Safeguard Groundnut from Aflatoxin Contamination

Vinod Kumar
M. P. Ghewande
M. S. Basu

NATIONAL RESEARCH CENTRE FOR GROUNDNUT
(INDIAN COUNCIL OF AGRICULTURAL RESEARCH)
P. B. 5, JUNAGADH - 362 001, GUJARAT, INDIA

October, 2005

Citation : Vinod Kumar, Ghewande, M. P. and Basu, M. S. 2005. Safeguard groundnut from aflatoxin contamination. National Research Centre for groundnut, Junagadh, India. pp 13.

Published by : DIRECTOR,
NATIONAL RESEARCH CENTRE FOR GROUNDNUT,
P. B. 5, JUNAGADH - 362001,
GUJARAT, INDIA

Telephone : 91-285-2673041/2672461

Fax: : 91-285-2672550

E-mail : director@nrcg.res.in

Printed at : NEW PRAGATI PRINTERS
2/10, Bhaktinagar Station Plot,
Near Meladi Maa Mandir,
Rajkot-360 002.
Phone:0281-2461965. 094260 40829

Preface

Aflatoxin contamination in groundnut is a perpetual problem and has become a major non-tariff trade barrier. Food safety is an important issue in food security and food trade and therefore it has become a major concern throughout the globe. The regulations concerning the permissible limits of aflatoxins in groundnut and its products are very stringent varying between 2 to 20 parts per billion (µg/kg). India is one of the largest groundnut growing countries of the world. The production systems and situations here are quite diverse and the crop is grown almost round the year. The fungal activities causing aflatoxin contamination are usually high in tropical countries like ours where high temperature is associated with high humidity. Often the crop suffers the end-of-season drought under rainfed conditions. This bulletin attempts to offer a handy guideline for farmers, traders, processors and consumers. Information contained in this publication is provided as a general advice.

The authors gratefully acknowledge the editorial support of Dr. J. B. Misra, Principal Scientist and Head Biochemistry and Dr. T. V. Prasad, Scientist, Entomology for scanning the photographs used in this publication. We thankfully acknowledge the cooperation of Dr. N. B. Bagwan, Mr. Diwakar Singh, and Mr. U. M. Vyas, Senior Research Fellows working under Mycotoxin Network Project in the compilation of this bulletin. One of the photographs, "Infection of Cotyledons by *A. flavus*" used in this bulletin has been taken from previous bulletin of N.R.C.G. "Diagnosis and Management of Major Diseases of Groundnut".

Authors

Contents

Safeguard Groundnut from Aflatoxin Contamination

1. What are aflatoxins?

Aflatoxins are a group of toxic compounds produced by fungi *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi invade several agricultural commodities and under certain conditions produce these toxins. Although aflatoxins do not affect the crop productivity as such but contaminate the produce, which have deleterious effects on human and animal health. Thus, the aflatoxins, if present in food commodities, reduce their quality and hence the market value. Occurrence of aflatoxin is the major impediment in the export of groundnut.

Pod infected by *A. flavus*

Kernels colonized with *A. flavus*

The problem of aflatoxin in groundnut was first reported in 1960 with an outbreak of Turkey X disease in England leading to death of about 1,00,000 chicks within a few months. The outbreak was due to use of groundnut meal imported from Brazil. This meal was later on found to be contaminated with aflatoxins.

Aflatoxins are produced as secondary metabolites by the fungi *A. flavus* and *A. parasiticus*. Among 18 different types of aflatoxins identified, major ones are B1, B2, G1 and G2. Aflatoxin B1 (AFB1) is normally predominant in amount in laboratory cultures as well as in food products. Aflatoxins find their way in the milk as aflatoxin M1 and M2, if the animals are fed on diet contaminated with aflatoxins B1 and B2, respectively.

