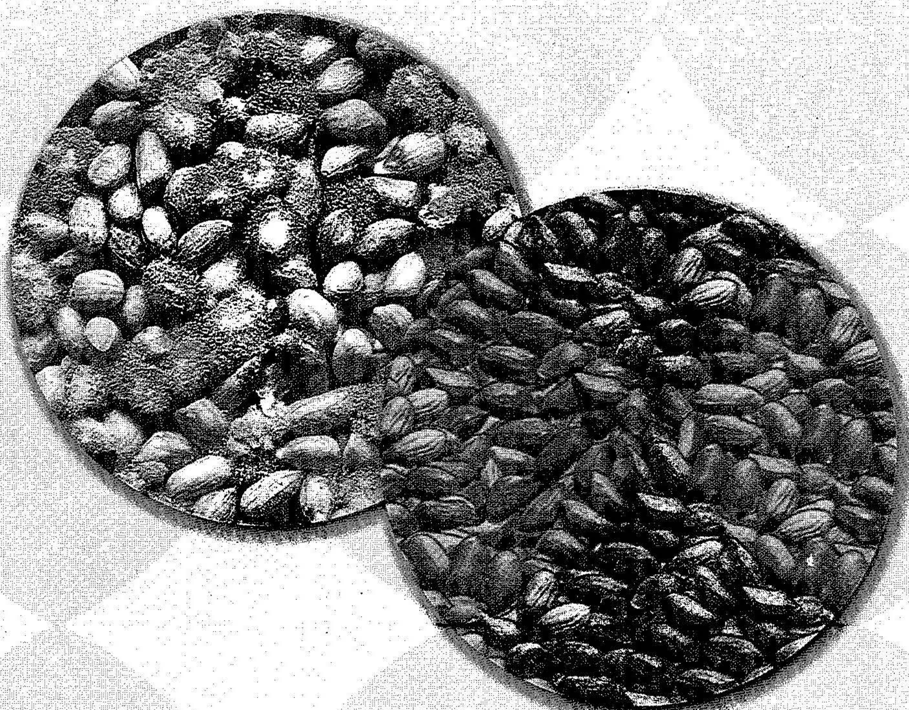
Information Bulletin

National Agricultural Technology Project

Mapping and Management of Aflatoxin Contamination in Groundnut in Gujarat, Andhra Pradesh and Karnataka



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International Crops Research Institute for the Semi-Arid Tropics
Patancheru, 502 324, Andhra Pradesh

NATIONAL AGRICULTURAL TECHNOLOGY PROJECT

Mapping and Management of Aflatoxin Contamination in Groundnut in Gujarat, Andhra Pradesh and Karnataka

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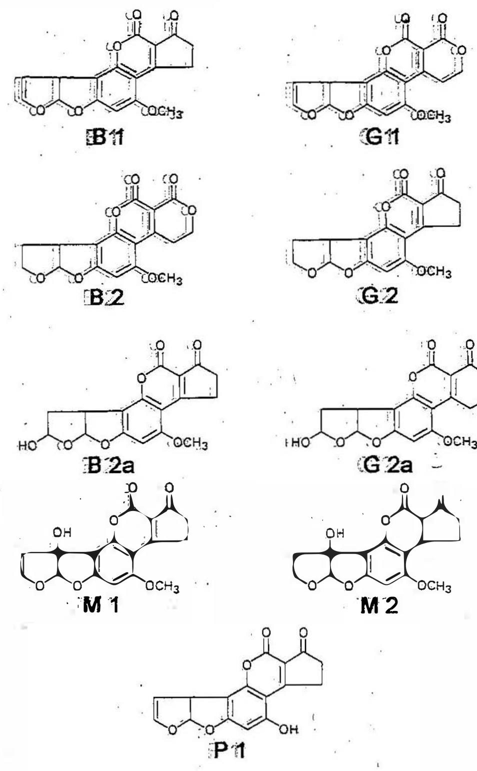
Preamble

Aflatoxins are non-additive food contaminants that occur in groundnut and its extractions under certain production systems and hence affect their value as food and feed. Aflatoxins are also considered a non-tariff trade barrier. The groundnut export industry is suffering to a large extent due to the stringent regulations of tolerance

importers. The finance and resource burden on farmers for detection and amelioration of these mycotoxins in ingredient rations and in the production environment is significant. The problem is further compounded due to the unorganized structure of the export set up and a general ignorance or inadequate knowledge of both the farmers and processors about aflatoxins. The impact of aflatoxin contamination in groundnut has slowly been assuming a larger dimension because the promotion of groundnut as food and feed is slowly becoming apparent in addition to its importance in export. The problem is not obvious like other diseases which produce visible symptoms

due to infection.



