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Aflatoxin contamination in groundnut under normal moisture and moisture stress field conditions

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Abstract
Aflatoxin contamination of groundnut is a serious quality problem in many tropical and sub-tropical countries in the world. India is no exception to this because groundnut is cultivated in varied environmental conditions in different states of India. With an objective to find out aflatoxin contamination resistant genotype, a total of 66 groundnut genotypes were screened for aflatoxin contamination under normal moisture and moisture stress condition. Analysis of variance has revealed that there is significant variation among genotypes tested even at 1 per cent level of significance. Average aflatoxin contamination under moisture stress condition was increased by 11.53 per cent. Heritability was 98.28 per cent with a genetic advance over mean of 260.79 per cent under normal moisture and 97.33 per cent with a genetic advance over mean of 226.30 per cent. Some of the genotypes were identified with no aflatoxin contamination under both normal moisture and moisture stress conditions *viz.*, Dh-86, Dh-101, Dh-216, Dh-246, SB-T2, SB-T13, SB-T14, VB-T31, K-9, ICGV-15119, Dh- 234 and J-11.

Keywords: Groundnut, aflatoxin, moisture stress, *Aspergillus flavus*, aflatoxin contamination

Introduction
In 1960, more than 1,00,000 young turkeys in poultry farms in England died in few months from a new disease that was termed "Turkey X disease". Histopathological examination of birds revealed the degeneration of liver cells and proliferation of bile duct epithelium. Subsequent surveys demonstrated that the toxic manifestations were caused by ingestion of certain mold contaminated seeds containing imported Brazilian groundnut meal. Allcroft *et al.* (1961) [1] isolated the toxin produced by molds growing on the groundnut meal. Sargeant *et al.* (1961) [2] for the first time isolated the toxin producing fungus, *Aspergillus flavus* and gave the name 'aflatoxin' to the toxin in view of its origin.

Many kinds of contaminants are found naturally in foods. Among these, mycotoxins are the major contaminants and 25 per cent of foods are contaminated with mycotoxins above safety limits. Among them aflatoxins are the major ones produced by toxigenic strains of *A. flavus* and *A. parasiticus* in the suitable environment. In August 1981, the Ministry of Agriculture in the United Kingdom banned the feeding of groundnut products to dairy cows because of the possible hazards of aflatoxins to the health of milk drinkers (Swindale, 1989) [3]. During 1983-1993 in India, 4818 samples of cereals, oilseed cakes, compound feeds and other ingredients showed high amounts of aflatoxin in groundnut cake, de-oiled groundnut cake, maize and mixed feeds showed high amounts of aflatoxin (Dhavan and Choudary, 1995) [4]. Surveys conducted in different parts of India (Ghewande *et al.*, 1993, Sahay and Rajan, 1990, Kolhe *et al.*, 1994 and Verma *et al.*, 1997) [5-8] have revealed that groundnuts and groundnut products were high-risk commodities for aflatoxin contamination. Levels of aflatoxin contamination varied from 0.8 to 2200 µg per kg in groundnut kernel, traces to 200 µg per kg in edible flour, 786 µg per kg in unrefined oil, 27 to 1122 µg per kg in cake. Ina survey, 18 per cent of groundnut based snack products carried aflatoxin B1 beyond permissible limit of 30 ppb (Rati and Santha, 1994) [9] in 21 per cent of groundnut samples (Bhat *et al.*, 1996).

Cole *et al.* (1993) [11] found that enhanced resistance of groundnut genotypes was partially associated with enhanced drought tolerance as indicated by the ability to maintain high kernel moisture under extended drought conditions. Anderson *et al.* (1995) [12] studied 12 potentially resistant genotypes for pre-harvest aflatoxin contamination and found that none of the genotypes were more resistant to pre-harvest aflatoxin contamination than the genotype Florunner. Nahdi (1996) [13] screened four groundnut genotypes TMV-2, NcAc-17090, Robust 33-1 and EC 76446 in two seasons by creating early and mid-season drought and found

