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Incidence of Mycotoxigenic Fungi on Peanut Seeds of Warangal district A.P.

KEYWORDS: Peanut, aflatoxin, ochratoxin A, zearalenone and citrinin

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ABSTRACT *A total of 300 peanut seed samples were collected from different places of Warangal district in Andhra Pradesh during 2011-2012. In all 45 fungal species belonging 20 genera were isolated. The dominant fungal genera in peanut seeds were Aspergillus (50%), Fusarium (40.32%), Cladosporium (16.42%), Alternaria (17.14%), Curvularia lunata (15.71%) and Penicillium (23.57%) sps. Aflatoxins, sterigmatocystin, ochratoxin A, citrinin and zeralenone were some of the mycotoxins detected in these samples.*

INTRODUCTION

Andhra Pradesh is one of the major groundnut growing states in India covering an area of 4505 ha with a production of 58273 tones. Peanut seeds are highly susceptible to fungi such as *Rhizopus, Aspergillus flavus, Pencillium* and *Fusarium*. Infection of groundnut by species of *Aspergillus* occurs both at pre-harvest and post harvest stage. (Dharmaputra *et al.*, 2003, Craufurd *et al.*, 2006 and Goncales *et al.*, 2008). Post-harvest conditions favour infestation during storage which may lead to production of mycotoxins specially aflatoxins (Bankole and Adebanjo, 2003). Mycotoxins contaminate groundnuts and affect their quality and cause economic losses during processing and export (Krishna Kishore *et al.*, 2002 and Wagacha and Muthomi 2008). There fore in the present investigation the mycoflora of peanut samples from Warangal district was investigated and its toxigenic potential of some of the dominant fungi was assayed.

MATERIALS AND METHODS

One hundred forty peanut samples from different store houses and forty samples from two oil mills of Warangal district were analyzed for mycoflora. Dilution plate method (Waksman, 1922) was adopted for isolation of mycotoxigenic fungi. Ten grams of each sample was taken in 250ml conical flask containing 100ml of sterilized water, and subjected to horizontal shaking for 30min. Form this solution dilutions were prepared as desired. 0.5ml of (10^{-4}) dilutant was pippeted in to the center of sterilized and cooled Petri plates and sterilized and cooled Asthana Hawkers medium (Glucose 5g, KNO_3 3.5gr KH_2PO_4 1.75gr $MgSO_4 7H_2O$ 0.75gr, Agar 16gr and distilled water 1liter) was poured by making gentle rotational movements of Petri plates so as to ensure uniform spreading of the sample. To suppress the bacterial growth and to restrict the fungal colonies, streptomycin and Rose Bengal were added. The Petri dishes were incubated in an inverted position at 27±2°C. The colonies were isolated and identified from 3rd day and continued up to 7th day. The fungi were identified with the help of standard monographs (Barnett and Hunter 1972 Singh *et al.*, 1999, Mathur and Kongsdal,2003 and Leslie and Summerel, 2006).The percentage of incidence, frequency and abundance of individual fungus were calculated using the following formula.

$$\text{Percentage of incidence} = \frac{\text{No. of colonies of a species in all the plates}}{\text{Total no. of colonies of all the species in all the plates}} \times 100$$

$$\text{Percentage of frequency} = \frac{\text{No. of observations in which a species appeared}}{\text{Total no. of observation}} \times 100$$

$$\text{Percentage of abundance} = \frac{\text{Total no of colonies of a species in all observations}}{\text{Total no of colonies in all observations}} \times 100$$

The fungi thus isolated were screened for their potential to produce different mycotoxins. Each fungus was grown individually in 25ml Czepak's medium ($NaNO_3$ 2g; KH_2PO_4 1.0g; $MgSO_4$. $7H_2O$ 0.5g; KCl 0.5g; Sucrose 30g and distilled water 1000ml) in Erlenmeyer conical flask and incubated at 27±2°C for 15 days. At the end of incubation period, the culture filtrate was employed for the detection and characterization of different mycotoxins. Different mycotoxins were detected with the help of Thin Layer Chromatography (TLC) under long wave UV light (360nm). They were further confirmed with help of colour tests and spray reagents (Surekha *et al.*, 2011).