Different kinds of aflatoxins

Considering the significance of the problem, the National Agricultural Technology Project has granted a project "Aflatoxin Contamination

Andhra Pradesh and Adjoining Areas" covering semi-arid tropic zones of Gujarat (Junagadh, Rajkot, Amreli, Jamnagar and Porbandar districts), Andhra Pradesh (Kurnool, Cuddapah, Ananthpur, and Chittoor districts) and two adjoining districts of Karnataka (Kolar and Tumkur).

Objectives

The objectives set for the project were

- To assess the extent of awareness among farmers about the aflatoxin contamination problem and its associated risks and to identify the farmers' cultural practices that may influence aflatoxin contamination.
- To document the current pre- and post-harvest practices that help to contain aflatoxin contamination

- On farm validation of integrated aflatoxin contamination management package (in Junagadh, Jamnagar, Ananthpur and Chittoor districts)
- To evaluate the load of aflatoxin of the groundnut produce of different growing seasons
- *In vitro* screening of released varieties for resistance against *Aspergillus flavus* infection and aflatoxin contamination.

Expected output

- Database on aflatoxin awareness and farmers' cultural practices
- Understanding farmers' practices that favour/contain development of *A. flavus* and aflatoxin contamination
- Influence of growing season on incidence of aflatoxin contamination
- Data on reaction of released varieties to *A. flavus* infection and aflatoxin contamination
- Aflatoxin management technologies

Participating Institutes

Lead Centre

National Research Centre for Groundnut (ICAR), Junagadh, 362 001, Gujarat

Participating Centres

Main Oilseeds Research Station (MORS), GAU, Junagadh 362 001, Gujarat

International Crops Research Institute for the Semi Arid Tropics, Patancheru, 502 324, A.P.

Significant achievements

Output 1. Database on aflatoxin awareness and farmers' cultural practices

Awareness survey

At farm level, unlike other pests, since aflatoxin contamination is not visible to naked eye, often it goes unnoticed. Considering this aspect, a survey was conducted to tackle the issues of awareness, database development and collection of pod- and soil-samples for mapping and the distribution of *A. flavus*. A total number of 3501 farmers were interviewed. It was observed that only about 1% of the farmers were aware of this problem. The reasons attributed for this were

- It was not visible (unless there is heavy infestation of seeds by the fungus (which is a rare situation) and
- Farmers do not get the premium for aflatoxin-free groundnuts

In addition, ancillary was collected during survey. Using this information a retrievable database in MSACCESS is being prepared.

Seed infection, aflatoxin contamination and soil population of *Aspergillus*

During survey pod samples were collected from the target districts and analysed for infection by *A. flavus* and aflatoxin content. A total number of 1570 samples (pods and soil) were collected from target districts of Gujarat and 1931 samples from Andhra Pradesh and Karnataka. The collected samples were analyzed for their seed infection, seed colonization by seed plating on Czapek's Dox Agar medium while, soil population of *A. flavus* was determined by using serial dilution methods on the same medium. The district-wise range of soil population of *A. flavus*, seed infection, seed colonization and aflatoxin content are given Plate 1.

Field examination in Tumkur

Soil sample collection in Tumkur

Across the states the infection was scattered. However, the range of infection varied across the districts suggesting that there is a possibility to get areas with low risk of aflatoxin contamination. In some cases, there was no relationship between load of soil fungal population and aflatoxin content. This suggests occurrence of non-toxigenic strains. More than 400 isolates of *Aspergillus* have been collected and they are being characterized for their aflatoxigenicity and other traits. The strains varied for thier sclerotial size, growth rate and sporulation.

