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Incidence of *Aspergillus flavus* L. Ex. Fries in groundnut (*Arachis hypogaea* L.) samples and its impact on nutritive value of kernels

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Abstract: Aflatoxins are problematic in groundnut due to infection of *Aspergillus flavus* L. Ex. Fries. In this context survey was conducted in 14 districts of Karnataka, India to assess the severity of *A. flavus*. The results revealed high incidence in Belligatti (taluk and dist- Dharwad) when assessed on agar plate. It was also found high in two other samples (Davanagere and Tumkur) by subjecting to blotter test. In samples collected from market, higher incidence of *A. flavus* was observed in Dharwad and Hubli. *Aspergillus flavus* produced greenish to brownish colonies, the conidia were pyriform to globbose, varied in size (8 x 7 to 5 x 4 µm). The fungus produced larger colony on potato dextrose agar and smaller one on host extract agar. In inoculated seeds, the infection led to the reduction in sugars (reducing, non-reducing and total sugars), proteins, oil content and seed germination.

Key words: *Aspergillus flavus,* Colonies, Conidia, Groundnut, Nutritive value

Introduction

Aspergillus flavus L. Ex. Fries contaminate groundnut, its products and Chilli with afla toxins (Ajith Kumar, 2003) and are health hazardous to human beings and livestock. It is a fungal microorganism which is ubiquitous and produces aflatoxins (B1, B2, G1, G2) in kernel and cake and it is secreted as M1 and M2 toxin in milk in animals fed with contaminated meal. The aflatoxins produced by the fungus in groundnuts are regarded as potent hepato carcinogens causing liver cancer and many other disorders. Other harmful effects are tetragenecity, deformation of developing fetus, reduction in RBC, WBC and haemoglobin content in blood and delayed blood clotting and suppression of immune system in case of chronic poisoning. Due to food safety problems posed by aflatoxins, The Food and Drug Administration of USA has set limit of 20 ppb for total aflatoxins in domestic human foods. However, in the European Union (EU) legislation and implementation with effect from 1999, the standards for aflatoxin tolerance were reset at 2 ppb for aflatoxin B1 and 4 ppb for total aflatoxin in human diet (Tushar Tanna, 2002).

India is the leading exporter of confectionery groundnut; loss of quality of produce affect export trade in international market. Karnataka is one of the important states producing groundnuts and it has export potential of HPS groundnut (mainly for confectionery purposes) and unfortunately aflatoxins database is lacking for the state.