increased infection of seeds by *A. flavus* and aflatoxin contamination was found only in second season. Holbrook *et al.* (2000) [14] evaluated 20 genotypes of groundnut including drought tolerant and susceptible types. He found that susceptible genotypes had greater pre-harvest aflatoxin contamination and drought tolerant genotypes had less pre-harvest aflatoxin contamination. Rao *et al.* (2001) [15] have suggested that management of drought by mechanisms like avoidance, tolerance or escape can have a significant impact on the ability of a genotype to reduce aflatoxin contamination. Rahmianna *et al.* (2004) [16] reported the evaluation of fourteen groundnut genotypes for drought tolerance and aflatoxin contamination. Three genotypes ICGV 86590, ICGV 93280 and ICGV 95322 had pod yields of more than 2.5 t per ha and low aflatoxin contamination was observed in spite of seed infection by *Aspergillus flavus*, which ranged between 3.3 and 14.7 per cent.

A survey by Vijay Krishna Kumar *et al.* (2001) [17] in Tumkur district of Karnataka revealed natural seed infection of groundnut by *A. flavus* was in the range of 10 to 22 per cent and aflatoxin content in commercial market samples were in the range of 3 to 18 µg per kg. In Japan, aflatoxin B1 was found in the exports from 20 out of 31 countries; five lots of large type raw shelled groundnuts and 269 lots of small type raw shelled groundnuts were rejected as they had aflatoxins above the regulation level (10 ppb) of aflatoxin B1 (Itoh *et al.*, 2001) [18]. Regulation was set by European Union (EU) for maximum permissible limits of aflatoxins in foodstuffs as presented below.

Table: Maximum permissible limits of aflatoxins in food stuffs and animal feeds

Sl. No.	Products	Aflatoxins : Maximum admissible level (µg/kg)	
1.	Human consumption: Commission regulation (EC) No. 1525/98 date-16/07/1998	B1	B1+B2+G1+G2
	➢ Peanuts intended for direct human consumption or as ingredient in food stuffs	2	4
	➢ Peanuts to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in food stuffs	8	15
2.	Animal and bird feed council directive: 1999/29/EC date- 22/04/1999		
	➢ Peanuts intended for direct usage as nutrient dietary for animal and bird (maximum content in µg/kg related to a feeding stuff with a moisture content of 12%)	20	
	➢ Peanuts to be subjected to further processing before animal and bird consumption or use as an ingredient in feeding stuff (maximum content in µg/kg related to a feed material with a moisture content of 12%)	200	

Craufurd *et al.* (2006) [19] conducted experiments on JL-24 with objectives 1. To examine the effects of sowing date and irrigation treatments on pod yield, infection with *A. flavus* and aflatoxin concentration; and 2. To quantify relations between infection, aflatoxin concentration and soil moisture stress, in Niger and West Africa. Seeds were sown at two to four different sowing dates under four different irrigation treatments (rain fed and irrigation at 7, 14 and 21 days intervals) between 1991 and 1994, giving 40 different 'environments'. In general, early sowing produced greater pod yields, as well as less infection and lower aflatoxin quantity. There were negative linear correlations between infection (r2 = 0.62) and the average simulated fraction of extractable soil water (FESW) between flowering and harvest, and between aflatoxin quantity (r2 = 0.54) and FESW in the last 25 days of pod- filling. This field study confirmed that infection and aflatoxin concentration in peanut should be related to the occurrence of soil moisture stress during pod-filling. Devaiah *et al.* (2011) [20] found that, as the seed moisture content decreases due to drought, the capacity of seed to produce phytoalexins decreases resulting in *Aspergillus* invasion and aflatoxin contamination. Drought stress and drought stress mediated-fungal infection compromise peanut defence and aggravate aflatoxin formation in the seeds. Rahmianna *et al.* (2015) [21] evaluated ten groundnut genotypes for aflatoxin contamination under drought conditions at later stage of reproductive growth (66-95 DAS) and found that only one genotype GH 51 had aflatoxin contamination under the safe level (≤ 10 ppb), with <15 per cent of seed number infected by *A. flavus.*

Waliyar *et al.* (2016) [12] evaluated ICRISAT's groundnut mini core collections with the objective to identify stable and reliable resistance sources to pre-harvest aflatoxin contamination. Field studies conducted during 2008 and 2009. Superior accessions (34) were selected and screened during 2010 and 2011. Seven best accessions with <1 µg kg-1 aflatoxin B1 levels were further selected and screened during 2012 and 2013. Based on the evaluation in 2008 and 2009, four accessions had aflatoxin contamination within 4 µg kg-1. Of the 34 selected accessions evaluated in 2010 and 2011, eight accessions had <1 µg kg-1. In total, 31 accessions had less aflatoxin contamination than the resistant check, 55–437. The seven best accessions, ICGs 13603, 1415, 14630, 3584, 5195, 6703 and 6888, over six years (2008– 2013) consistently accumulated very low levels of aflatoxin (<4 µg kg-1). These seven accessions can be utilized as potential sources for understanding the resistant mechanisms and can be further used in developing resistant cultivars.