Output 2. Suitability of produce of different growing seasons

A total of 550 (pre-monsoon - 92; monsoon - 323; and summer - 135) groundnut pod samples were collected from different villages across 27 talukas of five districts (Junagadh, Porbandar, Rajkot, Amreli and Jamnagar) of Saurashtra region of Gujarat during 2002-03 for assessing the suitability of groundnuts that are free from aflatoxins for food purpose (Table 1).

Among the pre-monsoon (2002) samples, 26% were free from infection, 44 % were free from colonization and none of the samples was free from aflatoxin. Less than 2% infection and colonization were recorded in 16% and 33% samples, respectively and only 12% samples showed less than two ppb aflatoxin. The ranges of infection, colonization and aflatoxin content in samples were 0-34%, 0-12% and 0.71-366.3 ppb, respectively.

From the monsoon (2002) crop samples, 21% and 36% samples were free from seed infection and colonization, respectively and none was free from aflatoxin. Whereas 11% and 22% samples showed less than two per cent seed infection and colonization, respectively, only 8% showed less than two ppb aflatoxin. The ranges of infection, colonization and aflatoxin content in samples were 0-50%, 0-21% and 0.037-8735.8 ppb, respectively.

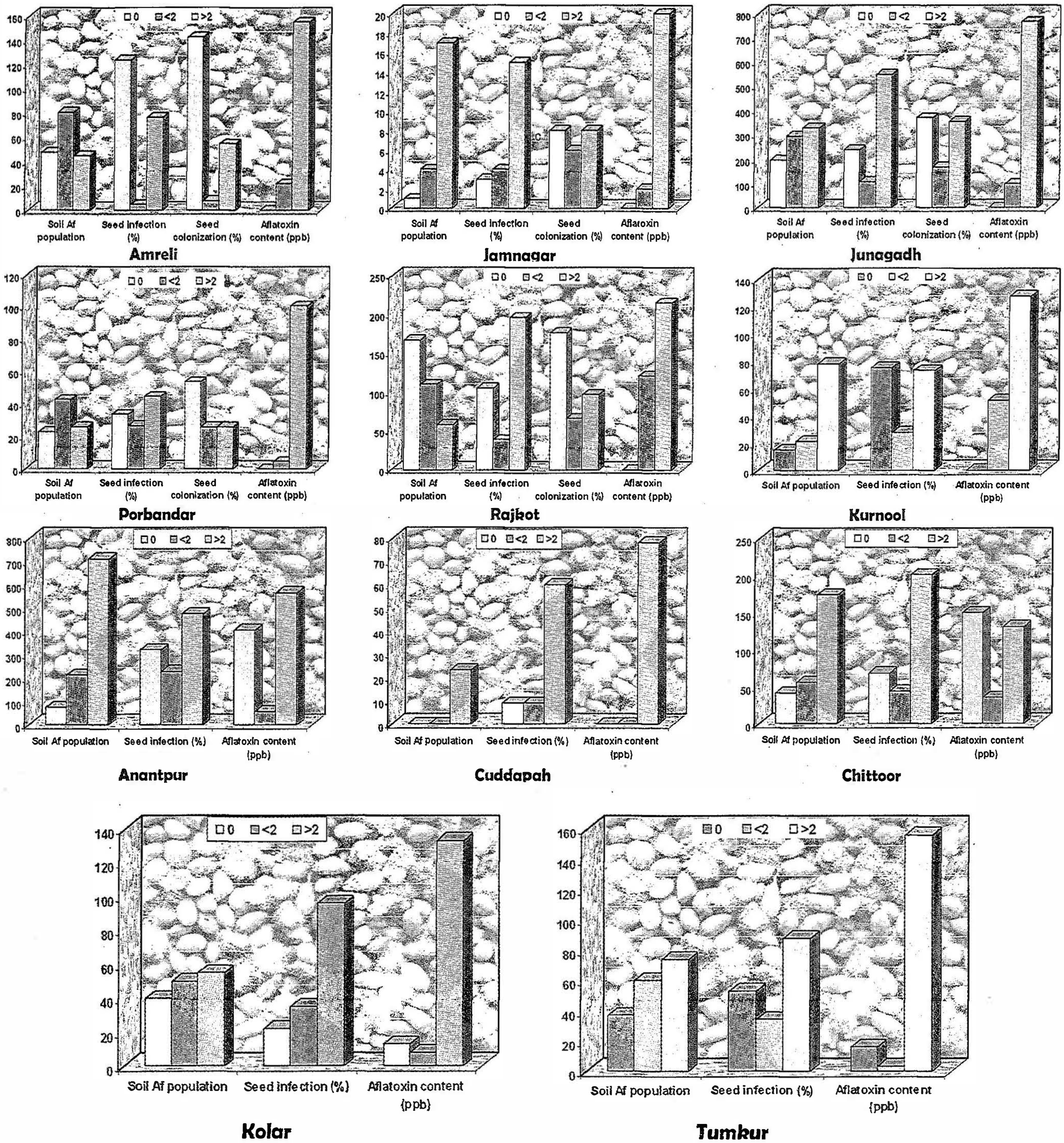
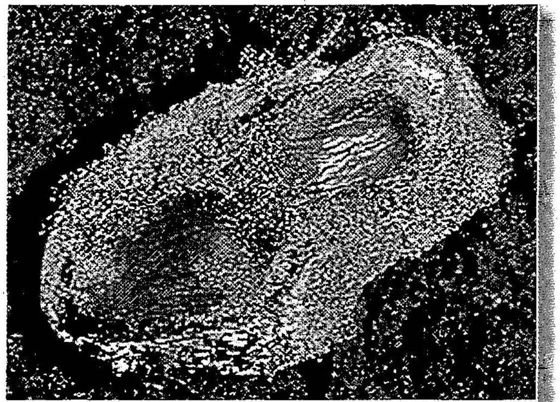