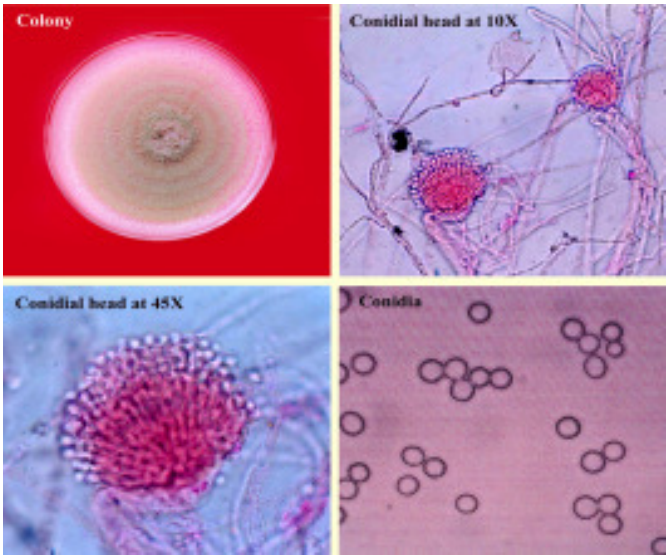

Plate 1. Morphology of *Aspergillus flavus*

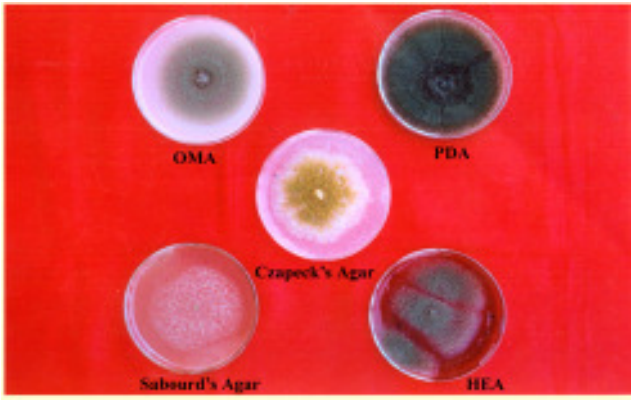

Plate 2. Growth of *Aspergillus flavus* on different solid media

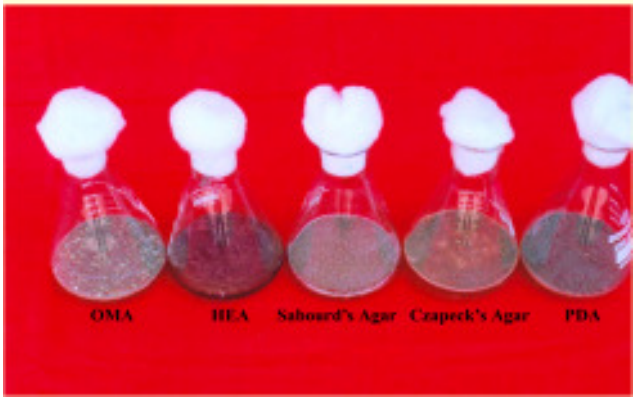

Plate 3. *Aspergillus flavus* in five different solid and liquid media

In view of health and economic implication the objectives for the present study were fixed as generation of database of aflatoxin contamination for the state by survey and sample collection and impact on quality of groundnut due to infection of *A. flavus.*

Material and methods

An intensive survey was conducted during from January to April to know the incidence and severity of *A. flavus* infection in groundnut in Karnataka. Farmers fields in Dharwad, Belagavi, Raichur, Gulbarga, Bidar, Bagalkot, Haveri, Uttar Kannada, Bengaluru, Davanagere, Shivamogga and Tumkur districts were selected to collect samples. Samples were also collected from markets of Dharwad, Hubli and Bengaluru. The pod samples were dried in shade accurately and stored in gunny bags and used for assessment immediately.

The apparently healthy seeds were surface sterilized with 0.1 per cent mercury chloride ($HgCl_2$) solution for one minute and washed thoroughly in distilled water and then transferred to sterile PDA plates and also on blotters and incubated. The seeds yielding *A. flavus* and other fungi were enumerated and per cent incidence was calculated. The location specific *A. flavus* isolates obtained were further studied for their morphological characters and compared with description of Thom and Raper (1945).

Cultural characters of isolates of *A. flavus* were studied on three non-synthetic (PDA, OMA, HEA) and two synthetic media (Czapeck's agar and Sabourd's agar) in *in vitro.* The composition and preparation of the above media was done as suggested by Hawksworth *et al.* (1983).

Twenty ml of each medium was poured into 90 mm diameter petriplate. After solidification, 5 mm of discs from fresh growth of *A. flavus* colonies were cut using cork borer and single disc was placed at the center of petridish. Each set of experiment was replicated twice and plates were incubated at 28 ± 1°C for eight days. Variability in cultural characters such as colony diameter, colony colour, type of margin and sporulation were recorded in each isolates of *A. flavus.*

For extraction of protein and sugar, hot alcohol was used. For estimating reducing sugar, DNSA method (Miller, 1959) was used. One gram crushed seed samples was extracted in hot alcohol and 0.5 ml of extract were drawn from each treatment in test tubes, 0.5 ml of distilled water was added to it. Standard were also included ranging from 10 to 100 mg concentration of glucose. DNSA reagent 0.5 ml was added to each sample mixed well and kept in boiling water for five minutes. The sample was cooled and final volume was made up to 25 ml using volumetric flask. Absorbance in terms of optical density of standards and the sample were read at 540 nm using Systronics UV Spectrophotometer-117. The standard curve was plotted on graph and sugar content in the sample was calculated.

For estimating total sugars, the representative sample of one ml from each treatment was taken in test tubes. One ml of 1 N HCl was added to each tube and placed them in boiling water for 15 minutes. Tubes were cooled and one drop of phenolpthalein indicator was added till pink colour appeared followed by addition of 0.1 N HCl till pink colour disappears. Finally, samples were mixed well and volume was made up to 5 ml with distilled water, 0.5 ml of representative samples were taken from these tubes and total sugar was estimated by DNSA method. Protein was estimated by Microkjeldhal method of nitrogen estimation. Oil content was estimated by nuclear magnetic resonance (NMR) Spectrometer at Agricultural College, Raichur.