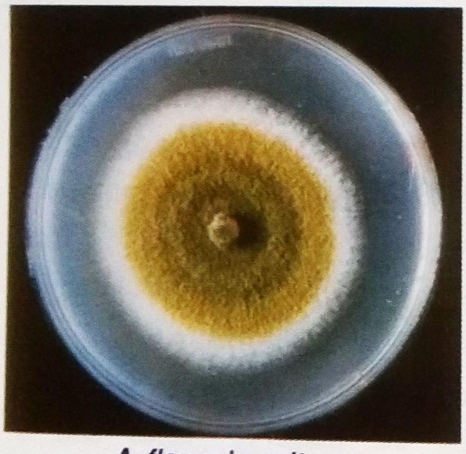
Aflatoxins enter human/animal system through ingestion of contaminated food or their food products.

2. How does *Aspergillus* sp. affect groundnut?

Infection of cotyledon by *A. flavus*

This fungus causes marked deterioration in surface appearance of kernel besides contaminating them with aflatoxins. The infected seeds generally do not germinate well and decay in the soil. Even the ones, which germinate do not produce a healthy plant and often suffer of *aflaroot disease*. The affected plants become stunted; their leaf lamina is drastically reduced with a pointed tip. Such plants do not produce flowers and hence become unproductive.

3. What are the risks associated with consumption of aflatoxin-contaminated foods to human and animal health?



A. flavus in culture

Aflatoxins are carcinogenic and cause cancer of liver. It lowers the body's immune response to invasion by foreign substances and acts synergistically with hepatitis viruses B and C. Indian childhood cirrhosis (ICC), a liver disorder found among children is caused by aflatoxins. Aflatoxin consumption by livestock and poultry causes the disease *aflatoxicosis*. As liver is the chief site of aflatoxin metabolism, consumption of high amounts of aflatoxins can lead to acute liver disease or even death within 72 hours. Presence of aflatoxins in low concentrations in diet of farm animals results in decreased feed efficiency, loss of weight, reduced egg production and contamination of milk.

Aflatoxins are so toxic that they can exert their harmful effects even if present in microgram quantities in the diet. Considering the extremely toxic nature and the practical problems associated with production and maintenance of food and feedstock completely free from aflatoxins, limits of tolerance have been prescribed for various foodstuffs for human and animal consumption (Table1).

Table 1: Effect on health at different levels of aflatoxin consumption

Limit*	Effects on human/animal health
Up to 20 ppb	No health hazard in humans
Up to 50 ppb	No health hazard in animals
Up to100 ppb	Slowed growth of young ones
200-400 ppb	Slowed growth of adults
400 ppb	Liver damage and cancer

* ppb = parts per billion or µg per kg

With the increasing awareness about the possible health hazards, in the past decade aflatoxin load in groundnut and other foodstuffs have become a major trade barrier. The permissible limits prescribed by various countries for aflatoxins are given in table 2.

Table 2: Permissible limits for aflatoxins under international trade

Country	Product	Limit (in ppb)
Australia	Groundnut	16
Belgium	All Foods	5
Canada	Nuts & Nut Products	15
China	Rice & Other Cereals	50
France	Groundnut	1
Japan	Groundnut	10
Denmark	Groundnut	10
Italy	Groundnut	50
European Union	Groundnut	4
U.K.	Nuts & Nut Products	4
U.S.	All Foods	20
India	All Foods	30

4. Why is awareness lacking among farmers, traders, processors and consumers in India?

Aflatoxin is not an innate constituent of groundnut kernels. It is present only in those pods/kernels, which have been invaded by aflatoxigenic fungi. In a given lot of pods/kernels, it is not likely that all the pods/kernels are infected. But even if a single

pod/kernel is infected, it has sufficiently high load of aflatoxin to raise the average level of aflatoxin in the sample beyond the permissible limit.

The groundnut farmers are well aware of damages caused due to insect-pests and diseases and associated economic losses but lack the awareness about aflatoxins because of the facts that

- *Aspergillus flavus* has, so far, not been attributed to cause any appreciable loss of yield in groundnut crop.
- Unless there is an appreciable mycelial growth on the surface of kernels, there are no visible marks of deterioration of surface quality. Thus damage is often hidden and the farmers at the most simply remove the fungus-infected pods or seeds if noticed.
- There is no premium paid to the farmer for the aflatoxin-free product in the domestic market
- In India, groundnut is generally regarded as a source of edible oil and during oil extraction most of aflatoxins, if present is left behind with the cake and does not remain with the oil in appreciable quantities. If the oil is solvent extracted, it becomes totally free from aflatoxins.