Results and Discussion

The incidence of seed borne fungi associated with groundnut seeds varied with the age and place of collection of sample. In all 35 fungal species representing 17 genera were recorded(Table-1).*A.flavus, A.flaviceps ,A.fumigatus, A.nidulens ,A.terreus ,Alternaria, Cladosporoum,* Species of *Fusarium* and *Pencillium ,Rhizopus* were recorded from all the samples. While *A. versicolor, A. parasiticus* were recorded from samples of store houses and oil mill-1(M1) respectively. *A.ochraceus, Mucor, P. islandicum* and *F. oxysporum* was isolated from samples of store house and oil mill 2(M2). While *C. brachyspor, F. graminearum* and *F. equiseti* was recorded in both M1 and M2 mills. Similarly *Neurospora* was confined to samples of store houses and M2. Where as *R. solani* and *D.*

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halodes could not be detected in samples of store house and M1 respectively. *Myrothecium, Nigrospora and Phoma* were isolated only from sample of store houses. Similarly *Stachybotrys, Trichoderma* and *Trichothecium* were significantly absent in samples of peanut collected from M1 and M2. The percentage of *A.flavus* was highest in all most all the samples, the species of *Fusarium* were next in their incidence.

Species *A.flavus* was highest (83.3%) in its percentage of frequency. While *A. niger, F. graminearum, C. lunata* were next in the order. On the other hand *Mucor, Trichothecium, Phoma, A. sydowii* were least in percentage of frequency. Rest of the fungi occurred with intermediate percentage of frequency. A.flavus was with highest percentage of abundance. While *A.flaviceps, A. niger, Cladosporium* and *F. equiseti* were next highest in their percentage of abundance. *A.sydowii, A.terreus, Mucor, Trichoderma, Trichothecium, Rhizoctonia* and *F. moniliforme* were with low percentage of abundance .Rest of the fungi occurred with intermediate percentage of abundance.

A critical observation of table 2 reveals that about 35%, 45% and 38% of strains *of A.flavus* and 30%, 40% of strains of *A.parasiticus* respectively elaborated aflatoxins. Four isolates (14.28%) of *A.nidulens* out of 14 screened were found to be positive for sterigmatocystin. Out of 12 isolates of *A. ochraceus* only three (25%) isolates elaborated *ochratoxin* A. Only one (11.11%) isolate of *A.terreus* could elaborate patulin out of 9 isolates screened. Two (18.18%) isolates of *F.moniliforme* out of 11 screened were found to be positive for DON production. Screening of 5 strains of *F.oxysporum* indicated that only one strain (13.33%) were positive for nivalenol (20%) production. Five isolates (13.33%) of *F. equiseti* out of 15 screened were found to be positive for zearalenone production. Out of 16 isolates of *P.citrinum* only 4(18.75%) isolates elaborate citrinin respectively. *T.roseum* isolated from store house could elaborate trichothecene (12.5%).

About 33% and 7% of *A.nidulens* and *A.terreus* isolated from oil mill -1(M1) were positive for sterigmatocystin and ochratoxin A respectively. Two (15.38%) isolates of F.graminarium out of 13 screened produced zearalenone. Only two (12.5%) isolates of *F.moniliforme* could elaborate DON out of 8 isolates screened. Similarly, 5(22.72%) isolates of *P.citrinum* out of screened produced citrinin. Varieties of moulds isolated from M2 sample were able to produce mycotoxins, the percentage of toxigenic isolates varied. Out of 34,18 and17strains of *A.nidulens, A.ochraceus* and *A.terreus* isolates screened 5(20.58%,)2(16.66%), and 14(11.76%) isolates respectively positive for sterigmatocystin, ochratoxin A and patulin. 14(34.61%) isolates of *F.moniliforme* out of 26 screened produced DON. Six(16.66%),3(27.27%) and 15(18.5%) isolates out of 12,11 and 27 isolates of *F.oxysporum, F.graminarium* and *F.equisiti* could elaborate zearalenone respectively. Different species of *Pencillium* elaborated citrinin (24%) and isalandicin (20%) respectively.

From the present investigations, groundnut seeds collected from store house and oil mills of Warangal district were infested by different seed borne fungi varying in percentage different sample conditions and place of collection. Creepy, (2002) reported that seeds and grains are more liable fungal infection particularly *Fusarium* and *Pencillium* species in tropical and subtropical regions dependent on the high levels of moisture content. Such seeds are may prove to be hazardous to human and animals depending on the use of stored groundnut seeds.

