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Aflatoxin contamination in stored seeds and fruits of forest tree species

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The arid region is characterized by low rainfall and high temperature conditions. The frequent drought conditions in this part of Rajasthan force people to use natural vegetations for their survival. In this context of plants, seed and pods *of Acacia senegal* and *Prosopis cineratia* are found abundantly. The local people stored these seeds / pods for longer time, to be used during famine period but unscientific and faulty storage practices produced aflatoxins in these alternative food materials and which caused serious diseases. The seeds and pods were normally surface washed but the mycotoxins produced inside these proved harmful for them. In *Acacia senegal* 27 plant materials out of 51 were found contaminated where as in *Prosopis cineraria* seed and pods 9 and 6 samples were found contaminated out of 21 and 18 samples examined respectively.

Key words: Aflatoxins, drought, famine food

INTRODUCTION

The name "Desert" itself gives a feeling of scarcity and hardships yet some of the oldest civilization have emerged in desert areas. In Indian arid zone rainfall is not only low (150 to 400 mm. annually) but it is also highly erratic, received in a few showers of high intensity and causes considerable run off. Rajasthan which is situated in the western part of India is the desert part in India. It is completely depended on the monsoon and when the monsoon fails then no agricultural crops can be grown. In such a severe time the local inhabitants of the regions depend on the natural plant products available in the region. They store them in their traditional storage structure for future use. Among them pods and seeds of *Prosopis cineraria,* seeds of *Acacia sanegal* are eaten raw as well as in other ways.

These samples are stored in traditional structures which are unhygienic and there is a greater chance of their contamination by different fungi present in the air, which ultimately produce mycotoxins by metabolic reactions. When these contaminated foods are eaten by local inhabitants they cause various disorder in the body and some times they may be proved fatal.

Mycotoxins are fungal secondary metabolites that are toxic to consumers. The problem is serious not only in food but also in various types of feed consumed by farm animals and poulty. Among the various mycotoxins produced by different fungi, aflatoxins are the most carcinogenic toxin produced by *Aspergillus flavus.*In the recent past aflatoxins have gained importance due to their hepatotoxic, carcinogenic and teratogenic properties. The occurrence and effects of aflatoxins in food and feeds have been elaborately studied and discussed by Anderson *et al.* (1975), Asquith (1983), Bilgrami (1984), Sinha (1990) and Sinha and Sinha (1988).

MATERIALS AND METHODS

Samples of seeds and pods of *Acacia senegal* and *Prosopis cineraria* were collected from different geographical regions with varying rainfall. Mycoflora associated with these seeds and pods was isolated by blotter paper technique (International seed testing Association 1966). Aflatoxin producing potential of *Aspergillus flavus* isolates obtained from different samples was tested in SMKY liquid medium (Diener and Davis 1966). The culture filtrate was extracted finally with chloroform and the concentrated chloroform extract was used for quantitative and qualitative estimation of aflatoxins. All the samples were observed under long wave UV light (365 nm) for BGYF test (Fennell *et al.* 1973) and the samples positive to

BGYF test were further ground and mixed to obtain a 50 g subsample for aflatoxin analysis. Qualitative analysis of aflatoxins was performed by thin layer chromatography (TLC) technique using toluene; isoamyl alcohol : methanol (90: 32: 2, v/v) solvent system. Chemical confirmation of aflatoxin B_1 was performed with trifluoro acetic acid and by spraying with 25% sulphuric acid. Quantity of aflatoxin B_1 was determined spectrophotometrically using a CAMAG TLC scanner.

RESULTS AND DISCUSSION

During observation 20 fungal species of 10 genera were isolated from seeds of *Prosopis cineraria* collected from different localities by using blotter techniques (Table 2). *Aspergillus flavus, Aspergillus niger* and *Aspergillus fumigatus* were the dominant fungi and were found associated with almost all samples collected. Besides these *Aspergillus ochraceous, Curvularia lunata* and *Fusarium sporotrichum* were the other well represented fungal species present in them. In the pods of *Prosopis cineraria* collected from different regions 16 fungal species of 6 genera were collected during investigation (Table 2). The incidence of *Aspergillus flavus, Aspergillus fumigatus, Aspergillus ochraceous, and Aspergillus niger* were dominant while other fungi were present in less percentage compare to them.

Similarly seventeen fungal species were isolated from seeds of *Acacia senegal* using the blotter technique (Table 1) *Aspergillus flavus, A. niger* and *A. ochraceous* were the dominent fungi and were associated with almost all samples collected from different regions. *A fumigatus, Chaetomium globosum* and *Curvularia lunata* were well represented also. In all 17 species of 8 genera were collected. It was clear from above observations that *A. flavus* was predominated in all and some seeds were completely covered. In certain samples a mixed infestation of *Aspergillus* and *Fusarium* species was noticed on the radical during emergence. In some samples *A. flavus* and *A. niger* infestation completely covered the seeds and did not allow any other fungi to grow. By the fungal infection caused the seed contents deteriorated gradually and in some cases, they completely discoloured and deformed. Predominance of fungi such as *A. flavus, A. fumigatus* and *A. niger* might be attributed due to their capabilities to derived nutrition from protein rich sources.