5. Which groundnut products may contaminate with aflatoxins?

Almost all products prepared from contaminated kernels are likely to have aflatoxins. Solvent extracted refined groundnut oil is however, one exception. The products that are most likely to have aflatoxins are:

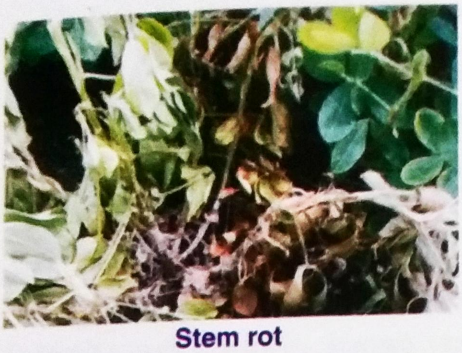
- Physically damaged or shriveled pods and/or kernels
- De-oiled groundnut cake
- Fodder left with contaminated immature pods.
- Peanut butter and other products of groundnut
- Filtered groundnut oil (to some extent if not efficiently filtered)

6. Which factors contribute to aflatoxin contamination in groundnut?

Groundnut pods are formed in soil. *Aspergillus flavus* is a ubiquitous soil fungus. This fungus however is essentially saprophytic in nature and under normal condition does not invade a healthy growing pod. Therefore, the entry of the fungi in the pods is facilitated by certain factors during the pre-harvest, harvest and post-harvest stages.

PRE-HARVEST

The soil population of *A. flavus* varies from farm to farm depending on soil types and crop rotations.



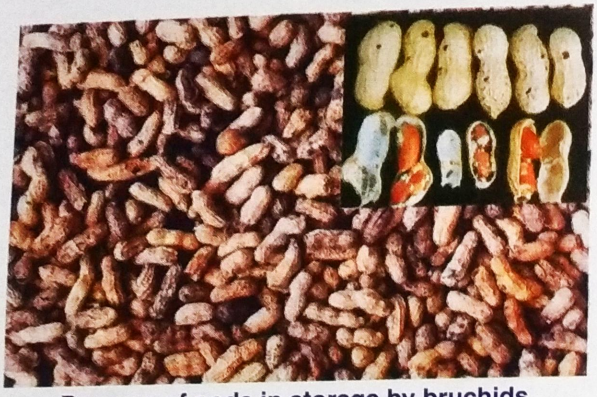
Stem rot

Pod rot

- Disturbances in soil-water-nutrient balance during the crop growth period activate these fungi leading to infection and subsequent aflatoxin production in the kernels.
- Development of cracks during pods growth (growth cracks)
- Mechanical injury to pods during intercultural operations
- Infestation of insect-pests (termites or pod borers) causing damage to pods.
- Death of plants caused by diseases (stem, root and pod rots) at pod maturity stages making the pods vulnerable to invasion by *A. flavus*.
- Nematode damage to the pod.
- High atmospheric temperature (30-40°C) in conjunction with reduced soil moisture availability.
- The crop undergoing protracted dry spell (more than 20 days) before harvest becomes highly vulnerable. Sandy soils become hot spots in such conditions.

POST-HARVEST

- Delayed harvest (over-mature crop)
- Mechanical damage to the pods during harvest.



Damage of pods in storage by bruchids

Bruchid damaged pod later developing *A. flavus*

- Harvesting the crop immediately after irrigation with high initial pod moisture at the time of processing and storage.
- Stacking the harvested plants before bringing the pod moisture level to less than 10%.
- Stacking the harvested plants under high humidity conditions.
- Damage to the pods by insects (such as bruchids) during storage.
- Storing haulms along with immature or small pods (this causes contamination of haulms which is used as forage)
- Gleaning pods from the soil after harvest (the pods left behind in the soils during harvest get infected very easily)
- Rewetting of stored pods due to factors like ground-moisture or roof leakage.