Plate 1. Distribution of soil *Aspergillus* population, infection, colonization and aflaoxin content in groundnut in target districts of Gujarat, A.P. and Karnataka

Among the summer (2003) crop samples, maximum samples were free from infection and colonization. While 64% samples were free from infection, 18% showed less than 2% infection. About 81% were free from colonization, 11% showed less than 2% colonization. Even though, none of the samples was free from aflatoxin, 23% samples showed less than two ppb aflatoxin. The ranges of infection, colonization and aflatoxin content in samples were 0-16%, 0-6% and 0.24-154.34 ppb, respectively. Hence, it is concluded that summer groundnuts could be diverted for food purpose where there is a probability of getting produce that could be within tolerance limits.



A single *A. flavus* infected pod split-open

Table: 1. Screening of the produce of different seasons from Gujarat

Observation	0	≤ 2	> 2
Pre-monsoon crop samples (92)			
Seed infection %	24 (26%)	13 (16%)	55 (60%)
Seed colonization %	39 (44%)	31 (33%)	22 (24%)
Aflatoxin content (ppb)	0	11 (12%)	81 (88%)
Monsoon crop samples (323)			
Seed infection %	67 (21%)	35 (11%)	221 (68%)
Seed colonization %	117(36%)	69 (22%)	134 (41%)
Aflatoxin content (ppb)	0	27 (8%)	296 (92%)
Summer crop samples (135)			
Seed infection %	87 (64%)	24 (18%)	24 (18%)
Seed colonization %	109 (81%)	15 (11%)	11 (08%)
Aflatoxin content (ppb)	0	31 (23%)	104(77%)

A total of 582 (monsoon - 445; and rabi - 137) groundnut pod samples were collected from different villages of Andhra Pradesh and Karnataka during 2002-03 for assessing the suitability of groundnuts that are free from aflatoxins for food purpose (Table 2).

