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Occurrence of Aflatoxin in Mustard Seeds, Cakes and Oil

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Mustard seedsamples, cake and oil were analysed for aflatoxin. Out of 27 oil samples contained aflatoxin see seed. Almost all the samples of cakes had fairly high aflatoxin contamination.

Key Words Aflatoxin Contamination Mustard Oil Seed

Mustard (*Brassica campestris*=Sarson) is an important oil seed crop grown in northern, central and eastern parts of India. Main use of sarson is its oil as cooking medium, seeds as source of spice and cake as animal feed.

Oil seeds are contaminated by aflatoxin (Basappa *et al.* 1977). We have no information on the natural occurrence of aflatoxin in mustard seeds and their products. The problem was investigated and the results are presented.

MATERIALS & METHODS

Isolation of mycoflora was done by the standard blotter and agar tests (ISTA, 1966). Percentage incidence of fungi was determined on the basis of occurrence of a particular fungal species in 100 seeds. Aflatoxin producing potential of *Aspergillus flavus*, isolated from the seeds and cakes was tested in liquid rice flour medium (Mishra & Sinha, 1979) and also in SMKY medium (Diener & Davis 1966). The mustard seed samples were examined under UV light far BGYF test (Fennell *et al.*, 1973) and samples positive to the test were extracted for aflatoxin (Thomas *et al.*, 1975) Aflatoxin from the oil was extracted according to Jones (1972).

Quantitative analysis of aflatoxin was done on TLC plates using toluene: isoamyl alcohol: (90:32:2) v/v) solvent system (Reddy *et al.*, 1970) and estimated in a spectrophotometer (Nebney & Nesbitt, 1965). Identity of the aflatoxin was confirmed chemically bytrifluro-acetic acid (Stack & Pohland, 1975).

RESULTS & DISCUSSION

Occurrence of fungi was influenced by the season (Table-1) samples collected in winter harboured 11 fungal species. *Fusarium moniliforme* was dominant while *A. flavus* had low incidence. In contrast, summer (April-May), samples were heavily infested by *A. flavus*. Presence of *F. moniliforme* was compratively low. In the monson samples, *A. flavus* in-

Table 1 Incidence of Fungi on Mustard Seeds

Season	Fungi	% incidence
Winter (J - M)	*Fusarium moniliforme*	70
	F. dimerum	40
	F. semitectum	30
	Alternaria alternata	22
	Aspergillus flavus	15
	Penicillium citrinum	10
	Cladosporium sp.	10
	A. niger	8
	Monilia sitophila	6
	Memnoniella echinata	2
Summer (A - M)	*Aspergillus flavus*	72
	F. moniliforme	30
	Alternaria alternata	25
	A. niger	20
	Rhizopus stolonifer	15
	A. ochraceous	12
	P. citrinum	10
	Chaetomium globosum	6
Monsoon (J - S)	*A. flavus*	85
	A. niger	45
	A. ochraceous	40
	A. candidus	25
	Curvularia lunata	15
	F. moniliforme	10
	Colletotrichum fenicola	9
	P. citrinum	8
	C. pallescens	5

creased but propagules of *Fusarium* sp. were few.

Out of 140 isolates *A. flavus*, 36 isolates were positive for toxin production and 12 of them produced aflatoxin B_1. Isolates producing both B_1 & B_2 were 14 while those which produced G1 along with B_1 & B_2 were 5. Aflatoxins B_1, B_2, G_1 and G_2 were produced by 5 isolates.

The capacity of the toxigenic isolates to produce aflatoxin substantially differed (Table 2). Highest amount of toxin (2230µg/kg) was produced by isolate 11 while isolate No.1 produced lower amount of the toxin.

Table 2 Quantitative Estimation of Aflatoxin B1 in Toxigenic Isolates

Isolate	Conc.(µg./kg)
1	10
2	1480
3	788
4	22
5	980
6	1640
7	20
8	2020
9	86
10	2220
11	2230
12	1040

Sarson seeds contained aflatoxin (Table 3). Out of 32 mustard seed samples screened, 20 gave BGY fluorescence under UV lamp but in only 12 samples out of 20, the quantity of aflatoxin ranged from "trace" to 750 µg/kg seed.

Mustard oil samples were also analysed for the presnce of aflatoxin. Out of 27 oil samples, 7 contained the natural occurrence of aflatoxin (Table-4). Oil cakes also contained the aflatoxin (Table-5), amount ranging from 105 to 1400 µg/kg.

Table 4 Aflatoxin in Oil Samples

	Sample No.	Quantity (µg/kg oil)
OSN	3	62
	7	70
	9	55
	13	60
	14	59
	22	87
	26	81

O.S.N. - Oil Sample Number.

Table 3 Natural Occurrence of Aflatoxin in Mustard Seeds

	Sample No.	Quantity (µg/kg seeds)
NEM	5	488
	6	Trace
	10	520
	13	750
	14	221
	18	20
	19	Trace
	21	88
	23	561
	25	Trace
	28	460
	30	Trace

Table 5 Aflatoxin in Mustard Cakes

Samp. No.	Quantity (µg/kg)	Samp. No.	Quantity (µg/kg)	Samp. No.	Quantity (µg/kg)
CSN 1	105	CSN 11	200	CSN 21	1190
2	720	12	420	22	650
3	1090	13	1100	23	900
4	1020	14	150	24	1230
5	501	15	700	25	1420
6	210	16	110	26	720
7	100	17	1400	27	220
8	108	18	1000	28	1220
9	1000	19	400	29	1200
10	320	20	510	30	240

C.S.N. Cake Sample Number

A. flavus occurred in high percentage during monsoon and summer.

Morphologically it has not been possible to distinguish between toxigenic and non-toxigenic strains. Possibly the toxigenic nature of some of the isolates is associated with their genetic make-up (Ciegler, 1977); Van Walbeek *et al* Non-toxigenic strains produce more ethanol while toxigenic strain produce more acetaldehyde. The latter has been suggested to act as precursor for aflatoxin synthesis (Mayura *et al* , Personal communition). Some of the isolates produced the aflatoxins (B_1, B_2, G_1 & G_2) others elaborated either B1 only or B_1 & B_2 only or B_1, B_2 and G_1 only. It is also possible that the strains differ in their capacity to interconvert the aflatoxins (Heathcote et al., 1978).

Some of the seed samples had aflatoxins as high as 750 µg/kg. This amount is more than the declared safe limit (30 µg/kg by WHO). A few of the *A. flavus* isolates elaborated aflatoxins in SMKY medium, in concentration ranging from 1,480 to 2,230 µg/kg, indicating the high toxigenic potentials of the isolates associated with mustard seeds. Oil samples also contained high concentration of aflatoxin. Consumption of such seeds and oil would pose health hazards to the consumer.

Furthermore, oil cakes showed the presence of aflatoxins in much higher quantity than the seed and oil samples, though all seed and oil samples did not show either the association of toxigenic strains of *A. flavus* or natural occurrence of aflatoxins in the oil. This could be attributed to secondary infection of the cakes after extraction of oil. The cakes are either stored as such exposed to the atmosphere or are stored in gunny bags. Consumption of such cakes by animals may result in aflatoxicosis.

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