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Studies on mycotoxin formation in different stored seeds

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Mycotoxin production in maize, groundnut and soyabean seeds stored in gunny bags for one year were estimated at three months interval. Six species of storage fungi commonly associated with stored seeds under natural conditions were grown in liquid culture and mycotoxin formation by them *in vitro* was also recorded.

The present study showed maize and groundnut seeds were very good substrate for mycotoxin formation by storage fungi.Aflatoxin B_1 was produced in higher quantities than ACT_1 in both seeds. Soybean seeds proved to be a poor substrate for mycotoxin production by any of the storage fungi tested under natural storage conditions. Environmental conditions also played important role in mycotoxin production.

Key words : Seeds, storage fungi, mycotoxin, Aflatoxin B_1, ACT_1

INTRODUCTION

Increasing interests are now being shown in mycotoxins in general in food and feedstuff, because of their carcinogenic properties (Christensen *et al.*, 1977) and the storage fungi were reported to be responsible for seed deterioration (Nandi *et al.*, 1988; Mukherjee *et al.*, 1992) under natural storage conditions. A number of workers suggested possible involvement of toxins, produced by the storage fungi in damaging the infected seeds (Harman and Nash, 1972; Lilliehoj *et al.*, 1975). The present investigation was undertaken to study the extent of mycotoxin formation in different seeds by storage fungi under natural storage conditions.

MATERIALS AND METHODS

Maize (*Zea mays* cv. Ganga), groundnut (*Arachis hypogaea* cv. Nizam) and soybean (*Glycine max* cv. Braggi) were stored for one year in gunny bags and the toxin metabolites produced in it by the storage fungi were periodically surveyed following ISTA (1988).

Aflatoxin B_1 used as standard set procurred from Sigma Chamicals. Another mycotoxin ACT_1 (*Aspergillus candidus* toxin) which was earlier isolated by Chattopadhyay *et al.* (1989) from paddy grains in this laboratory was used as the second standard set.

The screening experiments for toxin products *in vitro* was carried out following Jones (1972) and Chattopadhyay *et al.* (1989).

RESULT

Characteristic fluorescent band was detected under UV light in extracts of maize and groundnut seeds from sixth month onwards of storage. No fluorescent band was, however, detected from soybean seeds.

The blue fluorescence detected from chloroform : methanol fraction of the extracts was similar to the standard aflatoxin B_1 under UV (365 nm) and the Rf value of 0.52, when chloroform : methanol (95 : 5 v/w) was used as developing solvent which was more or less same as standard. Aflatoxin B_1 in maize and groundnut was 40 µg and 55 µg/kg seeds respectively after 6 months which increased to 215 µg and 244 µg/kg seeds in the same sequence (Table 1).

The bright-greenish blue fluorescence was identical to standard ACT_1 having the same Rf value and UV spectra. The amount of ACT_1 initially present 5 µg and 4 µg/kg of seeds in maize and groundnut respectively and thereafter increased gradually. (Table 1).

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In the screening experiment for toxin production *in vitro A. candidus* showed bright greenish-blue fluorescence similar to ACT_1 and *A.flavus*, a blue fluorescence identical to Aflatoxin B_1. All others gave –ve results (Table 2).

Table 1. Mycotoxin production in seeds during one year of natural storage

Seeds	Aflatoxin B_1 (µg/kg dry seed)					ACT_1 (µg/kg dry seed)				
	Storage period (months)					Storage period (months)				
	0[h]	3	6	9	12	0[h]	3	6	9	12
Maize	-	-	40	230	215	-	-	5	40	30
Groundnut	-	-	55	265	244	-	-	4	32	26
Soybean	-	-	-	-	-	-	-	-	-	-

0[h] = At the beginning of the study

Table 2. *In-vitro* production of mycotoxins by fungi isolated from seeds grown in liquid culture at 30 ± 1°C for one month

Fungi	Mycotoxins		
	Aflatoxin	$ACTS_1$	Any other
Aspergillus flavus	+	–	–
A. candidus	–	–	–
A. niger	–	–	–
A. ruber	–	–	–
A. versicolor	–	–	–
A. cheivalieri	–	–	–

"+" sign indicates +ve result

"–" sign indicates –ve result

DISCUSSION

The seed-borne fungi have the potentialities to produce toxin metabolites in storage seeds. Aflatoxin B_1 and ACT_1 are such toxins, which are often produced in agricultural commodities as secondary metabolites by certain strains of *A. flavus* (Diener and Davis, 1969) and *A. candidus* (Chattopadhyay *et al.*, 1989) respectively.

Production of Aflatoxin B_1 and ACT_1 in maize and groundnut seeds were recorded from sixth month of storage which increased gradually with time. This indicated that the toxins were produced slowly and gradually in storage (cf. Bilgrami, 1983). Another reason for late production of ACT_1 was probably due to late appearance of the responsible toxigenic strain of *A. candidus* in mycofloral succession (cf. Mukherjee *et al.*, 1988).

Bilgrami (1983) reported that moisture level in and around the substrate was a major contributory factor in toxin production. Thus, after 9 months of storage, which was coincidentally the rainy season of the year, the amont of Aflatoxin B_1 and ACT_1 increased considerably. This might be due to the fact that the prevailing high atmospheric RH and temperature during rainy season, increased the seed noisture above a level that favoured vigorous fungal growth and toxin production. Bilgrami (1983, 1984), Bilgrami and Sinha (1984) recorded extensive contamination of consumable commodities by mycotoxins in many parts of India, where temperature during monsoon was high. At later stages of storage, a small decrease in toxin content might be due to some degradation of the toxin.

Absence of any type of mycotoxin formation in soybean seed seems possibly due to the presence of some inhibitors or absence of essential elements. Moreover, soybean seed was earlier reported to be a poor substrate for aflatoxin production (Hesseltine *et al.*, 1966). Toxin production depends not only on the presence of a toxigenic strain but also on the nature of the substrate. It also depends on composition of saturated and unsaturated fatty acids. It may also prove wrong to assume, that mere presence of toxin producing fungi will indicate presence of mycotoxins. This is because the ability of microorganisms to produce toxins on a rich medium or natural substrate may be affected partly when the organism is present in a mixed population (Durbin, 1981). In such circumstances, toxin was either not produced at all as it was degraded by other organisms, present in the association, to harmless derivative (Christensen *et al.*, 1977).

Thus, toxin production was favoured not only by the presence of a toxigenic strain but also on the substrate as well as on the interaction of the organism with the environmental factors.

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