STUDY ON AFLATOXINS IN SOME FOODSTUFFS WITH SPECIAL REFERENCE TO PUBLIC HEALTH HAZARD IN EGYPT

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Summary

A total of 455 food samples representing 22 different food types were collected from several localities at Alexandria province in Egypt. Aflatoxin B1 and aflatoxin M1 were detected in 5 out of 455 (1.1%) of these food samples. From the same samples 206 fungal isolates were obtained. Thirty two of these isolates (15.5%) were found to be aflatoxin producers. *Aspergillus flavus* was the dominant isolate, while *Aspergillus parasiticus* was also isolated from a few other food samples. Among locally consumed foodstuffs. Peanut (7.5%) and Milk powder (6.6%) were found to be a suitable substrates for aflatoxin production. The hygienic and public health significance of the isolated aflatoxigenic strains were discussed.

(Key Words : Aflatoxin, *Aspergillus flavus*, *Aspergillus parasiticus*, Foodstuffs)

Introduction

Aflatoxins, a group of toxic metabolites produced by certain *Aspergillus species*, have been found to be carcinogenic, teratogenic and mutagenic to several species of experimental animals (Butler and Barnes, 1968; Gopalan, et al., 1972 and Adamson, et al., 1973). A total of four metabolites have been designated according to their fluorescent colours and chromatographic positions into aflatoxin B1, B2, G1 and G2.

Aflatoxins occur in a variety of crops and animal products such as meat, milk and eggs. The production of aflatoxins is affected by several factors which influence the mould growth such as moisture contents, relative humidity (RH), temperature, substrate composition and the presence of competing microorganisms (Ciegler, 1977). The common occurrence of aflatoxins in foodstuffs and their possible role in the incidence of liver cancer cause world wide concern and research in many countries.

The aim of this study was to investigate, detect and evaluate the presence of aflatoxins and aflatoxigenic fungi in some Egyptian foodstuffs marketed at Alexandria Province with special reference to its public health hazards, in Egypt country.

Materials and Methods

A total of 455 food samples were collected from different localities at Alexandria Province in Egypt. The samples included wheat (*Triticum aestivum*), barley (*Hordeum sativum*), corn (*zea mays*), crushed wheat (burghol), peanut, rice, broad beans (*Vicia faba*), hazelnuts, chickpeas (chicken feed), sesame seeds (sesame paste), milk powder, cowpeas, peas, vetch, dried figs, pine nut, liquorice (*urk-us-sus*), fennel seeds (*trigonella faenum-graecum*), spices, lentil and lupine. Each samples was divided into two parts, one for extraction of aflatoxins and the other for isolation of aflatoxigenic fungi.

Analysis of food samples for detection of aflatoxin :

Aflatoxins were extracted from the food samples according to either AOAC method (1975) or method of Seitz and Mohr (1977). The latter method was used for extracting only from samples such as corn (*zea mays*) and was found to be quicker, easier and less expensive than the first method. Thin layer chromatography of aflatoxins and samples extracts were performed on silicagel DG 254. The plates were developed first with diethyl ether and then with chloroform : acetone (9:1 v/v). Aflatoxins were identified on the basis of co-migration with aflatoxin standards (Fluka) and by their characteristic fluorescent colour under long Ultra Violet (UV) illumination at 360 nm.

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The fluorescent spots of aflatoxins were scraped off the TLC and eluted by chloroform:methanol (9:1 v/v). The solvent was evaporated under nitrogen to dryness and the residue was dissolved in methanol. The concentration of aflatoxins (B1, B2, G1, G2 and M1) in solution was determined by measuring its absorbance at 360 nm then calculated according to the method of Masri, et al. (1969).

Confirmatory tests for aflatoxin :

Three different derivatives were prepared by treating portions of the isolated toxin or the aflatoxin standard with formic acid thionyl chloride, acetic acid-thionyl chloride and tri-fluoroacetic acid. The test was then continued according to the method of Stoloff (1967).

Isolation and culturing of fungi :

Attempts to isolate fungi from the food samples were made aseptically on CZapek-Dox agar plates. The plates were incubated at $28 \pm 1^\circ\text{C}$ for 3-5 days. Suspected fungal colonies were transferred to CZapek slants and incubated for one week at $28 \pm 1^\circ\text{C}$ until sporulation. Identification of the fungal isolates obtained was confirmed by the Common Wealth Mycological Institute, Kew, England. Aflatoxigenic fungi were mostly found to be of the *A. flavus* and few of them were *A. parasiticus*.

