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FUNGAL FLORA AND AFLATOXIN PRODUCTION IN RELATION TO POST-HARVEST PRACTICES IN GROUNDNUT

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ABSTRACT

The influence of post-harvest drying practices of groundnut followed by the farmers in Tamil Nadu in relation to the development of mycoflora and aflatoxins has been studied. Samples from six farm holdings harvested under different weather conditions were studied for per cent moisture, mature/immature pod ratio, fungal flora and aflatoxin content. The moisture content of fresh pods ranged from 22-36%. The per cent incidence of fungi ranged from 4-34% depending upon the drying conditions and included toxigenic Aspergilli, Penicillia spp. and various field fungi. Aflatoxin B1 in detected from the 3rd day after stripping of the pods and the contents were in the range of 40-95 µg/kg and 75-215 µg/kg in the contaminated shells and kernels respectively. The immature and shrivelled pods constituted about 20-46% and were contaminated with the fungi 2-3 fold heavily than the matured pods.

KEY WORDS: Groundnut, Post-Harvest practices, Aflatoxin.

Aflatoxin contamination in groundnut due to fungi, especially Aspergillus flavus is a serious problem. The contamination may occur in the field at any stage of pod development due to stress conditions and especially during harvesting, drying and storage periods depending on the agronomic and post-harvest practices. Several studies have been made to investigate the fungal and mycotoxin contamination in groundnut during harvest and storage, drought, mechanical damage during harvest, fungal invasion and toxin production. Harvesting, drying and storage practices vary from region to region and have direct relevance to the fungal invasion and consequently toxin production in groundnut pods.

In Coimbatore and Periyar districts of Tamil Nadu, where this study was made, the harvested pods are usually heaped on the field itself in many cases at least for a few days, when the weather conditions are unfavourable for drying. If rain was anticipated during

harvesting, the pulled out plants as well as pods are covered with palmyrah leaves, gunny bags and/or fresh haulm themselves to prevent wetting of the pods. Further, it is a common practice to heap the drying pods every evening and to spread them out next morning for further drying. A study was therefore undertaken to investigate the influence of post-harvest practices in relation of fungal flora and aflatoxin production in this region.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

The samples of pods and haulms were collected from six farm-holdings at Sokkanur village, Palladam taluk, Coimbatore district (farm A to D) and at Kavilipalayam village, Sathyamangalam taluk, Periyar district (farm E and F). The study included both bunch (var TMV 2) and spreading (local) types grown rainfed during the August-December season of 1983. The samples were collected on the day of pulling and on every alternate days except in the case of holding A. Sampling of pod and haulm was done as described by Jones (1972).

The moisture contents of the pods and kernels were determined by drying to constant weight in an oven at 110°C. The mature/immature pod ratio was calculated by counting 100 pods in triplicate.

ISOLATION AND IDENTIFICATION OF FUNGI

The fungal flora associated with the shell, kernel and haulm were isolated, identified and their per cent incidence recorded. This was carried out after surface sterilization of the materials with 0.1% mercuric chloride solution for 2 minutes washing with sterile water thrice and plating them on 25 ml of Czapek's agar medium in petridishes. A minimum of 100 number in each of shells, kernels and haulms was plated. The inoculated petri dishes were incubated at room temparature for 5 days and the number of colonies of different fungi was recorded. The identification was carried out after bringing them into pure culture following the procedure of Raper and Fennel (1973). The toxin producing ability of the isolates was assessed on autoclaved rice medium according to Shotwell et al. (1966).

AFLATOXIN ANALYSIS

Fifty gram portions of defatted ground materials of kernels, shells or haulms were extracted with aqueous chloro-

form in duplicate as described by Jones (1972). The chloroform extracts were concentrated by distillation in vacuo and stored in refrigerator in amber coloured vials until analysis. The separation of aflatoxins was carried out on silica gel G by thin layer chromatography method. Initially, a qualitative screening of each extract was carried out to detect the presence of any UV fluorescing substance including aflatoxins on chromatoplates. The samples which showed fluorescence under UV light were cleaned on silica gel column according to Jones (1972). Estimation of aflatoxins was then carried out with internal and external standards, and by diluting the extracts to extinction.

RESULTS AND DISCUSSION

The inital moisture content of fresh pods ranged from 23 to 36 per cent except in the first sample of farm A which was collected from the heap 3rd day after harvest. There was North-East monsoon rain when farms A to D were harvested and dried, while farms E and F were harvested and dried during normal sunny days. The results of moisture (not shown) indicate that there was no uniform and rapid drying in the case of farms A to D. Pods of farms C and D were heaped for the first 2 days after striping due to rains and hence there was little reduction in moisture level at least for the first two days. It took eight days for the pods of farms A and B to reach safe moisture level (8%). Pods of farms C and D dried slowly and reached a moisture content of 12 per cent only after 10 days and subsequently sold out by the farmer. Pods of farms E and F dried rapidly and reached the safe moisture level in 6-7 days period.

