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SURVEY OF CORN FOR AFLATOXIN CONTAMINATION IN SOUTH WEST PAKISTAN

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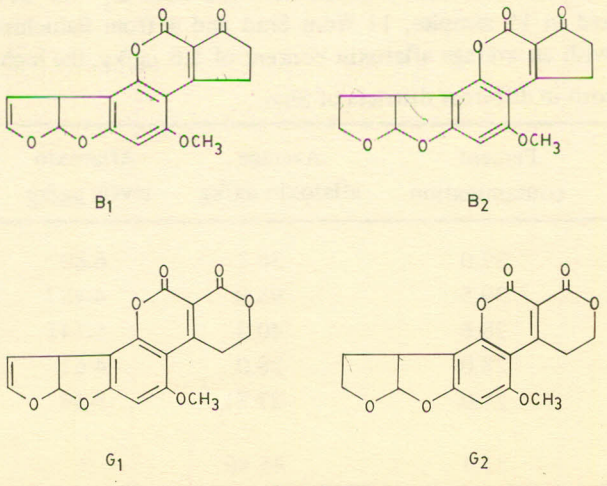
Two hundred and thirty-nine samples of corn collected from sixty locations in the Sind and Baluchistan provinces showed aflatoxin B_1 contamination in 28.8% of the samples analysed. Among the positive samples, 31 contained aflatoxin at less than 20 μg/kg, 28 between 21 to 100 μg/kg while only 8 samples had aflatoxin B_1 in excess of 100 μg/kg. The highest amount of aflatoxin B_1, 487 μg/kg, was found in a sample collected from Hyderabad city. The samples were analysed by the CB method as laid down in Association of Official Analytical Chemists and estimation was made by comparison with standards on thin-layer chromatography and confirmed by derivative formation, etc.

INTRODUCTION

Mushroom poisoning, due to the ingestion of *Amantia* spp., goes back to antiquity and ergot poisoning due to *Claviceps* spp. has been recorded through many centuries. Other toxic metabolites must have been present in our environment from times immemorial but there was no indication that saprophytic fungi could have been capable of toxin production.

Aflatoxins are naturally occurring environmental pollutants of biological origin, produced by certain ubiquitous fungi, viz. *Aspergillus flavus* and *A. parasiticus*, as secondary metabolites. Among the various aflatoxins, the most widely occurring is aflatoxin B_1 with the empirical formula of $C_{17}H_{12}O_6$, molecular weight of 312 and a melting point of about $268^\circ - 269^\circ$. The structures of the four major aflatoxins, which were determined in 1963 [1], are shown in Fig. 1.



B_1 B_2

G_1 G_2

The importance of aflatoxins was realized after an outbreak of poisoning of turkeys in Britain in 1960 in which 100,000 birds died after ingesting a diet containing contaminated peanut meal. The disease became known as "Turkey disease", since the aetiology was unknown [2]. Pheasant and partridge poults were also badly affected [3] along with calves and pigs [4]. It was demonstrated that fungus *A. flavus* was responsible for the production of these toxins, hence the name aflatoxin [5].

Hepatomas in epidemic form were simultaneously observed by US research workers in commercial hatcheries of the rainbow trout [6]. The source of aflatoxin in this case was mouldy cottonseed meal incorporated in the trout feed. Aflatoxin can be produced in most of the agricultural commodities in various amounts depending upon envrionmental factors such as temperature and humidity which play an important role in its bioproduction. *A. flavus* can produce aflatoxins on rice over temperatures ranging from 11° to 37°C [7], however, the problem can be acute when cereal grains are stored in humid, warm conditions such as experienced in tropical and sub-tropical regions.

Association of *A. flavus* with corn was established some 83 years ago [8]. Soon after the discovery of aflatoxins, a major part of literature was published in the early and mid-seventies and the toxin was identified as a major problem in corn in United States. In 1977, it was reported that 90% corn in Georgia had aflatoxin in excess of 20 μg/kg. [9]. It was further observed that 90% corn grown in southeastern USA was contaminated with aflatoxin and in numerous samples the amount exceed 1000 μg/kg [10]. Corn is among several agricultural commodities like cottonseed, peanut and other nuts which are highly susceptible to aflatoxin production.

A preliminary survey was carried out on various agricultural commodities in and around Karachi to detect the levels of aflatoxin contamination. Corn was one of the several grains which were found contaminated with aflatoxin. Authors have previously shown that *A. flavus* was the most predominant fungus on most of the stored grains studied (11 – 13). In view of previous findings, a survey of corn was conducted in different districts of Sind and Baluchistan. Corn samples of all grades were randomly collected from wholesale markets, shops and houses in big cities along with small towns, villages and fields. Corn is stored in different ways after sun drying. In villages mud bins are used for the storage of grains and unshelled corn is stored in heaps. In cities corn is stored in house-type godowns and stacked jute bags are placed on wooden or cemented platforms. It is harvested immediately after the monsoons and occasionally stored with high moisture content which results in *A. flavus* proliferation and subsequent production of aflatoxins. Often grains remain on cobs for several days before being shelled by hand, dried and stored. Delayed shelling and drying results in the production of aflatoxins in grains still on cobs. These secondary metabolities, which are carcinogenic, mutagenic and teratogenic, have been found, on assaying, in significant quantities in the corn collected from various areas of these provinces. Aflatoxin B_1 was found in all positive samples while aflatoxins B_2, G_1, G_2, were detected occasionally (Fig. 1).

