5.6	2.3	7.9	4.3	1.9	6.2	14.1
			6	4.1	0.76	8.0	3.6	11.6	5.6	2.3	7.9	19.5
			12	3.6	0.89	4.3	1.9	6.2	3.6	3.1	6.7	12.9
		Shake	3	5.0	0.51	6.5	3.2	9.7	6.0	2.3	8.3	18.0
			6	4.3	0.88	11.1	5.6	16.7	12.7	3.7	16.4	33.1
			12	4.2	0.92	6.3	2.6	8.9	5.6	2.1	7.7	16.6
105	RC	Static	3	3.5	0.95	7.4	8.0	15.4	13.3	11.7	25.0	40.7
			6	3.8	1.24	23.2	10.4	32.6	19.5	17.1	36.6	69.2
			12	5.2	4.88	6.7	2.6	9.3	13.2	15.2	28.4	37.7
	Cy	Static	3	4.1	1.04	4.6	4.1	8.7	5.0	2.2	7.2	15.9
			6	3.6	1.48	6.8	5.8	12.6	6.1	3.5	9.6	22.2
			12	3.5	1.65	4.7	3.1	7.8	3.2	1.6	4.8	12.2
		Shake	3	4.4	1.03	6.6	4.8	11.4	5.0	3.1	8.1	19.5
			6	4.1	1.56	10.8	5.1	15.9	10.6	4.2	14.8	30.7
			12	3.8	1.66	5.7	2.3	8.0	4.6	1.5	6.1	14.1

RC = Rice-corn steep liquor Cy = Czapek-yeast extract Intial pH of RC = 4.2 and Cy =6.4

desiccation at 100°C for 24 hours (Walbeek et al., 1969). Corn steep liquor containing 45% dry substances or total solid were supplied by Egyptian Company for Industrial Starch and Glucose.

Aflatoxins analyses

Aflatoxins in the mycelia and culture filtrates were determined separately. Aflatoxins were extracted from mycelia with chloroform according to the method of G, according to the method of Nabney and Nesbitt (1965) using the solvent system diethyl ether for purification, followed by 2% methanol in chloroform. Individual aflatoxin band was eluted with methanol and estimated by spectrophotometer, using the extinction coefficients reported by Nabney and Nesbitt (1965). Aflatoxins B_1 and B_2 were estimated as aflatoxin B and aflatoxins G_1 and G_2 as aflatoxin G. Aflatoxin standards: Aflatoxins B_1, B_2, G_1 and G_2 standards were provided by Sigma Chemical Company, England.

Results and Discussion

Aspergillus flavus can grow and produce aflatoxins on a wide variety of synthetic medium. Aflatoxins production is known to depend upon the strain of the organism and many factors, the most important of which are medium and time of incubation.

It appeared from Tables 1 and 2 that the maximum amount of aflatoxins was produced at the 6th day of incubation in all cases on both media. Other investigators obtained maximum amounts of aflatoxins by *A. flavus* after 5-8 days of incubation (Davis et al., 1966; Shotwell et al., 1966; Gunasekaran, 1980). On the other hand, the maximum mycelial dry weight, as a function of fungal growth, was reached after 12 days of incubation indicating that the highest levels of aflatoxins did not coincide with the maximum mycelial growth. Similar results have been shown by Hayes et al. (1966) and Mabrouk and El-Shayeb (1981). RC medium proved to be the best and suitable for aflatoxins production

Table 2. Formation of aflatoxins by 3 isolates of *A. flavus* from raisins using tow nutrient media at different incubation periods