The per cent incidence of fungi in the samples of different farm holdings at various stages of drying are given in Table 1. The incidence of fungi was greater on kernels followed by shells and haulms. The predominant fungi observed on kernels were Aspergillus flavus, A.parasiticus, A.niger, Penicillium spp. Rhizopus stolonifer and Diplodia natalensis. The per cent incidence was higher in heaped pods which were dried slowly (C and D) followed by normally dried pods (A and B). The pods (E and F) which dried rapidly showed lesser incidence of fungi and included only D.natalensis, A.niger and Penicillium spp.

A.niger, R.stolonifer, Sclerotium rolfsii, D.natalensis and **Macrophomina phaseolina** were identified on shells. Field fungi such as **S.rolfsii** and **Rhizoctonia bataticola** were noted on haulms besides stray occurrence of **A.flavus.** It is interesting to note that the fungal incidence in general was greater in the pods collected on the 3rd and 5th days of drying. The higher incidence of aflatoxin producing fungi can be attributed to various factors such as drought stress as these crops were grown rainfed, the favourable moisture content and temperature inside the heap. Storing of under-dried pods usually increased the temperature inside the heap.

The immature and shrivelled pods constituted about 20-48% of the samples collected in all cases (A-F). It has been found that the immature pods were, in general, affected 2-3 fold heavier than the matured ones by fungi such as **Macrophomina phaseolina, S.rolfsii, R.stolonifer, A.niger** and **Penicillium** spp. (Table 2).

The aflatoxin levels in various samples are shown in Table 3. Pods of farm C and D that were initially heap stored and got accidently wet due to rain showed aflatoxin Bi up to 215 µg/kg. The toxin was present both in the kernels and shells, the former containing at least twice the amount as that on the shells. The samples collected initially from all the holdings did not show any toxins; however storage of wet produce in heaps during initial two days led to heavy fungal incidence and elaboration of toxin due to favourable moisture and temperature conditions. Despite the presence of several fungi there was no toxin produced on the pods of farm A and B which were dried normally. Samples of farm E and F harvested and dried rapidly under sunny weather did not show even traces of toxin when compared to other cases. Subramanian and Rao (1974) and Mehan and McDonald (1983) found citrinin in a number of damaged parts of rainy season groundnuts. However, no citrinin was detected in our study as there was little mechanicle damage to the pods. Under tropical conditions aflatoxins were detected 48 h after harvest (Bampton,1963) whereas aflatoxin Bi was detected by the 3rd day of drying in this study.

The results of this study corroborate the observations of Jackson (1967) and McDonald and Harkness (1964)

Table 1. Mycoflora of groundnut as influenced by post - harvest drying practices

Materials	Farm	Per cent fungal incidence at different days						Predominant fungi recorded
		1	3	5	7	9	Total	
I Kernel	A	12	16	12	14	9	63	A. flavus, D. natalensis, Rhizopus stolonifer
	B	16	25	14	17	11	83	A. flavus, A. niger, D. natalensis
	C	14	33	29	30	24	130	A. flavus, A. parasiticus, A. niger
	D	20	31	45	38	36	170	A. flavus, A. parasiticus, A. niger
	E	0	3	5	5	-	13	D. natalensis, R. stolonifer
II Shell	A	14	18	13	22	18	85	A. niger, S. rolfsii
	B	19	10	16	15	22	82	M. phaseolina, D. natalensis
	C	21	16	21	19	14	91	S. rolfsii, A. flavus, D. natalensis
	D	19	14	25	23	25	106	A. flavus, A. niger, A. parasiticus
	E	5	7	6	2	-	20	D. natalensis, S. rolfsii
	F	3	2	4	4	-	13	S. rolfsii, D. natalensis
III Haulm	A	3	9	9	3	8	32	S. rolfsii
	B	3	3	5	2	9	22	R. bataticola
	C	2	8	6	7	7	30	A. flavus, A. niger, S.rolfsii
	D	9	6	8	8	6	37	A. niger, A. flavus
	E	0	3	8	4	5	20	R. bataticola
	F	2	4	1	2	4	13	Corticium sp.

Table 2. Per cent maturity and fungal incidence in groundnut pods

S.No.	Farm	Pod maturity		Per cent fungal incidence	
		mature %	immature %	mature pods	immature pods
1.	A	70.0	30.0	25.0	51.7
2.	B	71.2	28.8	38.1	61.5
3.	C	80.4	19.6	25.7	18.9
4.	D	51.9	48.1	20.4	64.0
5.	E	60.2	39.8	12.3	43.0
6.	F	60.6	39.4	16.5	23.9

Correlation coefficient between immature pods and fungal incidence = 0.5588**
(Significant at P = 0.01)

Table 3. Aflatoxin level in drying groundnuts

Days of harvest	Aflatoxin Bi µg / kg*			
	Farm C		Farm D	
	Kernel	Shell	Kernel	Shell
3	117	60	75	41
5	175	86	132	85
7	202	91	165	90
9	215	94	170	88

* Mean of two analysis

that the slowly dried pods had high levels of aflatoxin and higher percentage of seed invasion by A. flavus than the rapidly dried pods.

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