7. Risk factors for groundnut growers

The farmers may consider the following to assess risk levels:

Risk factors	Indicators
Soil moisture availability	No rains for 30 or more days before harvest
Soil (and air) temperatures	Above average during pod filling
Kernel moisture	In a range of 15 to 30 %
Soil population of aflatoxigenic fungi	More than 1000 colonies per gram of soil

8. Strategies for groundnut growers

Although commercial cultivars show some variations in the extent to which their seeds may be invaded by fungus yet the available resistance is grossly inadequate to ensure freedom from aflatoxin contamination. Therefore, among the currently available commercial cultivars none is reported to have resistance against aflatoxin contamination. However, cultivars such as J 11, Karad 4-11, Kopergaon 1, S 230, Chitra, GG 11, Koyana and TKG 19A with moderate level of resistance to *in vitro* seed colonization can be adopted in areas where aflatoxin contamination is a serious problem.

Having assessed the degree of risk and the factors, which leads to *A. flavus* infection and aflatoxin contamination, the growers can make the decisions about the extent to which they implement the following strategies or practices.

(a) Pre-sowing stages

1. Remove stubbles of previous crops/weed flora and keep the field clean.
2. Undertake deep ploughing (8-10 inches) to invert the soil and expose the soil to sun for 2-3 weeks for soil solarization (this reduces soil pests and fungal colonies).

***Trichoderma* in culture**

3. Apply neem (*Azadirachta indica*) cake or castor (*Ricinus communis*) cake @ 500 kg/ha in furrow at the time of sowing. Mix 2.5 kg of commercial formulation of *Trichoderma* with the neem/castor cake, sprinkle a little water, if cakes are too dry, before mixing the *Trichoderma* and keep the

mixture in shade for about a week before applying to the soil.

4. Apply farmyard manure/compost @ 5-10 tons/ha, if available.
5. In rainfed production system, practice inter-row water harvesting adopting paired row method of planting.

Caution

1. Avoid shallow tillage and immediate planking of soils.
2. Do not apply un- or under-decomposed green manure or crop residues in the field.

(b) Sowing and Post-sowing Operations

1. Select short/medium duration variety, which can escape end of season drought at maturity. Advance sowing by a fortnight with a pre-sowing irrigation / pre-monsoon showers helps to evade end- of- season drought
2. Sow only sound seeds and treat them with Carbendazim 50WP (Bavistin) @ 2g/kg one week before sowing. And also treat the seeds again with commercial formulations of *Trichoderma harzianum* or *T. viride* @ 4g/kg seed just before sowing.
3. Apply gypsum @ 400-500 kg/ha at flowering.
4. Spray the crop once with Clorothalonil 75 WP (Kavach) @ 0.2 to 0.3% (or 3g/litre of spray solution) just after the appearance of the first visible symptoms of foliar diseases like rust and leaf spots.
5. Avoid end-of-season drought by providing supplemental irrigation, if possible.
6. Harvest the crop at right maturity (blackening of inner surface of shell).

Caution

1. Do not grow long duration variety, as it would be exposed to drought if cessation of rains occur before pod maturity.
2. Do not delay the sowing much beyond the onset of monsoon.
3. Do not allow rainwater to run off. Rather capture and conserve it *in situ* to avoid protracted dry spells.
4. Avoid use of cultivars with poor seed filling property.
5. Control the development of diseases and build up of insect pest populations by appropriate spray.

6. Do not delay the harvesting for the sake of allowing maturity to a small fraction of pods that are immature.
7. Do not mix the over matured pods, which remain in the soils due to weak pegs strength and are collected later while ploughing the fields as these may contain a high load of aflatoxin.