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Table 1. Incidence of different fungal genera of groundnut from store house and oil mills

	Storage house			Oil mill samples			
			M1			M2	
Name of the fungus	Incidence (In %)	Frequency (In %)	Abundance (In %)	Incidence (In %)	Frequency (In %)	Abundance (In %)	Incidence (In %)	Frequency (In %)	Abundance (In %)
Alternaria alternata	0.45	17.14	3.59	0.05	8.33	0.7	0.24	8.33	1.94
Aspergillus flavipes	0.6	15	6.6	0.28	8.33	13.54	0.46	33.33	6.79
A. flavus	42.93	50	26.04	4.12	83.3	46.8	3.1	66.66	33
A. fumigatus	0.29	11.4	1.65	0.28	8.33	1.41	0.1	8.33	1.94
A. nidulans	0.41	12.8	3.2	0.5	33.33	3.54	0.63	16.66	10.6
A. niger	1.21	27.1	12.24	1.49	58.33	7.8	1.24	41.66	11.65
A. ochraceous	0.25	5.71	1.16	-	-	-	0.1	8.33	1.94
A. parasiticus	0.55	11.4	3.01	0.05	8.33	0.7	-	-	-
A. sydowii	0.08	0.71	0.09	0.28	8.33	2.12	0.85	8.33	0.97
A. terricola	0.41	8.57	3.01	-	-	-	-	-	-
A. terreus	0.02	1.42	0.19	0.11	16.66	2.12	0.34	8.33	3.88
A. versicolor	0.2	7.14	2.04	0.15	16.66	1.41	-	-	-
Cladosporium cladosporioides	2.04	36.4	18.85	1.36	33.33	12.76	0.86	16.66	8.73
Curvularia brachyspora				0.08	8.33	1.41	1.02	8.33	1.68
Curvularia lunata	0.56	15.7	3.01	0.04	8.33	0.7	0.04	8.33	0.97
Drechslera maydis	0.3	10.7	1.55	-	-	-	0.51	16.66	3.36
Fusarium graminearum				0.28	16.66	4.25	0.61	25	4.2
Fusarium moniliforme	0.17	8.57	0.68	0.04	8.33	1.41	0.06	8.33	0.84

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F. oxysporum	0.21	6.42	1.26	-	-	-	0.15	16.66	2.52
F. equiseti				0.41	25	5.67	0.29	16.66	5.04
Neurospora crassa	0.07	2.85	0.68	-	-	-	0.22	16.66	1.94
Nigrospora oryzae	0.1	2.85	0.38	-	-	-	-	-	-
Mucor varians	0.05	1.42	0.48	-	-	-	0.05	8.33	0.97
Myrothecium roridum	0.02	0.71	0.29	-	-	-	-	-	-
Penicillium chrysogenum	0.16	9.28	1.36	-	-	-	-	-	-
P. citrinum	0.31	10	1.94	0.04	8.33	0.7	0.06	8.33	0.97
P. islandicum	0.17	6.42	2.23	-	-	-	0.06	16.66	1.94
P.notatum	0.47	13.57	2.52	-	-	-	-	-	-
Phoma humicola	0.01	0.71	0.09	-	-	-	-	-	-
Rhizoctonia solani				-	-	-	0.04	8.33	0.97
Rhizopus stolonifer	0.17	5	0.68	0.09	8.33	0.7	0.25	16.66-	3.88
Stachybotrys atra	0.02	1.42	0.29	-	-	-	-	-	-
Trichoderma viride	0.18	3.57	0.58	-	-	-	-	-	-
Trichothecium roseum	0.01	0.71	0.09	-	-	-	-	-	-
M1- Mamidi yellosa oil mill									
M2 - Uma maheshwara oil mill									

Table 2. Toxigenic potential of fungi of groundnut in storage and oil mills

			M1		M2		
Name of the fungus	Store house		Oil mills				Name of the toxin
	S.S	T.S(%)	S.S	T.S(%)	S.S	T.S(%)	
Aspergillus flavus	70	35.71	106	45.28	83	38.55	Aflatoxin
A. nidulans	14	14.28	21	33.33	34	20.58	Sterigmatocystin
A. ochraceus	12	25	-	-	18	16.66	Ochratoxin A
A. parasiticus	10	30	22	40.9	-	-	Aflatoxin
A. terreus	9	11.11	11	27.27	17	11.76	Patulin
Fusarium graminearum	-	-	13	15	38	11	Zearalenone
F. equiseti	15	13.33	-	-	27	18.51	Zearalenone
F. moniliforme	11	18.18	8	12.5	26	34.61	Deoxynivalenol
F. oxysporum	5	13.33	-	-	12	16.66	Zearalenone
Penicillium citrinum	16	18.75	22	22.72	33	24.24	Citrinin
P. griseofulvum	16	12.5	-	-	-	-	CPA
P. islandicum	11	9.09	-	-	15	20	Islandicin
Trichothecium roseum	24	8.33	-	-	-	-	Trichothecene
S.S - Strains Screened	and T.S - Toxigenic strains						

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