Isolate No.	Culture medium	Culture condition	Incubation period (days)	Final pH	Dry wt. (g/50ml)	Aflatoxins (µg/50ml)						Total aflatoxins
						Culture filtrate			Mycelium			
						B	G	Total	B	G	Total	
170	RC	Static	3	3.4	1.25	10.9	9.5	20.4	29.6	23.3	52.9	73.3
			6	3.6	1.40	20.8	7.2	28.0	38.5	25.0	63.5	91.5
			12	4.5	2.88	12.0	11.5	23.5	29.0	22.7	51.7	75.2
	Cy	Static	3	5.2	1.05	5.6	4.2	9.8	4.3	3.3	6.7	16.5
			6	4.8	1.54	9.2	5.1	14.3	8.6	5.8	13.9	28.2
			12	3.6	1.63	5.3	3.1	8.4	4.3	1.8	6.2	14.5
		Shake	3	5.0	1.03	7.8	5.2	13.0	6.8	4.6	11.4	24.4
			6	4.6	1.45	11.1	6.7	17.8	10.1	5.3	15.4	33.2
			12	4.6	1.65	4.3	2.9	7.2	5.3	2.6	4.9	15.1
184	RC	Static	3	3.3	1.13	14.3	12.6	26.9	4.1	5.0	9.1	36.0
			6	4.2	1.34	15.0	19.5	34.5	8.2	16.5	24.6	59.1
			12	5.8	5.00	8.7	4.8	13.5	3.9	4.9	8.7	22.3
	Cy	Static	3	4.8	0.59	6.1	3.6	9.7	5.3	3.7	9.0	18.7
			6	4.4	1.82	8.3	4.7	13.1	7.8	4.4	12.7	25.8
			12	3.5	1.88	3.2	3.1	6.3	4.0	2.1	6.1	12.4
		Shake	3	4.6	0.85	8.3	5.3	13.6	7.8	4.7	12.5	26.1
			6	4.5	1.45	10.3	5.8	16.1	9.3	5.6	14.9	31.0
			12	3.6	1.56	5.2	3.7	8.9	4.2	2.7	6.9	15.8
195	RC	Static	3	4.2	1.72	16.7	0.0	16.7	21.5	0.0	21.5	38.2
			6	5.0	2.03	32.9	0.0	32.9	26.7	0.0	26.7	59.6
			12	6.1	2.88	10.8	0.0	10.8	16.5	0.0	16.5	27.4
	Cy	Static	3	3.5	1.05	5.4	3.6	9.0	3.6	3.6	7.2	16.3
			6	4.2	1.48	7.3	4.8	12.1	5.2	4.3	9.5	21.7
			12	3.6	1.62	4.2	2.0	6.2	2.3	1.1	3.4	9.6
		Shake	3	5.6	1.08	6.8	4.3	11.1	4.1	3.8	7.9	19.0
			6	4.5	1.42	10.2	5.3	15.5	5.3	5.1	10.4	25.9
			12	3.5	1.57	4.3	2.3	6.6	2.6	1.0	3.7	10.3

RC = Rice-corn steep liquor Cy = Czapek-yeast extract Intial pH of RC = 4.2 and Cy = 6.4

by all the tested *A. flavus* isolates. The suitability of corn steep liquor (in static culture) for aflatoxin production has been shown by Shotwell et al. (1966), Gupta et al. (1971), Bullerman (1974) and Mabrouk and El-Shayeb (1981). The amounts of aflatoxins produced by CY extract medium in shake cultures were higher than that produced in the same medium by using static culture. Payne and Hagler (1983) found that in shake cultures, but not in stationary cultures, increased growth was generally associated with increased aflatoxins production by *A. parasiticus* and *A. flavus*.

With the exception of *A. flavus* isolate No. 184, all the other isolates under investigation, i.e. No. 1, 51, 108, 170 and 195 which were grown in RC medium produced higher amounts of aflatoxins in mycelia extract than that in the culture filtrates. On the contrary, these 5 *A. flavus* isolates produced higher amounts of aflatoxins in culture filtrates when grown on CY medium. In this concern, Mabrouk and El-Shayeb (1981) found that the total aflatoxin content of *A. flavus* mycelia highly exceeded that of culture filtrates. Generally, more aflatoxins B were produced in both culture filtrates and mycelia with all the tested *A. flavus* isolates, except isolate No. 184 which showed more aflatoxin G in RC medium. The highest amount of total aflatoxins, 91.5 µg/flask was produced by *A. flavus* isolate No. 170 (isolated from raisins) in RC medium followed by *A. flavus* isolate No. 51 (isolated from smoked herrings) which produced 81.1 µg/flask in the same medium. This indicates that aflatoxin production depends upon the *A. flavus* isolate. Schindler et al. (1967) and Sharma et al. (1980) demonstrated that aflatoxins formation depend on the strain of *A. flavus*.

By following the change of the initial unbuffered pH (6.4 for CY medium and 4.2 for RC medium), it can be seen that it remained acidic during all periods of incubation. The final pH value of the medium was approximately 3.2-6.1 in case of RC medium, whereas it decreased slightly to about 3.4-5.6 in case of CY medium.

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