Among the monsoon (2002) samples, 41% were free from infection and 13 % of the samples were free from aflatoxin. Less than 2% infection and aflatoxin content were recorded in 19% and 31%

Table: 2. Screening of the produce of different seasons from Andhra Pradesh and Karnataka

Observation	0	≤2	>2
Monsoon samples (445)			
Seed infection (%)	184 (41 %)	86 (19%)	175 (39%)
Af population	163 (37%)	158 (35%)	124 (28%)
Aflatoxin content (ppb)	57 (13%)	136 (31%)	252 (57%)
Rabi samples (137)			
Seed infection (%)	19 (14%)	41 (30%)	77 (56%)
Af population	29 (21%)	30(22%)	78 (57%)
Aflatoxin content (ppb)	33 (24%)	46 (34%)	58(42%)

samples, respectively and 39% and 57% samples having more than 2 % infection and two ppb aflatoxin content, respectively. In case of soil population of *A. flavus,* 13% samples were free from infection, 31 % samples showed less than 2 and 57 % samples were recorded for more than 2 cfu per gram of soil. From the rabi (2002) crop samples, 14 % samples were free from infection, 30 % samples showed less than 2 % infection and 56 % samples were recorded for more than 2 % seed infection. In case of soil population of *A. flavus*, 21% samples were free from infection, 22 % samples showed less than 2 and 57 % samples were recorded for more than 2 cfu per gram of soil. Where as 24 % samples were free from aflatoxin content and 34 % and 42 % samples were recorded for less than two ppb and greater than two ppb aflatoxin content, respectively.

Output 3. Host plant resistance

Ninety-two released cultivars were evaluated in concrete blocks under artificially inoculated conditions. During screening, the population of Af 11-4 was constantly monitored to ensure sufficient load of fungus, (Figure 1). After harvest, seed infection ranged from 0 to 28% and seed colonization ranged from 0-20%. Aflatoxin content ranged from 1.17 (ICGV 86325) to 196.59 (ICGS 44) ppb. The genotypes that supported less than 5 ppb aflatoxin are shown in Table 3.

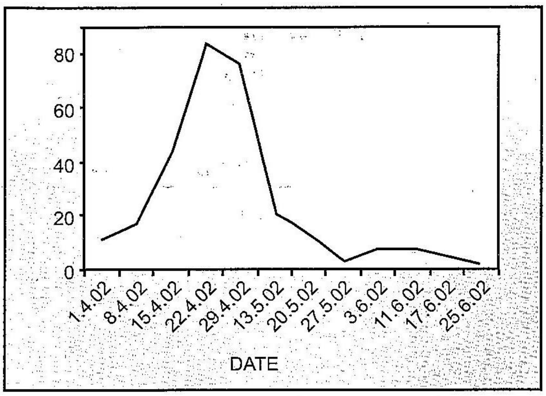


Figure 1 Monitoring of the population of *A.flavus* in artificially infested concrete blocks

One hundred advanced breeding lines supplied by ICRISAT were screened under artificially inoculated conditions. The promising genotypes (19) were identified and screened in a replicated trial subsequently. Among them, 11 genotypes did not show infection again. Aflatoxin content in

Table 3. Reaction of selected released cultivars of groundnut against *A. flavus* (crop raised in artificially infested concrete blocks)

Cultivar	Seed infection %	Seed colonization %	Aflatoxin content (ppb)
CO-1	0	0	3.89
GG-12	9	6	3.38
ICGS-11	0	0	5.09
S-230	4	0	5.09
ICGV-86325	10	5	1.17
M-148	3	0	3.06
ALR-3	3	3	4.85
Tirupati-1	0	0	
Punjab-1	0	0	3.73
CSMG-884	8	4	1.59

these genotypes ranged from 0.06 (ICGV 01149) to 75.96 ppb (ICGV 01119). The genotypes that showed consistently low infection and colonization levels and less than 5 ppb aflatoxin contamination are presented in Table 4.

Table 4. Seed infection, colonization and aflatoxin content of elite accessions of groundnut raised under artificially inoculated conditions

Genotype	Infection%			Colonization%			Aflatoxin
	2001	2002	Mean	2001	2002	Mean	(ppb)
ICGV 01096	0	10	5	0	10	5	4.99
ICGV 01161	0	0	0	0	0	0	2.21
ICGV 01162	0	4	2	0	0	0	3.89
ICGV 01163	0	0	0	0	0	0	3.88
ICGV01158	0	0	0	0	0	0	3.96
ICGV 01105	0	8	4	0	8	4	1.66
ICGV01092	0	6	3	0	6	3	4.08
ICGV 01149	0	0	0	0	0	0	0.06
ICGV 01115	0	0	0	0	0	0	3.32
ICGV 01126	0	2	1	0	0	0	3.29
ICGV 01093	0	0	0	0	0	0	2.20
GG-20 (control)	40	40	40	20	20	20	366.39

As seen from the above table, these accessions appear to be promising and could be used as part of integrated management strategy, once the farmers accept them as agronomically desirable varieties. Interestingly, some of these genotypes are agronomically superior too with high pod yield.