MATERIALS AND METHOD

Since aflatoxin distribution in grains is heterogenous, representative samples of at least 2 kg corn were randomly collected. However, drawing of samples (amount) would depend on lot size in a godown, shiphold etc. Subsamples for analysis were prepared after passing through sample divider to reduce it to about 100 g. The subsample was grounded in a laboratory mill and 50 g. of the grounded sample were analysed according to the CB method of the American Association of Analytical Chemists [14]. Aflatoxin was determined through thin layer chromatography and quantification was achieved through comparison with aflatoxin standards and confirmation by derivation with trifluoroacetic acid and H_2SO_4 spray of TLC plates.

Fungi were isolated from corn samples on Czapek's Dox Agar Medium to determine the extent of infection by *A. flavus* and *A. parasiticus*. To screen out aflatoxigenic strains, isolates of *A. flavus* were inoculated on outoclaved corn with moisture content 18 to 20% and temperature $28^{o} \pm 2^{o}$ and a relative humidity around 85% for seven days.

RESULTS AND DISCUSSION

Out of the 239 samples collected from 60 locations from Sind and Baluchistan, 68 samples (28.8%) were aflatoxin positive. The number of aflatoxin contaminated samples was higher in Sind (32.3%) than in Baluchistan (23.85) (Tables 1 and 2). The average amount of aflatoxin present in the Sind samples was higher (45.46 μg/kg of B_1) than in the samples from Baluchistan (28.3 μg/kg B_1). 42 (33%) aflatoxin positive samples with an average content of 45.46 μg/kg of B_1 were detected out of 130 samples from 37 locations in five districts of Sind. The highest average (95 μg/kg) of AFB_1 was noted in samples from Hyderabad district and the lowest (27.81 μg/kg) in Tharparkar. The number of positive samples (39.5%) was also highest from Hyderabad district. Out of the 5 samples which had aflatoxin in proportion of more than 100 μg/kg, 4 were from Hyderabad district and the maximum amount of aflatoxin (487 μg/kg) in any one sample was also from this district.

So far as Baluchistan was concerned, samples were collected from 23 locations in five districts. 26 samples were contaminated with an average of 28.3 μg/kg of aflatoxin B_1. The number of positive samples (37.5%) was highest in Sibi district while the lowest number of contaminated samples (15.38%) was in Quetta district. Also the maximum amount of AFB_1 (271 μg/kg) was detected in samples from Sibi district. The aflatoxin G_1 was detected in 15 samples, 11 from Sind and 4 from Baluchistan with an average aflatoxin content of 5.6 μg/kg, the highest

Table 1: Occurrence of aflatoxin B_1 in corn in different districts of Sind

District	No. of collected samples	No. of aflatoxin positive samples	Percent contamination	Average aflatoxin μg/kg	Aflatoxin levels μg/kg
Nawabshah	27	10	37.0	36.2	6-89
Hyderabad	38	15	39.5	95.0	4-487
Badin	21	6	28.6	40.3	5-141
Sanghar	25	7	28.0	28.0	4-61
Tharparkar	19	4	21.0	27.81	5-54
	130	42	32.3	45.46	–

Table 2: Occurrence of **aflatoxin** B_1 in corn in different districts of Baluchistan

District	No. of collected samples	No. of aflatoxin positive samples	Percent contamination	Average aflatoxin μg/kg	Aflatoxin levels μg/kg.
Quetta	26	4	15.38	19.25	7-31
Lorallai	18	6	33.33	29.6	16-46
Pishin	15	3	20.00	21.0	5-39
Sibi	24	9	37.50	69.7	8-271
Fort Sandiman	26	4	15.40	23.0	12-51
	109	26	23.85	28.3	–

being 16 μg/kg. 7 samples, 5 from Sind and 2 from Baluchistan were found to contain aflatoxin B_2 while aflatoxin G_2 could only be detected in one sample collected from Hyderabad city.

Aspergillus flavus was the most dominant fungus associated with 59% corn kernels collected from different locations. *A. flavus* isolates were tested for their aflatoxigenic potential. Some *A. flavus* isolates from aflatoxin contaminated grains, on inoculation to sterilized corn, produced aflatoxins. However, others did not show any aflatoxin producing ability. Furthermore, few isolates of *A. flavus* from corn samples which did not have any aflatoxin contamination, demonstrated high potential for aflatoxin production. 48% isolates from positive samples were aflatoxigenic and some produced as high as 6.3 mg/kg of aflatoxin of the same autoclaved corn which was previously found free from aflatoxin. This demonstrates that under favourable conditions these *A. flavus* isolates could produce substantial amount of aflatoxins which could prove hazardous to human health and to animals as well. However, the mere presence of *A. Flavus* on grains does not necessarily indicate aflatoxin contamination.

The present survey, although not conducted on a comprehensive scale, clearly shows aflatoxin contamination problem in corn and the situation can further deteriorate if optimal conditions exist for the biosynthesis of this toxin as is evident from reported cases from South East Africa [15] and India [16].

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