AFLATOXIN PRODUCTION IN SUNFLOWER (*HELIANTHUS ANNUUS*) SEED VARIETIES

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ABSTRACT

Sunflower seeds have been for the first time shown to support aflatoxin production. The toxin production in five varieties of sunflower seeds has been studied. Under laboratory conditions, considerable variation in toxin production was seen among the five varieties. The possible causes for the low toxin production on whole seeds have been examined. The hard seed coat of the whole seeds impedes the peretrability of the fungus thus resulting in low toxin production.

AFLATOXIN contamination has been encountered in several argicultural commodities and more so in oil seeds[1]. Extensive research has been in progress on several aspects of aflatoxin problem in peanuts and to some extent in soyabeans and cotton seeds. However, this problem has not been adequately investigated on other oil seeds. Aflatoxin production on sunflower seeds (*Helianthus annuus*) has so far not been studied. It is perhaps important to investigate this as well since sunflower seed holds the second place in international oil production and is the fourth largest source of oil seed protein in the world[2,3]. Russia had been the largest producer of sunflower seeds so far, though efforts are currently being made to cultivate this oil-seed crop extensively in U.S.A.[4] and other regions of the world. In India, there has been a crash programme to boost the production of several oil-seed crops including sunflower seeds. Current estimates indicate that at least 3,50,000 hectares are under sunflower seed cultivation in the southern regions of the country[5,6]. Sunflower seed is known to possess certain unique advantages over soyabeans since its oil yield is greater and its meal is practically devoid of any toxic material and will therefore be useful as protein-rich feed for poultry and human consumption as well. In fact preparations made from deoiled meal have been successfully used as protein-rich supplements to pre-school children[2]. In view of the importance of sunflower seed as source of oil and protein-rich meal, it was of interest to investigate the aflatoxin production on some promising varieties currently released for extensive cultivation. The present communication deals with the toxin production on five varieties of sunflower seeds.

EXPERIMENTAL SECTION

Material : Sunflower seeds.—Five authentic varieties of sunflower seeds (EC. 68413, EC. 68414, EC. 68415, EC. 69874 and sunrise selection) were obtained from Oil Seed Specialist, Andhra Pradesh Agricultural University, Hyderabad.

Fungal isolates.—Two isolates of *Aspergillus flavus* (NIN. 169 and NIN. 195) and three isolates of *Aspergillus parasiticus* (NRRL. 2999, RIB. 4002 and NIN. 204) were used.

Methods.—Twenty gram lots of each variety of sunflower seeds (whole seed or broken seeds) were rehydrated with 10 ml of water, sterilised by autoclaving at 15 lb pressure per sq. in. for 15 min. The flasks were then inoculated with a uniform spore suspension of the fungal isolates and incubated at 28° C for seven days. At the end of the incubation period, the samples were sprayed with 95% alcohol and dried overnight at 80° C. The dried samples were first defatted with *n*-hexane and then extracted with methanol. The aqueous methanolic extracts were treated with 20% lead acetate to remove interfering pigments. The filtrates after lead acetate treatment were extracted with chloroform. The chloroform extracts were concentrated and used for thin layer chromatography using chloroform : methanol (95 : 5) as the developing solvent system. The aflatoxin B_1 content was quantitated by the method described by Pons *et al.*[7] using a pure reference standard toxin B_1.

RESULTS AND DISCUSSION

The aflatoxin B_1 production by different fungal isolates on five varieties of sunflower seeds are indicated in Table I. The toxin production was usually related to the toxigenic potential of the isolates of *A. flavus* and *A. parasiticus*. The varieties also exhibited variation in toxin producing capacity.

A comparative study of the toxin production potential of three categories of oil seeds are presented in Table II. Using the same strain (NRRL. 2999), the toxin production on peanuts and soyabeans have been examined in earlier studies[8,9]. This indicates that the aflatoxin producing capacity of sunflower seeds is markedly lower than peanuts and soyabeans.

An attempt was, therefore, made to examine why sunflower seeds support only minimum toxin elaboration even under optimal conditions. In this study

TABLE I
Aflatoxin production on varieties of sunflower seeds

Isolate	Toxin (B_1) produced in parts per million				
	EC. 68413	EC. 68414	EC. 68415	EC. 69874	Sunrise selection
NIN. 169, *A. flavus* ..	0·62	0·62	0·80	1·56	1·56
NIN. 195, „ ..	Nil	Nil	< 0·1	< 0·1	< 0·1
NRRL. 2999, *A. parasiticus* ..	15·6	6·13	15·6	15·6	6·13
NIN. 204, „ ..	< 0·6	< 0·1	< 0·3	< 0·1	< 0·1
RIB. 4002, „ ..	3·12	3·12	0·06	0·06	0·25

TABLE II
Aflatoxin production on oil seeds

Oil seed	Protein %	Oil %	Range of aflatoxin yield in ppm
Soyabean ..	40–45	20–28	20–30
Sunflower ..	18–21	45–50	6–16
Groundnut ..	20–25	47–49	25–50

four varieties of sunflower seeds were used. It was thought that thick seed coat in sunflower seeds might be responsibe for the low toxin production. In a fresh series, whole seeds and broken seeds of the four varieties were again infected under identical conditions as described. The toxin production under these conditions is given in Table III. These data appear to suggest strongly that the "armoured seed coat"[10] perhaps interferes with the penetration of the invading fungus and subsequent ability to produce the toxin. This is indicated by the fact that toxin production in broken seeds is markedly higher than on the whole seeds and is almost comparable as on other oil seeds such as peanuts and soyabeans.

TABLE III
Aflatoxin production on whole and broken seeds of different varieties of sunflower

Sunflower varieties	Aflatoxin B_1 produced * in parts per million	
	Whole seed	Broken seed
EC. 68413 ..	15·62	39·05
EC. 68414 ..	6·25	39·05
EC. 68415 ..	15·62	39·05
Sunrise selection ..	6·25	15·62

* Isolate NRRL. 2999.

These observations suggest that aflatoxin contamination can be encountered in sunflower seeds and accumulation of the toxin could be considerably minimised by storing the sunflower seeds with the seed coat and could be dehulled just prior to extraction of oil. The hardy nature of the seed coat offering resistance to the penetration of fungi is perhaps not an uncommon phenomenon. Such instances have been observed in certain varieties of peanuts[11] and cotton seed varieties[12].

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