Groundnut is affected by several production constraints. Since the crop is mostly grown under rain-fed and low input condition, it is essential that new groundnut varieties carry resistance to multiple stresses operating in a region in the appropriate maturity backgrounds. Since groundnut is also used as food, it is essential that quality traits receive adequate attention in genetic enhancement. Management of aflatoxin contamination requires both preventive and curative approaches starting from sowing, harvesting up-to processing and storage. Hence, resistant cultivars will be a useful, low-cost and eco-friendly part of an integrated aflatoxin management program and it is the most viable and economical solution to aflatoxin problem in groundnut.

Material and Methods

Totally, 66 genotypes were used for field screening. The seeds were sown in two replications each for moisture stressed and normal moisture plots in a randomized complete block design. Each genotype was sown in 2 rows per replication with spacing of 30 cm between rows and 15 cm between plants within a row. For normal moisture plots, irrigation was given as per package of practices (once in 5-6

days). For moisture stressed plots, water stress was imposed by withdrawing irrigation for 15 days at pod development stage. ICGV- 91114 was used as tolerant check for drought tolerance. Observations were recorded from 5 randomly tagged plants from each replication for each genotype in moisture stressed and normal moisture plots. Root zone soil of both moisture stressed and normal moisture plots were made sick for *Aspergillus flavus* by pouring a broth culture of *Aspergillus flavus* to root zone of plants once at flower initiation stage and second at peg initiation stage using a spore concentration of 4×106 spores ml-1in the broth. Pathogen population in root zone was confirmed by isolation of the pathogen at 106 times dilution levels.

Aflatoxin content of the seed samples of each treatment in each replication was estimated by using Indirect ELISA at ICRISAT, Hyderabad which was expressed as µg/kg.

Results

Analysis of variance was done to test the significance of differences among genotypes for aflatoxin contamination under both moisture stress and normal moisture condition. Analysis of variance revealed that the genotypes under study differed significantly even at one per cent level of probability for aflatoxin contamination in both moisture stress and normal moisture plots. The mean sum of squares is presented in the Table 1 for normal moisture and moisture stress plots.

The mean aflatoxin content under normal moisture condition was 0.26µg/kg with a range of 0 to 1.70µg/kg. The phenotypic and genotypic coefficients of variation were 128.81 and 127.70 per cent, respectively. The heritability for the trait was 98.28 per cent with a genetic advance over mean of 260.79 per cent. However, under moisture stress condition, the mean aflatoxin content was 0.29µg/kg with a range of 0 to 2.08µg/kg. The phenotypic and genotypic coefficients of variation were 112.87 and 111.35 per cent, respectively. The heritability for the trait was 97.33 per cent with a genetic advance over mean of 226.30 per cent (Table 2).

Table 1: Analysis of variance for aflatoxin contamination under normal moisture and moisture stress condition

Source	d.f.	Normal moisture	Moisture stress
Replications	1	0.00	0.00
Genotypes	65	0.23**	0.22**
Error	65	0.00	0.00
S. Em		0.02	0.03
C.V. (%)		16.89	18.44
C.D. @ 5%		0.09	0.11
C.D. @ 1%		0.12	0.14

Where
**: Significance at 1%
S.Em: Standard error of mean C.V- Coefficient of variation C.D- Critical difference
D.F.: Degrees of freedom

Table 2: Genetic components of variation for aflatoxin contamination under normal moisture and moisture stress condition

Aflatoxin contamination (µg/kg)	Average	Range	GCV (%)	PCV (%)	h2bs (%)	GAM (%)
Normal moisture	0.26	0.00-1.70	127.70	128.81	98.28	260.79
Moisture stress	0.29	0.00-2.08	111.35	112.87	97.33	226.30