Two aflatoxin-producing liquid media were employed to investigate the potential of isolated fungi to produce aflatoxins. One media was recommended by Reddy, et al. (1971), while the other was developed by Salhab and Edwards (1978). The media were inoculated by ($\text{Ca. } 10^6$) spores and incubated in a closed rotary shaker and shaken at 100-150 r.p.m. for 3-8 days. By the end of the incubation period an equal volume of chloroform was added and the aflatoxins were extracted according to the method of Salhab and Edwards (1978).

Results and Discussion

Out of 455 food samples of 22 different types, only 5 (1.1%) were found to contain aflatoxins. The aflatoxin contaminated samples included: peanut, rice and milk powder (table 1). The highest incidence of contamination with aflatoxin was found to occur in peanut (7.5%) while the incidence of contamination in rice and milk powder were (2.0%) and (6.6%), respectively, shown in table 1. No aflatoxins were detected in the other 19 samples. Aflatoxin B1 levels in the peanut samples varied from 98 to 1056 p.p.b., and was 28 p.p.b. in rice. Aflatoxin M1 in milk powder was 15 p.p.b. (table 2).

The data obtained through this preliminary study indicated the presence of aflatoxin in only 1.1% of the

TABLE 1. INCIDENCE OF AFLATOXINS IN SOME FOOD SAMPLES AT ALEXANDRIA PROVINCE IN EGYPT

Sample Type	Total samples examined	Total samples contaminated with aflatoxin	%
Peanut	40	3	7.5
Rice	50	1	2.0
Wheat (Triticum aestivum)	50	0	0.0
Barley (Hordeum sativum)	50	0	0.0
Milk powder	15	1	6.6
Corn (Zea mays)	50	0	0.0
Broad beans (Vicia faba)	40	0	0.0
Vetch	10	0	0.0
Lupine	10	0	0.0
Crushed wheat (Burghol)	10	0	0.0
Lentil	15	0	0.0
Sesame seeds	10	0	0.0
Chicken feed	10	0	0.0
Ferngreek seeds (T.F.G.)	15	0	0.0
Chick peas (Chicken feed)	10	0	0.0
Nuts	15	0	0.0
Dried figs	10	0	0.0
Green pea	15	0	0.0
Sesame paste	5	0	0.0
Cowpea	15	0	0.0
Pine nut	5	0	0.0
Liquorice (Urk-us-sus)	5	0	0.0
Total	455	5	1.1

TABLE 2. LEVELS OF AFLATOXIN IN CONTAMINATED FOOD SAMPLES IN EGYPT

Sample Type	Total sample contaminated	Country of origin	Main Toxin found	Levels of aflatoxins (p.p.b.)
Peanut	3	Egypt	B1	934
		Egypt	B1	1056
		Egypt	B1	98
Rice	1	Italy	B1	28
Milk powder	1	Denmark	M1	15

food samples tested. This incidence of aflatoxin contamination compared with the reported findings in other countries for similar studies is quite low. In Poland 8.7% of 150 samples examined yielded aflatoxins (Juszkiewicz and Piskoka, 1977). In Egypt, aflatoxins were found in 33% of 42 samples examined (Girgis, et al., 1977) and in New Zealand, 5.3% of 38 samples were aflatoxin contaminated (Staton, 1977). Although the incidence of aflatoxin in our foodstuffs was low the level of contamination varied from 15 to 1056 p.p.b. knowing that a level of 15 p.p.b. of aflatoxin B1 causes liver tumor in rat and rainbow trout (Lee, et al., 1971), levels reported in this study can be dangerous.

Aflatoxin incidence in peanut samples (7.5%) table 1 compared to that reported from Thailand and the Philippines (Shank, et al., 1972; Campbell and Stoloff, 1974) is quite low.

Out of the samples studied small grains were found to be a very poor source of aflatoxins. This observation is substantiated by studies in different countries (Shank, et al., 1972; Shotwell, et al., 1975). Moreover, all bean samples were found to be aflatoxin free. This finding is in

close agreement with other reported studies (Liewellyn and O' Rear, 1977; Gupta and Venkatasubramanian, 1975).

Although only 1.1% of the tested samples were found to be contaminated with aflatoxin, only 7.2% of these samples comprising 12 different foodstuffs were contaminated with aflatoxigenic fungi. Incidence of aflatoxigenic fungi in Egypt is close to the incidence rates reported from other countries, such as India (Rao, et al., 1965). Iraq (Al-Adil, et al., 1976) and Russia (L' vova, et al., 1978).

