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Aflatoxin Elaboration In *Brassica Campestris* Under Storage

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From seed samples of *Brassica campestris* obtained from different godowns, 20 fungi and 140 isolates of *Aspergillus flavus* were isolated. Thirty six isolates of *A. flavus* were toxigenic producing aflatoxins B_1,B_2,G_1 & G_2. Concentration of B_1 was high.

Brassica campestris (sarson) is an important oil seed crop grown in northern, central and eastern parts of India. Part of the crop is stored. The seeds are kept in bags and stacked and also stored in earthen structures known as "Kothi".

During storage, the seeds are exposed to fluctuations in temperature and humidity. Heat produced by the seeds during storage leads to mustiness and association/infection of several fungi. Christensen (1957) reported the manifestation of moulds on seed lots during storage. Some of these fungi are mycotoxic and produce toxin in the seeds. So far no information is available on aflatoxin production in mustard seeds. This problem has been investigated and the results are reported.

MATERIALS & METHODS - Isolation of mycoflora was done by the standard blotter and agar tests (ISTA, 1966). Percentage incidence of the fungi was determined on the basis of occurrence of a particular fungal species in 100 samples.

Aflatoxin producing potential of *A. flavus* isolates was tested in liquid rice flour medium (Misra & Sinha, 1979). Mustard seed samples were examined under UV light for BGYF test (Fennell *et al.*, 1973) and only positive samples were extracted chemically for aflatoxins (Thomas *et al.*, 1975). Qualitative analysis of aflatoxin was done on TLC plates using toluene: isoamyl alcohol: methanol (90:30:2 v/v) solvent system (Reddy *et al.*, 1970) and quantitative estimation of aflatoxin B_1 was done spectrophotometrically (Nabney & Nesbitt, 1965). Identity of the aflatoxin was confirmed chemically by trifluoroacetic acid (Stack & Pohland, 1975).

RESULTS - During winter season, 11 fungal species were recorded of which highest per cent incidence was of *Fusarium moniliforme* (Table-1). *A.flavus* had only 15 per cent incidence. During summer (April - May) *A. flavus* was dominant whereas the presence of *F. moniliforme* was low. During monsoon, when the humidity increased, incidence of *A. flavus* increased to 85 per cent. In contrast, the occurrence of *Fusarium* sp. was low.

The dominance of *A.flavus* in kothi was lesser than in gunny bag (Table-2). However the difference was not remarkable during the winter and summer seasons but significant in monsoon.

Out of 140 isolates, 36 were positive to toxin production and 12 produced aflatoxin B_1. Isolates producing both B_1, B_2 were 14. Five isolates produced G_1 along with B_1& B_2. Only 5 isolates produced the aflatoxins - B_1,B_2, G_1 & G_2. The 12 isolates produced variable amounts of the toxin (Table-3), which ranged from 10 to 2230 ppb.

Table 1 Incidence of Fungal Flora from *Brassica Campestris* seeds

Season	Fungi	Incidence (%)
Winter (Jan - March)'	Altemaria altemata	22
	Aspergillus flavus	15
	A. niger	8
	A. ochraceous	5
	Cladosporium sp.	10
	Fusarium dimerum	40
	F. moniliforme	70
	F. semitectum	30
	Memnoniella echinata	5
	Monilia sitophila	6
	Penicillium citrinum	10
Summer (April - May)	A. flavus	72
	A. niger	20
	A. ochraceous	12
	P. citrinum	10
	Alternaria alternata	25
	Chaetomium globossum	6
	Rhizopus stolonifer	15
	F. moniliforme	30
Monsoon (July - Sept)	A. flavus	85
	A. niger	45
	A. ochraceous	40
	A. candidus	25
	Curvularia lu ata	15
	C. pallescens	5
	Colletotrichum acutatum	9
	P. citrinum	8
	F. moniliforme	10

Table 2 - Occurrence pf *A. flavus* on mustard seeds in storage

-Storage system	Season	Occurrence (%)
Kothi	Winter	8
	Summer	55
	Monsoon	65
Gunny bag	Winter	10
	Summer	72
	Monsoon	85

Table 3 Quantitative Analysis of Aflatoxin B_1 in toxigenic isolates

Isolate No.	Concentration (ppb)
1	10
2	1480
3	788
4	22
5	980
6	1640
7	20
8	2020
9	86
10	2220
11	2230
12	1040

Table 4 Natural Occurrence Of Aflatoxins In Mustard Seeds

Sarson samples	Total Number	Per cent
Samples	32	
BGY flourescent samples	20	67
Toxigenic samples	12	60.00

Sample No.	Quantity in ppb
NEM - 5	488
NEM - 6	Trace
NEM - 10	520
NEM - 13	750
NEM - 14	221
NEM - 18	20
NEM - 19	Trace
NEM - 21	88
NEM - 23	561
NEM - 25	Trace
NEM - 28	460
NEM - 30	Trace

The seeds were tested for the aflatoxin. Out of 32 mustard seed samples, 20 gave BGY fluorescence (Table-4). Further screening revealed that 12 had natural occurrence of aflatoxin which ranged from "trace" to 750 ppb.

DISCUSSION - The variation in per cent incidence of *A. flavus* on mustard seeds is due to fluctuation in atmospheric humidity. This is clearly evident by the increase in *A. flavus* during monsoon. Surprisingly, during summer also high population was recorded. The internal moisture released on account of respiration by the seeds may have favoured the fungal growth.

Dominance of *A. flavus* in gunny bag in the monsoon is apparently due to easy access of atmospheric moisture to the seeds. But, this is restricted by the thick wall of kothi which is also made air tight to some extent by closing the exit and entry points.

Potential importance of mycotoxins in the health of man and his domestic animals has been well realized by the investigations in the recent past (Wyllie & Morehouse, 1977).

All the isolates of *A. flavus* were not toxigenic. Only 5 isolates produced aflatoxin B_1,B_2, G_1 & G_2. Toxigenic and non-toxigenic strains could not be distinguished morphologically.

Because mustard is used throughout the country, there is likelihood of the danger of aflatoxicosis to the consumer. This is more so because it is thermostable (Kush & Gupta, 1982).

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