Output 4. Integrated management module: On-farm evaluation

For developing an effective integrated management package, there is a need to identify the stages at which technology interventions are essential. To arrive at the same Hazard Analysis Critical Control Point Analysis of aflatoxin contamination was done.

Hazard Analysis of Critical Control Point for Aflatoxin Contamination

Hazard Analysis and Critical Control Point (HACCP), pronounced hassip, focuses on preventing hazards that could cause food-borne illnesses by applying science-based controls, from raw material to finished products. HACCP involves seven principles viz.

i) Analysis of hazards
ii) Identification of critical control points
iii) Establishing preventive measures with critical limits for each control point
iv) Establishment of procedures to monitor the critical control points
v) Establishment of corrective actions to be taken when monitoring shows that a critical limit has not been met
vi) Establishment of procedures to verify that the system is working properly and
vii) Effective record keeping and documentation of the HACCP system.

The advantages

- Focuses on identifying and preventing hazards from contaminating food
- Is based on sound science
- Record keeping allows investigators to see how well a system is complying with food safety laws over a period rather than how well it is doing on any given day
- Places responsibility for ensuring food safety appropriately on the concerned and
- Helps in developing healthy competition in the world market and reduces barriers to international trade.

Some critical stages of aflatoxin contamination in groundnut

Density of Fungal population and cropping sytem

Aspergillus distribution varies significantly across agro-ecological locations and hence the degree of infection also could vary. Across A.P., Gujarat and Karnataka, it was observed that the soil fungal population varied from 0-730x10^3 cfu/g. In addition, the toxigenicity of the isolate is also important. Cropping system also influences the survival of the fungus.

Soil temperature & soil moisture deficit stress

During severe soil moisture deficit, 75-80% pod infection could occur. The optimum conditions for the fungal development are 28-30.5°C in pod zone & 20-30 days of end of season drought. However, at 26.3°C less contamination is reported. Extended drought at low temp has similar effect as that of short spells of drought at optimum temperature for fungal development. 45% infection was reported in irrigated soils at 34.5°C but only 2-3% in cool soils at 25.2°C.

Infection via peg, pod and seed

Aerial pegs are vulnerable showing 1.5 to 3% infection. Similarly, over mature and damaged pods are also prone to fungal infection.

During harvest and processing

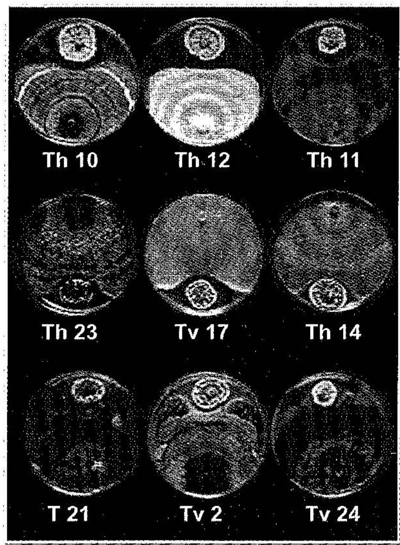
Unseasonal rains as they occur in some parts of the country cause wetting of pods during harvest and drying. Such pods get easily infected due to prevailing warm and humid conditions. Damage to the seed coat during mechanical shelling and moistening of pod during mechanical shelling predispose the kernels to fungus.

During storage and transshipment

High humidity in the store and during shipment are congenial conditions for the fungus to proliferate and infect seeds.

Infection via flower

About 7% of field-collected flowers were shown to be infected including that of developing ovaries. Warm and humid weather at blooming could lead to infection through flowers.