In the light of the aforementioned obtained results showed in table 3, the common types of aflatoxin produced were B1, B2 and G1 respectively. Out of 206 total isolates of aflatoxigenic fungi there are 32 toxigenic isolates with a contamination percentage of 15.5%. This result agree with Reddy, et al. (1971).

The data presented in this study (table 4) showed the levels of aflatoxins produced by aflatoxigenic isolates and by *Aspergillus parasiticus* from different feed samples. This results are in agreement with these obtained by Wogen and Newbrene (1967).

TABLE 3. INCIDENCE OF ISOLATED AFLATOXIGENIC FUNGI

Sample Type	Total sample	Total isolates	No. of Toxigenic Isolates	Contamination (%)	Aflatoxin produced
Peanut	40	17	5	12.5	B1, B2
Rice	50	23	3	6.0	B1
Wheat	50	21	3	6.0	B1
Barley	50	17	2	4.0	B1, B2, G1
Corn	40	18	4	10.0	B1, B2
Broad beans	40	23	6	15.0	B1, B2
Lupine	10	4	0	0.0	
Vetch	10	6	1	10.0	B1
Lentil	15	3	0	0.0	
Green pea	15	8	0	0.0	
Crushed wheat	10	7	1	10.0	B1
Dried figs	10	6	0	0.0	
Sesame seeds	10	6	0	0.0	
Ground pepper	20	12	2	10.0	B1
Spices	10	7	0	0.0	
Chicken feed	10	4	0	0.0	
Chick pea	10	2	0	0.0	
Cowpea	15	5	1	6.6	B1
Ferngreek seed	15	7	2	13.3	B2
hazelnuts	15	5	1	6.6	B1
Pine nut	5	5	1	20.0	B1
Urk-us-sus	5	0	0	0.0	
Total	455	206	32	15.53	

TABLE 4. TYPE AND LEVELS OF AFLATOXINS PRODUCED BY AFLATOXIGENIC ISOLATES AND *ASPERGILLUS PARASITICUS* FROM SOME FOOD SAMPLES, IN EGYPT

Food type	Aflatoxin produced	Levels of Aflatoxins ($\mu\text{g}/100$ ml of media) ($\mu\text{g}/\text{g}$ substrate)	
Peanut	B1	622	76
	B2	103	13
	G1	— ve	6
	G2	— ve	Trace
Rice	B1	821	100
	B2	— ve	7
	G1	— ve	11
	G2	— ve	Trace
Pine nut	B1	— ve	71
	B2	— ve	11
	G1	— ve	5
	G2	— ve	Trace
Liquorice	B1	— ve	0.95
Wheat	B1	512	— ve
Corn	B1	1004	— ve
	B2	918	— ve
Broad bean	B1	1315	— ve
Vetch	B1	918	— ve
Barley	B1	721	— ve
	B2	214	— ve
	G1	12	— ve
Crushed wheat (Burghol)	B1	115	— ve
Black pepper	B1	427	— ve
Cowpea	B1	1584	— ve
Ferngreek seeds	B2	215	— ve
Hazelnut	B1	284	— ve

From public health point of view, aflatoxin B1 is the most potent hepatocarcinogenic agent so far recognized (Wogen and Newbrene, 1967). It is suspected of being a primary cause of human cancer in certain areas in Africa, South China, Hawaii, India, Rumania and elsewhere (Newbrene and Rogers, 1973). In addition, numerous reports from different laboratories appeared recently in the literature concerning a possible role of aflatoxin B1 in the etiology of Reye's syndrome (Bourgoois, et al., 1969; Oslon, et al., 1971 and Reyan, et al., 1979). It is likely

that the earlier disease outbreak labelled as mouldy corn toxicosis and aspergillus toxicosis were mainly caused by aflatoxins in the feed.

For the prevention of aflatoxicosis in man, the following instructions, precautions and suggested recommended points must be taken into consideration which includes: Avoidance of contaminated feeds by monitoring batches for aflatoxin content is suggested. Young nearly weaned, pregnant and lactating animals require special protection from suspected toxic feeds. Consequently all food products which are spoilt through mould and yeast must be totally condemned. Moreover, mould and yeast must be considered as indication of food sanitary quality and hygienic status. This demands more strict hygienic measures on foodstuffs to minimize the risk of aflatoxins on the consumers.

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