Table 1: Percentage incidence of fungal species on pods of *Acacia senegal*

Fungi	Different localities						
	Jodhpur	Jaipur	Jaisalmer	Pokran	Barmer	Sirohi	Nagaur
Ailtenaria alternata	12	08	05	06	02	15	05
A. solani	—	05	—	—	—	08	—
Aspergillus flavus	30	35	28	25	20	38	32
A. fumigatus	22	20	23	15	17	26	18
A. niger	27	30	27	25	22	30	18
A. ochraceus	20	28	22	20	25	28	20
A tamarii	05	06	03	02	10	15	08
A terreus	02	03	02	05	07	08	05
Chaetomium globosum	10	12	04	—	08	10	04
Cladosporium neroarum	20	25	22	15	12	25	23
Curvularia lunata	05	08	05	03	02	10	07
C. pallesceus	02	04	—	—	—	05	—
Drechslera tetramera	04	05	—	—	—	08	—
Fusarium moniliforme	15	08	—	12	10	15	07
F. oxysprum	14	10	12	11	08	18	12
F. equiseti	08	05	03	02	05	08	—
Memnoniella echinata	05	04	02	05	—	10	2

Table 2: Percentage incidence of fungal species on surface stefilized seeds of *Prosopis cineraria* collected from different localities

Fungi	Different localities						
	Jodhpur	Jaipur	Jaisalmer	Pokran	Barmer	Sirohi	Nagaur
Ailtenaria alternata	06	1	—	—	—	03	—
A. solani	—	—	—	—	—	01	—
Aspergillus flavus	38	42	40	44	31	47	42
A. fumigatus	31	45	17	29	23	37	15
A. niger	43	30	42	51	33	27	42
A. ochraceus	12	10	09	11	17	21	09
Chaetomium globosum	09	07	03	06	06	04	04
Curvularia lunata	17	24	11	09	13	—	07
Fusarium moniliforme	03	07	01	03	—	05	02
F. sporotrichum	12	14	14	23	17	22	10
Rhizopus stolonifer	02	03	—	—	—	—	—
Stachybotrys atra	01	01	03	04	02	05	02

Table 3: Aflatoxins producing potential of *A. flavus* isolates in *Prosopis cineraria* and *Acacia senegal* in SMKY medium

Name of species	No. of isolated screened	No. of toxigenic isolates	% of toxic isolates	Range
Acacia Senegal	51	27	52.94	Trace to 2470
Prosopis cineraria (seed)	21	9	42.85	Trace to 985 micro gm/kg
Prosopis cineraria (fruit)	18	6	33.33	Trace to 1610 micro gm/kg

Table 4: Aflatoxins producing potential of *A. flavus* isolates in *Prosopis cineraria* and *Acacia senegal* in SMKY medium

Name of species	No. of isolated screened	No. of toxigenic isolates	% of toxic isolates	Range
Acacia senegal	78	38	48.71	Trace to 1290 micro gm/kg
Prosopis cineraria (seed)	54	23	42.59	Trace to 1690 micro gm/kg
Prosopis cineraria (fruit)	21	09	42.85	Trace to 1956 micro gm/kg

Aflatoxins producing potential of *A. flavus* was tested in SMKY liquid medium. Out of 78 isolates of *Acacia senegal* 38 were toxigenic and produced aflatoxin in liquid medium. Aflatoxin B_1 was elaborated by 30 isolates while 6 isolates produced aflatoxin B_1 & B_2 and only 2 isolates elaborated afloatoxin B_1, B_2, G_1 & G_2 (Table 3). The toxin producing range was from trace to 1290 microgm/kg. In *Prosopis cinenraria* seeds out of 54 isolated samples screened only 23 produced aflatoxins. Out of them 21 produced Aflotoxin B_1 and 2 produced Aflatoxin B_2 (Table 4). The toxin producing range was from trace to 1690 microgram/kg. In *A. senegal* out of 51 seed samples screened naturally only 27 were found contaminated with aflatoxins (25 with Aflatoxin B_1, 1 with aflatoxin B_2 and 1 with aflatoxins B_1, B_2, G_1 and G_2).The toxin producing range was from trace to 1370 microgram/kg. Thirteen samples produced aflatoxin B_1 above 20 ppm the limit set by the world health organisation (WHO). In *Prosopis cineraria* seeds 9 out of 21 samples screened found contaminated with aflatoxins (7 produced aflatoxin B_1 and 2 produced all aflatoxin B_1, B_2, G_1 & G_2). The range of toxins production was trace to 985 microgm/kg. In *P. cineraria* fruits out of 18 samples screened 6 produces aflatoxins. Aflatoxin B_1 and one produces aflatoxin B_1 and B_2 both. The toxin producing range was from trace to 1610 microgram/kg.

The results indicated that seeds of *A. senegal* and *Prosopis cineraria* were prone to aflatoxin contamination in storage. The high incidence of toxigenic isolates and the high aflatoxin contamination might be due to heavy pre & post monsoon rain, as well as cloudy and foggy nights alternating with moderate hot days in these areas, besides faulty and unscientific traditional storage practices used by rural especially tribal people.

There was increase in fungal flora with increase in storage period, which might be due to seasonal variation. In this region there was a marked fluctuation of temperature and relative humidity in different seasons, especially in rainy season. In rainy season there was an increase in relative humidity and temperature which favoured the growth of mycoflora and thus increased the fungal incidence.

Although these seeds and pods were used by local inhabitants from time immemorial but the changing climate and scientific discoveries clearly indicated that they were not safer in the traditional storage structures. The aflatoxins B_1 beyond the limit prescribed by WHO proved to be dangerous in the region.

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