(c) Harvest and Post-harvest Operations

1. 'Hot spots', the patches of field that have undergone stress or harbored diseases, should be harvested, dried, stocked and disposed off separately.
2. Avoid mechanical damage to the pods during harvesting. Inserting the blade in the pod zone causes the damage.
3. Dry the uprooted plants along with the pods in small heaps by keeping them up side down i.e. foliage towards ground and pods upwards. This facilitates rapid drying and thus shortens the risk-period of invasion by the fungi. Dry the plants till the leaf/pegs become brittle. This takes 6-7 days.
4. Pick the immature pods first and do not mix them with the main lot of mature pods. If mechanical thresher is used, appropriate sieves should be used to isolate immature pods.
5. Remove all the pods showing mechanical or insect damage.
6. Dry thoroughly the sound pods to a safe moisture level of 8%. Well-dried pods produce rattling sound on shaking a handful of pods.
7. Store the produce in new/clean polyethene lined gunny bags and stack them on wooden planks keeping a metre gap from the walls in a well-aerated and well-covered space.
8. Keep the storage space free from any kind of seepage or leakage water that may lead to build up of moisture.
9. Prevent insect damage to the pods in storage by fumigating with phosphene (use 3-5 aluminum phsophide tablets for every 100 kg of pods for 7-8 days).

Caution

1. Do not apply *Khurpi* (hand hoe) / spade to harvest groundnut crop.
2. Do not detach the pods immediately after uprooting the plants. Allow them to dry first along with vines.

3. Do not dry, store or process the immature, damaged, diseased or pest infested pods along with the healthy ones.
4. Do not put freshly harvested plants in the hopper for threshing.
5. Do not mix the gleaned pods (left behind in the field while uprooting the plants and collected much later during ploughing) with the main produce.
6. Do not store the bags by placing them directly on the floor.
7. Do not open the doors of the warehouse or any other vent for at least a week after fumigation.

9. Care to be exercised by traders and exporters

Aflatoxin contamination renders groundnuts and its products unfit for entering the major import markets much more than any other factors. Importers are required by law to systematically test the incoming consignments for the total amount of aflatoxins and reject those exceeding the maximum permitted levels. Hence the exporters need to be aware that each country's regulatory standards for aflatoxin limits are as good as laws, and non-conformity of their consignments may have direct legal consequences. The non-conformity not only results in rejection or downgrading of shipments, but also may even lead to blanket ban of import from particular origins. As stated earlier, the maximum permissible limits for aflatoxins in food and feed products varies from market to market as shown in table 2.

Therefore, it is essential for the trading, processing and export houses to exercise utmost care while handling and storing the farmers' produce. If adequate care is not taken, a good lot purchased from the farmers may build-up aflatoxin during subsequent handling and trans-shipment. The following measures will greatly reduce the chances of build up of aflatoxins during storage and trans-shipment.

Continue to exercise the same practices for storing the produce in warehouse or factory premises as have been outlined for the farmers.

1. Use pre-cleaner, destoner etc. to clean the produce and process it under dust free condition.
2. Do away with the practice of sprinkling water on the dry pods to reduce splitting/breakage of kernels during shelling by manually or motor driven decorticators. The breakage can instead be reduced by adjustment of the space

between blade and the sieve according to pod size.

3. Bring down its moisture level below 8% by sun drying the kernels at the time of bagging for shipment. At this moisture level, the skin (testa) comes off easily with slight rubbing.
4. Remove shriveled, discolored, and damaged kernels including the nuts with broken skin (testa) from the bulk either by handpicking or electronic sorting machine or a combination of both before putting them in new gunny bags.
5. Try to maintain warehouse temperature between 25-27°C and remove all causes and sources that may lead moisture build up.
6. Perform a regular inspection of the stored material and if by any chance some bag shows marks of deterioration, remove the same from the warehouse.
7. If required, fumigate the warehouse with phosphene to prevent insect damage to the pods in storage. Use 3-5 aluminum phsophide tablets for every 100 kg of pods and keep the doors and other ventilators closed for at least 7-8 days.

Tips for Processors

1. Use electronic sorting machines, if available otherwise handpick damaged, immature or *A. flavus* infested pods to reduce the contamination of the end product. In every 5000 kernels in the farmers produce, one is likely to be infected with *A. flavus*. By sorting, the proportion of infected kernels may be brought down to level of one in 10,000-30,000.
2. Separate the fully mature large pods (to be used for raw consumption) from the remaining produce (used for oil extraction).
3. Equip the processing unit with suitable analytical facilities or have access to some accredited quality control laboratory to get the adequate number of randomly drawn samples analyzed for aflatoxin load. This would prevent rejection of the processed material at a later stage.