Results and discussion

The survey indicated that the groundnut produced in the state is potentially contaminated with *A. flavus.* The market samples also showed high inoculum load. The results of agar plate and blotter paper method revealed the presence of *A. flavus* and it varied from location to location. It ranged from traces to 25 per cent in agar plate method and 3-20 per cent in blotter technique. In case of field samples, maximum fungal incidence was recorded in Belligatti (25%) in agar plate. On other hand, Davanagere and Tumkur samples recorded maximum colonies of *A. flavus* (20%) in blotter technique. Among market samples, maximum fungal incidence was recorded in samples of Dharwad (25%) and Hubli (16.60%), on agar plate and blotter technique, respectively. Higher incidence of fungi in fields of Dharwad and Dharwad market during January to April months may be attributed to prevalence of favourable conditions like severe drought, optimum temperature (27-30°C), relative humidity and moisture content of pods that might have helped the infection of *A. flavus* on groundnut which resulted in. high proportion of *A. flavus* (Table 1).

Aspergillus flavus appeared yellowish green colony on artificial laboratory media, it is a key factor in identification of the fungus. The isolates produced green to dark green coloured colony on PDA, OMA, Sabourd's agar and HEA media. However, fungus produced yellowish green to whitish yellow colony on Czapeck's agar (Table 2, Plate 1 and 2). The conidia and conidiophores imparted typical colour to the colony. Seven out of 22 isolates produced sclerotia on PDA, five on OMA, six in Czapeck's agar (Plate 3). None of the isolates produced sclerotial on Sabourd's and HEA. The conidia produced in PDA were of larger size (8 x 7 µm) compared to one produced in other media (5 x 4 µm). All the isolates of *A. flavus* did not produce sclerotia and sclerotial production depends on media, strain and environmental factor (Mehan *et al.,* 1995).

Reduction in total sugar, reducing sugar and non-reducing sugar was noticed in inoculated seeds of 15 cultivars (Table 3). In total sugar, it was to the extent of 0.28 to 7.21 per cent, in reducing sugar 0.84 to 9.09 per cent and in case of non-reducing sugar it was from 0.16 to 6.59 per cent. The reason for reduction in sugar might be due to utilization of sugar by fungi (Jamaluddin *et al.*, 1987). Sugars play a major role for disease resistance and they are the precursor for synthesis of phenolics and phytoalexins. The investigation also revealed that reduction in oil content (2.35-6.11%) in *A. flavus* inoculated seeds compared to apparently healthy ones (Table 3). While, invading fungus causes the oxidation of fatty acids and inactivation of enzymes. This may be the reason for the reduction in oil content. A reduction in the proteins content was to the extent of 7.06-

Table 1. Composition of mycoflora of groundnut seeds collected from different parts of Karnataka

Location	Seed mycoflora of groundnut (%)									
	Agar plate					Blotter technique				
	Aspergillus flavus	*Aspergillus niger*	*Rhizopus* spp.	*Fusarium* spp.	Others	*Aspergillus flavus*	*Aspergillus niger*	*Rhizopus* spp.	*Fusarium* spp.	*Others***
Bhalki	-	36.60	23.30	10.00	20.00	13.33	23.30	16.66	-	10.00
Bangalore	20.00	43.30	33.30	3.30	10.00	3.00	26.67	23.30	-	10.00
Dharwad	3.33	56.67	33.30	3.30	13.33	13.30	10.00	23.33	-	6.67
Ranebennur	3.30	60.00	26.67	-	3.30	3.33	13.30	6.60	6.60	23.30
Chitradurga	-	50.00	56.67	-	16.67	16.60	10.20	10.00	-	23.30
Tiptur	-	33.33	26.60	-	13.30	10.00	20.20	26.66	3.30	13.30
Bailhongal	-	36.66	23.33	13.33	20.00	6.67	20.00	30.00	10.00	10.00
Shivamogga	6.67	33.33	56.67	10.00	13.30	3.30	20.00	30.00	-	6.67
Ballari	6.67	26.67	56.67	3.33	33.33	13.30	6.60	26.67	-	13.30
Mudhol	-	60.00	26.67	-	16.67	10.00	10.00	10.00	-	23.30
Davanagere	16.60	26.60	26.67	-	10.00	20.00	26.60	13.33	3.3	20.00
Ankola	-	73.30	26.67	-	16.67	10.00	20.00	23.30	-	16.60
Raichur	-	23.30	23.30	6.67	3.33	6.67	13.30	10.00	-	16.67
Belagavi	-	20.00	26.67	23.30	10.00	13.30	13.30	-	3.30	13.33
Tumkur	-	53.33	-	23.30	3.30	20.00	13.30	23.30	-	10.00
Kittur	13.30	33.33	-	-	13.30	3.30	23.30	23.70	3.30	13.33
Manavi	3.30	36.67	-	-	13.30	6.67	13.37	20.00	-	13.30
Belligatti	25.00	40	-	-	5.00	3.30	16.60	10.00	-	13.30
Hubli (market)*	-	70.00	26.67	-	16.67	16.60	16.60	13.33	-	6.67
Magdi*	0.37	50.00	26.67	6.67	6.67	10.00	13.30	6.67	3.30	10.00
Dharwad (market)*	25.00	20	-	-	-	10.00	16.60	20.00	-	10.00