Where
GCV- Genotypic coefficient of variation PCV- Phenotypic coefficient of variation h2bs – Heritability (broad sense)
GAM- Genetic advance as per cent over mean

Plate 1: General view of experimental plot

Comparative mean performance of groundnut genotypes for aflatoxin contamination under normal moisture and moisture stress conditions is presented in Table 3. It was observed that, the overall mean aflatoxin contamination of all the genotypes under moisture stress (0.29µg/kg) was more than that of normal moisture (0.26µg/kg). Among the test genotypes, the mean aflatoxin contamination under normal moisture was in the range of 0 to 1.70µg/kg against 0 to 2.08µg/kg under moisture stress condition. Twelve genotypes *viz*., ICGV-15119, Dh-234, Dh-246, Dh-216, K-9, Dh-101, Dh-86, VB-T31, SB-T14, SB-T2, SB-T13 and J-11
did not show any aflatoxin contamination (0µg/kg) under both the situations (normal moisture and moisture stress) as indicated in the Table 4.

Under normal moisture, maximum aflatoxin contamination was recorded in case of ICGV-15146 (1.70µg/kg) followed by ICGV-15151 (1.23µg/kg) and ICGV-15152 (1.19µg/kg). However, under moisture stress, ICGV-15141 (2.08µg/kg) recorded highest aflatoxin load followed by ICGV-15143 (0.98µg/kg) and TMV 2 (0.7µg/kg).

Discussion

In the present study, aflatoxin contamination generally increased under moisture stress condition as compared to normal moisture condition. The total number of genotypes affected and the amount of aflatoxin production in each infected genotype was generally high under moisture stress condition as compared to normal moisture condition. Overall mean was increased by 11.53 per cent under moisture stress condition as compared to normal moisture condition. This clearly demonstrates that, aflatoxin contamination would increase under moisture stress conditions especially during pod maturation stage of the plant growth. This would be possibly due to formation of cracks in the pod walls under moisture stress which would facilitate fungal invasion and aflatoxin contamination.

Devaiah *et al.*, (2011) [20] reported that as seed moisture content decreases during drought, the capacity of seed to produce phytoalexins decreases resulting in *Aspergillus* invasion and subsequent aflatoxin production. Some of the enzymes that are induced in response to fungal attack such as

chitinases, osmotins, peroxidases, and proteases are also adversely affected during drought stress through cell membrane-mediated mechanisms. Drought stress and drought stress mediated-fungal infection compromise peanut defence and exacerbate aflatoxin formation in the seeds (Guo *et al.*, 2006) [26].

Table 3: Comparative performance of groundnut genotypes for aflatoxin contamination under normal moisture and moisture stress conditions