10. Information on sampling and analytical methods for aflatoxins

(a) Sampling

Sampling is the most important contributor to the variation in analytical values for aflatoxins in groundnut. Since the invasion of fungi may occur in either isolated

pockets in bulk materials, or on individual seeds, the distribution of aflatoxin in a kernel lot is non-homogenous. The following factors are required to be considered for drawing a representative sample:

- Samples should be drawn by qualified, suitably trained, and motivated person, who understands the importance of sampling for obtaining an accurate test result.
- Transportation and storage facilities for samples must be adequate, and storage times must be as short as possible.
- Good sampling practice necessarily requires clear and secure labeling of samples, giving essential information such as name of sampler, place of sampling, lot number, conditions, applied tools, time, date and other observations.
- Each single lot has to be sampled separately. Large lots should be sub-divided into sub-lots (if possible). It is important to ensure that sample contains portions of the entire lot or sub-lot.

The decisions based on small samples tend to give negative results even from a lot with a high mean aflatoxin concentration, and a reasonably small risk of a false decision can be reached only if very large samples are analyzed.

In groundnut lots composed of mixed loads from different sources, a larger initial sample should be taken. Although it would be a costly exercise, very large samples of many kilograms of groundnuts must be taken to obtain a low risk of wrong decision for both the consumer and the producer. These large samples are required to be subdivided into sub samples to permit adequate analysis. For example, the Peanut Administration Committee (PAC) in the USA adopts the sampling procedure that involves multiple sampling and assay for representative units of three 22kg samples from each groundnut lot. Proper grinding and sub sampling is essential before performing analysis. Devices such as the **Dickens-Salterwhite sub sampling mill** (which can simultaneously grind and subdivide the samples) are available for preparing the sample. Alternatively, the sample may be ground and subdivided in separate operations. Rotary sample dividers such as "**Spinning riffles**" and the "**Cascade sampler**" are capable of producing several representative sub samples, which can be useful in aflatoxin analysis. The size of the sub sample is further reduced until a test portion, in size generally ranging from 20-100 g is obtained. The compromise between solvent economy and a representative sample appears to be 50 g.

(b) Methods for analysis

Many analytical (*viz.* TLC, HPLC, HPTLC) and immunological methods (ELISA tests) are available for estimation of aflatoxins in agricultural commodities. The development of highly automated HPLC systems has afforded very precise, selective and sensitive quantification techniques for aflatoxin analysis. HPLC methods have been developed using both normal and reverse phase systems in conjunction with UV adsorption and fluorescence detection techniques. Reverse phase HPLC separations of aflatoxins are more widely used than normal-phase separations. However, for routine analysis Indirect Competitive ELISA method is the most commonly used method for estimation of aflatoxins. This method is simple, rapid, sensitive method (up to 0.09 ppb quantity can be estimated) and very little sample cleanup is required. Readymade standards are available from several commercial companies at reasonable prices. The commercial success of ELISA kits shows that the value of immunoassays for aflatoxins is being more widely recognized.

11. Strategies to grow 'Safe to Eat' groundnut

As 75% of the total groundnut production in India is used for the extraction of edible oil (shortly after harvest) there is little concern about the prevalence of aflatoxin in oils. However, in recent years with the increased direct consumption of groundnut (kernels) the issue needs to be addressed keeping the producers, processors and consumers interests in view. The strategies to produce aflatoxin free groundnut in major Indian production systems must be based on:

a) Identification of potential production systems that support zero to negligible toxigenic strains of *A. flavus* and *A. parasiticus*.

b) Promotion of new generation confectionery grade varieties with better seed coat/ testa resistance to *A. flavus* colonization.

c) Localized intense cultivation with critical inputs and protective irrigation - preferably through contract farming, and finally

d) Introduction of better/efficient processing and packaging equipments and improvements in cargo handling.