* Market samples ** Others includes: *Pencillium* and *Mucor*

Table 2. Radial growth of *Aspergillus flavus* isolates on different solid media (mm)

Location and isolates	Potato dextrose agar	Oat meal agar	Czapeck's agar	Sabourd's agar	Host extract agar	Mean
Bhalki (AFL BHL)	72.50	61.50	64.00	55.31	40.83	58.82
GKVK (AFL GKV)	72.81	73.63	70.50	60.00	39.33	63.25
Dharwad campus (AFL DWC)	73.31	58.00	69.50	54.50	45.50	60.16
Ranebennur (AFL RBN)	80.50	66.33	69.50	62.33	44.50	64.63
Chitradurga (AFL CDG)	73.81	71.83	68.16	63.31	29.50	61.32
Tiptur (AFL TPR)	80.00	64.66	72.50	70.33	43.00	66.09
Bailhongal (AFL BLG)	76.67	64.33	64.83	68.66	47.00	64.28
Shivamogga (AFL SMG)	74.83	71.66	66.50	66.33	50.00	65.86
Ballari (AFL BLY)	80.16	78.16	62.81	68.33	43.50	66.59
Hubli market (AFL HBM)	84.66	74.50	75.13	77.66	43.50	71.09
Mudhol (AFL MDL)	74.16	71.50	76.50	66.16	45.13	66.69
Davanagere (AFL DVG)	70.50	67.33	70.50	69.50	47.81	65.13
Ankola (AFL ALK)	73.31	66.50	63.50	67.83	44.50	63.12
Raichur (AFL RCR)	80.00	64.83	66.16	62.16	42.50	63.13
Magadi (AFL MGB)	82.33	66.33	67.33	66.31	35.50	63.61
Belagavi (AFL BGM)	80.83	68.16	67.31	58.50	34.83	61.92
Tumkur (AFL TMR)	70.50	64.16	64.00	61.00	35.33	58.99
Kittur (AFL KTR)	80.83	63.83	63.50	62.66	37.66	65.69
Manvi (AFL MNV)	80.00	64.33	68.00	66.66	30.33	61.86
Belligatti (AFL BGT)	80.66	65.33	66.00	56.00	43.33	61.26
Dharwad market (AFL DWM)	75.83	64.98	73.00	62.00	43.16	63.72
Junagadh (AFL JGR)	75.81	63.98	59.15	64.33	43.33	61.32
Mean	77.00	67.08	67.66	64.08	42.17	
	Isolates (A)		Media (B)		Interaction (AxB)	
S.Em.±	0.45		0.22		1.01	
C.D. at 1%	1.82		0.80		3.77	

24.64 per cent over apparently healthy ones. The maximum reduction of 24.64 per cent was recorded in case of Dh-40 followed by JL-24 (19.95%) and J-11 (18.52) (Table 3). The fungus also affected the seed viability significantly. The germination percentage varied from zero to 90.00 per cent in various genotypes inoculated with *A. flavus* while reduction over

Table 3. Changes in the oil content of groundnut kernels as influenced by infection of *Aspergillus flavus*