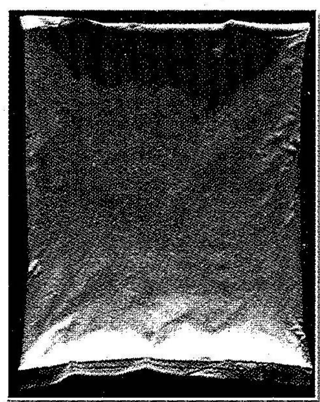


In vitro* screening of *Trichoderma* against *A. flavus

On-farm trials

For the management of aflatoxin contamination, it is necessary to identify the critical control points that are congenial for multiplication of *A. flavus* and the stages of the crop that are predisposed to the infection by the fungus. Based on the published literature, such critical points have been identified and for each of the critical points the management options were identified. These options were configured into an integrated aflatoxin contamination management package. This package targeted at pre-and post-harvest aflatoxin contamination and was evaluated for two years under farmers' conditions. The package included

- Summer ploughing
- Healthy seed @120 kg/ha
- Seed treatment with carbendazim @ 2g/kg
- Furrow application of *Trichoderma* (biocontral agent tested at the NRCG) based in castor cake/FYM @ 500 kg/ha
- Harvest at right maturity
- Drying to reduce pod moisture quickly to less than 9%
- Sorting of diseased pods



Formulated *Trichoderma*

- Plant protection esp. management of leaf spots, rust, and stem rot, managing insect pests. During last three years (2001-2004) under this project, 244 on-farm trails in 56 villages of 16 districts of Gujarat, Andhra Pradesh and Karnataka were conducted to evaluate the effectiveness of improved package over farmers' practices.

From the results of three years of on-farm trials, it was found that the integrated aflatoxin management package was useful in reducing the infection, colonization and aflatoxin content (Table 5). The overall reduction in the trials ranged from 0-88%. During kharif2001, in Gujarat, out of36 trials, seed infection was low in 20 trials in treated plots, seed colonization was low in 17 plots and the soil population of *Aspergillus* was reduced in 24 plots. Aflatoxin content was reduced in 28 trials. During 2002, there was a considerable variation in response in the on-farm trials for infection, colonization and aflatoxin content. In 28 trials the infection levels reduced whereas in 21 trials seed colonization was reduced. In 28 trials, there was a reduction in soil *A. flavus* population ranging from 0.8 to 7.4 $x10^3$ propagules per gram of soil as compared to the control plots where the population ranged from 1.6 to $17x10^3$ propagules per gram of soil. In 38 plots, aflatoxin content was reduced ranging from 0.87 to 29.19 as compared to 2.27 to 132.71 ppb which substantiates possibility of encountering atoxigenic strains. In 18 trials, there was a reduction in all three parameters viz. seed infection (0-80%), colonization (0-18%), aflatoxin content (0.87-19.26ppb) as against control plots where seed infection was 4-88%, colonization was 0-30% and aflatoxin content was 4.11 to 21.32 ppb.

Table 5 : On-farm evaluation of aflatoxin contamination

	Gujarat		
Reduction in	***Kharif* 2001 (36 trials)**	***Kharif* 2002 (50 trials)**	***Kharif* 2003 (50 trials)**
Soil population of *A.flavus*	24	27	37
Seed infection	20	28	36
Seed colonization	17	20	34
Aflatoxin content ppb	28	38	41
	Andhra Pradesh		
Reduction in	***Kharif* 2001 (39trials)**	***Kharif* 2002 (50 trials)**	***Kharif* 2003 (50 trials)**
Soil population of *A.flavus*	*31*	*33*	*37*
Seed infection	22	06	36
Aflatoxin content ppb	19	35	41
Yield benefit			
Gujarat	**11.64%**	**12.38%**	**28.36%**
Andhra Pradesh	**29.29%**	**Marginal**	**10.51%**

Similarly, during kharif 2003, in 36 trials the infection levels reduced ranging from 0-10% whereas in 34 trials seed colonization was reduced (0-6 %). In 37 trials, there was a reduction in soil *A. flavus* population ranging from 1-13.8 x10^3 propagules per gram of soil and in 41 plots, aflatoxin content was reduced ranging from 0.06 to 27.4 as compared to 2.27 to 1235.96 ppb over the control. The package was also beneficial in reducing aflaroot incidence as well as stem rot, which is a major problem in majority of farmers' fields. In as many as 74% of the plots reduction in stem rot was observed which was a spin-off of the treatment, probably due to the effectiveness of the combination of castor cake coupled with *Trichoderma.* Aflaroot infection was decreased in 78% of the trials where the improved package was adopted.

