

DETECTION OF AFLATOXIN IN SUNFLOWER SEED

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Abstract

TLC technique was used for the detection of aflatoxin in sunflower seeds. Of the 24 samples of sunflower seeds collected from different localities of Pakistan, 13 samples showed the presence of aflatoxin B₁ including 5 in which aflatoxin B₂ was also produced. The amount of aflatoxin B₁ and B₂ detected was upto 437 ug/kg in seed samples collected from Sindh and upto 14 ug/kg in samples collected from Punjab. Out of 41 strains of *Aspergillus flavus* Link., isolated from sunflower seeds, 29 strains produced aflatoxin B₁ including 8 which also produced aflatoxin B₂. Upto 467 ug/l of aflatoxin B₁ and from traces-234 ug/l of aflatoxin B₂ was produced by *A. flavus* isolates.

Introduction

Sunflower (*Helianthus annuus* L.) an oil seed crop is planted over 25899 hectares in Pakistan (Anon., 1990). Sunflower seed contain 25-32% edible oil which is a rich source of polyunsaturated fatty acids used for human consumption (Neergaard, 1977). Sunflower seeds also provide a nutritious food for cattle, poultry hogs and cage birds. The seeds are also consumed roasted, salted and a coffee substitute is prepared from roasted seeds (Shastri, 1956). The quality of seed is destroyed by the growth of mold fungi (Singh & Prasad, 1978). Of the different fungi isolated from sunflower seed, *Aspergillus flavus* Link., was found to be most predominant (Dawar & Ghaffar, 1991). The fungus is known to produce four major metabolites like aflatoxin B₁, B₂, G₁ and G₂ (Diener & Davis, 1969) where aflatoxin B₁ is usually the most abundant. The toxic member of the group has showed a high correlation between the incidence of human hepatomas and aflatoxin consumption (Goldblatt, 1969). As the sunflower seed is used for human consumption and its cake is used in the manufacture of poultry feed, studies were therefore carried out to investigate the frequency of aflatoxin contamination in sunflower seeds.

Materials and Methods

Twenty four samples of sunflower seeds collected from different localities of Pakistan viz., Sindh (18), Punjab (5) and Balochistan (1) were used for the detection of aflatoxins. Seed samples were ground in a grinder and aflatoxin extracted using the method as laid down by the Association of Official Analytical Chemists (Anon., 1975). Quantification of aflatoxin was done by comparing with standard through thin layer chromatography (TLC) where precoated Silica gel (TLC) plates were inoculated and developed in Chloroform-Xylene-Acetone (6:3:1) solvent system. The de-

Table 1. Aflatoxin concentration in sunflower seeds.

Location	Aflatoxin B ₁ (µg/kg)	Aflatoxin B ₂ (µg/kg)	Location	Aflatoxin B ₁ (µg/kg)	Aflatoxin B ₂ (µg/kg)
Sindh					
Sample 1	24	--	14	--	--
2	--	--	15	--	--
3	Traces	--	16	29	36
4	Traces	--	17	--	--
5	437	437	18	--	--
6	73	Traces	Punjab		
7	35	--	1	--	--
8	4	6	2	14	--
9	--	--	3	--	--
10	36	Traces	4	--	--
11	Traces	--	5	Traces	--
12	--	--	Balochistan		
13	45	--	1	--	--

veloped plates were sprayed with 50% sulphuric acid for confirmation. Forty one strains of *A. flavus* isolated from 24 samples of sunflower seed were also tested for their ability to produce aflatoxin. The cultures grown in 50 ml yeast extract sucrose broth for 10 days at 28°C were extracted and quantified for the presence of aflatoxin by using the method described above for the detection of aflatoxin in sunflower seeds.

Results and Discussion

Results of the present study shows that incidence of aflatoxin contamination in sunflower seed is quite high. Of the 24 samples of sunflower seed tested 13 samples were found to be contaminated with aflatoxin B₁ which included 5 samples that showed the presence of aflatoxin B₁ and B₂ (Table 1). The amount of aflatoxin B₁ and B₂ recorded was from Traces-437 µg/kg of seed. Similarly, 41 strain of *A. flavus* isolated from sunflower seeds were tested for their ability to produce aflatoxins in liquid culture. Of these, 29 isolates produced aflatoxin B₁ which included 8 isolates that also produced aflatoxin B₂ (Table 2). The EEC has set a tolerance limit of 20 µg/kg for feed and feed ingredients (Anon., 1977). Since sunflower is an important source of edible oil and the sunflower seed cake is also used in poultry feed, sunflower seeds contaminated with aflatoxin thus poses a potential threat for the life of human beings and poultry birds. There is therefore need for regular monitoring of

Table 2. Production of Aflatoxin by *Aspergillus flavus* isolated from sunflower seeds.

Location	Aflatoxin B ₁ (µg/l)	Aflatoxin B ₂ (µg/l)	Location	Aflatoxin B ₁ (µg/l)	Aflatoxin B ₂ (µg/l)
Sindh			22	14	--
Strain 1	57	47	23	12	--
2	47	--	24	14	--
3	467	234	25	--	--
4	Traces	--	26	--	--
5	Traces	--	27	12	--
6	18	--	28	--	--
7	Traces	--	29	--	--
8	9	--	30	--	--
9	--	--	31	Traces	--
10	233	112	32	--	--
11	Traces	--	33	--	--
12	117	Traces	34	--	--
13	Traces	--	35	--	--
14	2	4	Punjab		
15	4	--	1	467	234
16	5	--	2	Traces	--
17	11	--	3	234	--
18	5	--	4	3	--
19	14	--	5	19	Traces
20	--	--	Balochistan		
21	--	--	1	Traces	Traces

aflatoxin contamination in sunflower seeds for quality control, and to develop methods which can reduce the chances of aflatoxin production during storage and transit.

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