Genotypes	Kernel oil content (%)		Per cent reduction over healthy	Kernel protein content (%)		Per cent reduction over healthy	Reducing sugar (%)		Per cent reduction over healthy	Total sugar (%)		Per cent reduction over healthy
	Healthy	Inoculated		Healthy	Inoculated		Healthy	Inoculated		Healthy	Inoculated	
TGLPS-3	39.31 (38.72)	37.55 (37.49)	4.47	21.81 (27.835)	19.25 (26.02)	11.73	1.07 (6.02)	1.01 (5.57)	5.60	9.40 (17.85)	8.80 (16.95)	6.38
TKG-19 A	41.11 (39.87)	39.80 (39.11)	3.18	21.96 (27.93)	19.16 (25.91)	12.75	0.71 (4.96)	0.67 (4.78)	5.63	7.20 (15.61)	6.92 (15.22)	3.88
R-9251	39.41 (38.95)	38.18 (38.17)	3.10	23.18 (28.79)	19.07 (25.91)	17.73	1.09 (5.99)	1.01 (5.40)	7.33	7.72 (15.62)	7.45 (15.83)	3.49
R-8808	44.36 (41.78)	41.65 (40.19)	6.11	21.00 (27.27)	17.93 (25.02)	14.58	0.96 (5.49)	0.91 (5.40)	5.21	8.58 (17.05)	8.12 (16.53)	5.39
Dh-40	40.52 (39.52)	38.81 (38.55)	4.22	23.53 (29.03)	17.73 (24.42)	24.64	1.24 (6.42)	1.17 (56.28)	5.64	8.19 (16.63)	7.8 (16.21)	4.76
J-11	43.11 (41.23)	41.92 (39.76)	2.72	23.88 (29.26)	19.52 (26.34)	18.52	0.77 (4.94)	0.70 (4.78)	9.09	7.20 (15.55)	7.0 (15.34)	2.77
ICGV-92242	44.63 (41.93)	42.77 (40.80)	4.16	22.97 (28.66)	19.86 (26.48)	13.53	0.91 (5.44)	0.90 (5.44)	1.01	7.05 (15.38)	7.03 (15.34)	0.28
JL-24	43.44) (41.24	41.42) (40.08	4.65	21.96 (27.93)	17.58 (26.38)	19.95	1.41 (6.68)	1.34 (6.67)	4.96	8.31 (16.74)	8.01 (16.42)	3.84
TAG-24	43.32 (41.18)	40.93 (39.67)	5.51	23.62) (29.06	19.95 (24.74)	15.54	1.19) (6.12	1.18 (6.27)	0.84	7.28 (15.61)	7.25 (15.62)	4.41
Dh-102	40.48 (39.51)	39.29 (38.82)	2.93	22.22 (28.14)	20.03 (26.52)	9.87	0.63 (4.60)	0.59 (4.35)	6.34	7.76 (16.16)	7.20) (15.56	7.21
GPBD-4	42.53) (40.48	41.53 (40.32)	2.35	21.51 (27.58)	19.77 (26.59)	8.07	1.14 (4.15)	1.10 (6.15)	3.50	8.23 (16.69)	8.00 (16.42)	2.79
Dh-3-30	43.63 (41.35)	42.23 (40.54)	3.28	23.01 (28.68)	19.86 (26.42)	13.68	0.65) (4.62	0.60 (4.42	)	8.33	7.34 (15.72)	7.15) (15.57	2.58
Dh-54	39.22) (38.78	38.21 (38.17)	2.58	23.98 (29.58)	19.59 (26.53)	18.30	1.32 (6.52)	1.29 (6.41)	2.27	7.28 (15.67)	6.91 (14.65)	4.84
TMV-2	43.58 (41.15)	41.77 (40.21)	4.15	23.97 (29.20)	19.59 (26.27)	18.27	1.15 (6.14)	1.10 (5.74)	4.35	7.30 (15.67)	7.00 (15.34)	4.10
Dh-86	40.67 (39.61)	38.24 (38.20)	5.98	20.77 (27.58)	19.19 (25.95)	7.06	1.26 (6.42)	1.22 (6.02)	3.17	7.73 (16.16)	7.34 (15.73)	5.04
S.Em.±	0.69	0.33		0.29	0.42		0.09	0.18		0.49	0.35	
C.D. at 1%	2.93	1.38		1.24	1.77		0.39	0.78		2.05	1.49	

Figures in parentheses indicate arcsine transformed values

apparently healthy seed was in the range of 29.76 to cent per cent. In TKG-19A variety the reduction in germination was 100 per cent. TMV-2, Dh-86, TAG-21 and J-11 also exhibited higher viability loss.

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