Discussion meet

A discussion meet of the farmers was held at the Gujarat Agricultural Unviersity on 20.09.2003 to bring together the farmers who have been participating in the on-farm trials and also other farmers so that they share their experiences to each other and also express their views about the package. Drs. R.R. Khanadar, S. Desai, R.L. Savalia, S.N. Nigam, M.S. Basu and others participated in the discussion meet. The meet was successful as the farmers felt that the package was useful but needed more awareness programmes to encourage the farmers to take up production of aflatoxin-free groundnut production.

Future prospects

In the present study, the low-risk plots were scattered, probably due to sampling pattern. If scaled up, there is a possibility to get low-risk areas. The formulation technology appears to be viable and worth scaling up. The HACCP based management strategy appears feasible and thus has to be further studied for further improvement.

Significant findings

1. There were wide variations in seed infection, soil population among the target districts. In general, in Gujarat, the infection levels were low as compared to Andhra Pradesh and Karnataka.
2. The farmers in general were not aware of aflatoxin contamination and they were enthusiastic to know about it.
3. Considering the significance of the problem, NGOs, Government Organizations and Private Processing Entrepreneurs are willing to put their together for production of aflatoxin-free groundnuts.
4. During many meetings of the farmers, trade houses and processors, the clientele are interested in not only knowing about the problem but also get their samples tested.
5. With further more in depth analyses, there appears to be a scope for identifying the no risk zones aflatoxin contamination and also segregation and development of specific groundnut production systems for various purposes as against the present practice of post-mortem approach of cultivating groundnuts and then deciding as to where to be consumed.

Publications from the Project

Desai, S, Thakur, R.P., Rao, V.P., and Anjaiah, V. 2000. Characterization of isolates of *Trichoderma* for biocontrol potential against *Aspergillus flavus* infection in groundnut. International Arachis Newsletter 20: 57-59.

Desai, S. and Bandyopadhyay, A. 2001. Cost Effective Tools for Estimation of Aflatoxins and Other Mycotoxins in Groundnut. At the Stakeholders' meet on aflatoxins, ICRISAT, A.P.

Vijay Krishna Kumar, K., Desai, S., Rao, V.P., Nur, H.A. and Thakur, R.P. 2002. Evaluation of an integrated aflatoxin contamination package to reduce pre-harvest seed infection by *Aspergillus flavus* in groundnut. International Arachis Newsletter 22: 42-44.

Vijay Krishna Kumar, K., Thakur, R.P. and Desai, S. 2001. Prevalence of aflatoxin contamination in groundnut in Tumkur district of Karnataka, India. International Arachis Newsletter 21: 37-39.

Invited lectures in seminars/symposia:

Desai, S., R.P. Thakur, A. Bandyopadhyay, R.R. Khandar, and I.U. Dhruj. 2002. An Integrated Approach to Manage Aflatoxin Contamination, a Non-tariff Trade Barrier, in Groundnut and its Extractions: Application of Principles of HACCP. Paper presented at the National seminar on Crop protection in WTO perspective, 22-25, January 2002 held at CPCRI, Kasargod, India.

Desai, S. and M.S. Basu. 2002. Aflatoxin Contamination in Groundnut- A propos Non-tariff Trade Barrier and Food Safety. Paper preseneted at the "Asian Congress of Mycology and Plant Pathology" in the session on Quarantine, SPS and WTO, Mysore, 1-4 Oct. 2002.

Desai, S., R.R. Khandar, I.U. Dhruj, R.D. Yeole, D.S. Kelaiya, U.M. Vyas and N.B. Bagwan (2002). A survey for soil inhabitation and seed infection of groundnut by *Aspergillus flavus* in the Saurashtra region of Gujarat. Poster presented at the "Asian Congress of Mycology and Plant Pathology" Mysore, 1-4 Oct. 2002.