Sl. No	Genotypes	Aflatoxin content (µg/kg)			
		Normal moisture	Moisture stress	Mean reduction	Change in % mean
1	ICGV-15114	0.82	0.45	0.37	45.20
2	ICGV-15119	0.20	0.20	0.00	0.00
3	ICGV-15120	0.45	0.10	0.35	77.78
4	ICGV-15122	0.65	0.30	0.35	53.85
5	ICGV-15123	0.00	0.45	-0.45	-100.00
6	ICGV-15124	0.00	0.60	-0.60	-100.00
7	ICGV-15138	0.65	0.00	0.65	100.00
8	ICGV-15141	0.00	2.08	-2.08	-100.00
9	ICGV-15143	0.00	0.98	-0.98	-100.00
10	ICGV-15145	0.30	0.35	-0.05	-16.66
11	ICGV-15146	1.70	0.00	1.70	100.00
12	ICGV-15148	0.75	0.20	0.55	73.22
13	ICGV-15149	0.59	0.40	0.19	32.09
14	ICGV-15151	1.23	0.60	0.63	51.02
15	ICGV-15152	1.19	0.00	1.19	100.00
16	ICGV-15153	0.57	0.00	0.57	100.00
17	ICGV-15154	0.00	0.70	-0.70	-100.00
18	ICGV-15158	0.00	0.25	-0.25	-100.00
19	ICGV-15159	0.25	0.00	0.25	100.00
20	ICGV-15161	0.00	0.50	-0.50	-100.00
21	Dh-241	0.25	0.00	0.25	100.00
22	Dh-235	0.25	0.55	-0.30	-123.06
23	Dh-234	0.00	0.00	0.00	0.00
24	Dh-243	0.00	0.30	-0.30	-100.00
25	Dh-245	0.00	0.30	-0.30	-100.00
26	Dh-246	0.00	0.00	0.00	0.00
27	Dh-247	0.72	0.40	0.32	44.14
28	Dh-216	0.00	0.00	0.00	0.00
29	K-6	0.25	0.15	0.10	39.42
30	K-9	0.00	0.00	0.00	0.00
31	ICGV-91115	0.25	0.45	-0.20	-82.30
32	Dh-101	0.00	0.00	0.00	0.00
33	G2-52	0.00	0.45	-0.45	-100.00
34	GPBD-4	0.30	0.00	0.30	100.00
35	Dh-86	0.00	0.00	0.00	0.00
36	TMV-2	0.00	0.70	-0.70	-100.00
37	GPBD-5	0.00	0.25	-0.25	-100.00
38	LOCAL-1	0.45	0.65	-0.20	-43.66
39	R-2001-3	0.20	0.00	0.20	100.00
40	VB-T4	0.00	0.40	-0.40	-100.00
41	KCG-6	0.00	0.40	-0.40	-100.00
42	SB-T1	0.25	0.00	0.25	100.00
43	SB	0.10	0.45	-0.35	-335.21
44	VB-T11	0.25	0.00	0.25	100.00
45	VB-T14	0.25	0.51	-0.26	-102.04
46	SB-T7	0.20	0.00	0.20	100.00
47	SB-T14	0.00	0.00	0.00	0.00
48	LOCAL-2	0.40	0.20	0.20	49.94
49	LOCAL-3	0.00	0.40	-0.40	-100.00
50	SB-T15	0.40	0.40	0.00	0.11
51	ICGV-91114	0.35	0.25	0.10	28.57
52	VB-T31	0.00	0.00	0.00	0.00
53	SB-T12	0.45	0.45	0.00	-0.23
54	SB-T13	0.00	0.00	0.00	0.00
55	KCG-2	0.15	0.20	-0.05	-33.16
56	VB-T13	0.25	0.40	-0.15	-60.32
57	VB	0.30	0.40	-0.10	-34.03
58	VB-T18	0.00	0.65	-0.65	-100.00
59	SB-T3	0.25	0.00	0.25	100.00
60	SB-T40	0.25	0.25	0.00	-0.62
61	VB-T35	0.70	0.45	0.25	35.71

62	SB-T2	0.00	0.00	0.00	0.00
63	SB-T10	0.45	0.70	-0.25	-55.56
64	J-11	0.00	0.00	0.00	0.00
65	JL-24	0.50	0.25	0.25	50.00
66	GKVK-5	0.00	0.35	-0.35	-100.00
Average		0.26	0.29	-0.03	-11.53

-Sign indicates increase in value under moisture stress condition over normal moisture condition
+Sign indicates decrease in value under moisture stress condition over normal moisture condition

Table 4: Genotypes having tolerance to aflatoxin contamination under both normal moisture and moisture stress condition

Sl. No	Genotype	Aflatoxin content (µg/kg)	
		Normal moisture	Moisture stress
1	Dh-86	0	0
2	Dh-101	0	0
3	Dh-216	0	0
4	Dh-246	0	0
5	SB-T2	0	0
6	SB-T13	0	0
7	SB-T14	0	0
8	VB-T31	0	0
9	K-9	0	0
10	ICGV-15119	0	0
11	Dh-234	0	0
12	J-11 (RC)	0	0

Susceptible check TMV-2 has shown 100% increase in aflatoxin contamination under moisture stress condition as compared to normal moisture condition. Similar results were reported by Mehan *et al.*, (1986) [23], Craufurd *et al.*, (2006) [19] and Waliyar *et al.* (2016) [12].

Breeding for drought tolerance can be accepted as one of the strategies for developing aflatoxin tolerant peanut cultivars, which would not only minimize water usage but also help expand peanut production in marginal and sub-marginal soils. These results are in accordance with Davidson *et al.*, (1983) [24] and Blankenship *et al.*, (1985) [25].

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