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# A systematic approach to monitoring high preharvest aflatoxin levels in maize and peanuts in Africa and Asia

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**OPINION ARTICLE**

## Abstract

Aflatoxin in maize and peanuts remains a critical problem in much of Africa and Asia. Many countries in these regions lack a systematic preharvest approach for providing government agencies with warnings of a potential threat to human and animal health resulting from excessive levels of aflatoxin in crops at harvest. This paper sets out an approach to such a system. It is based on the establishment of a surveillance system in each community to monitor aflatoxin contamination resulting from drought stress before harvest and advise on remedial actions. The system should be under the control of a central government coordinator. If severe drought stress occurs, the coordinator would arrange for samples of the affected crop to be provided to a central aflatoxin laboratory established and controlled by the relevant government department. Assays from the central laboratory would be sent via the central coordinator to a government scientific advisory body, which would recommend appropriate remedial action to be taken at government level.

**Keywords:** aflatoxin, maize, peanuts, surveillance, warning

## 1. Introduction

The formation of aflatoxin in foods and feedstuffs remains a critical problem in tropical and subtropical Africa and Asia, where maize or peanuts are commonly staple foods. Aflatoxin $B_1$ is the most powerful known liver carcinogen, causing a significant proportion of worldwide annual deaths from liver cancer (Liu and Wu, 2010). Low levels of aflatoxin in the diet have also been implicated in growth impairment in children and possibly in immunosuppression (Wu *et al.*, 2014). Higher concentrations of aflatoxin may cause an acute disease known as aflatoxicosis, and have resulted in deaths in several tropical countries (Azziz-Baumgartner *et al.*, 2005; Krishnamachari *et al.*, 1975, Lye *et al.*, 1995).

The species of major concern for aflatoxin production are *Aspergillus flavus*, which commonly occurs in both maize and peanuts, and the closely related *Aspergillus parasiticus*, which occurs in peanuts worldwide, although it is rarely found in Southeast Asia (Pitt and Hocking, 2009). *A. flavus* produces aflatoxin in other commodities, especially tree nuts and figs, but with less concern for public health as they are not commonly staple foods.

The current food safety paradigm is to move away from sampling and analysis of finished product and to focus on controls that reduce or prevent the occurrence of foodborne hazards. However, although good agricultural practice can reduce the preharvest formation of aflatoxin in maize or peanut crops, control steps are lacking (Pitt *et al.*, 2012a). Good drying and storage systems will prevent further increases in aflatoxin levels, and sorting of dried product will reduce levels, especially for maize but sampling and analysis remain the only effective control measures. Infection by *A. flavus* of peanuts (Pitt, 1989) or maize crops (Siriacha *et al.*, 1994) occurs in the field during kernel or nut development. Drought stress or insect damage (exacerbated by drought stress) may then result in extensive fungal growth, leading to aflatoxin formation in the kernels or nuts. Severe drought stress, which farmers cannot control

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and which is normally accompanied by high temperatures, is the major cause of the formation of very high levels of preharvest aflatoxin in these crops (Hill *et al.,* 1983; Milićević *et al.*, 2016; Payne and Widstron, 1992). In the absence of drought stress, fungal infection is reduced and aflatoxin formation is rarely a serious issue. Preharvest *A. flavus* infections will often result in unacceptable increases in aflatoxin formation when drying, transport and storage procedures are not optimal (Pitt *et al.,* 2012a).

Many strains of *A. flavus* do not produce aflatoxin and those that do show wide variation in toxin production. Moreover, only a small percentage of peanuts or maize kernels are infected in any particular crop, so contamination of individual particles with aflatoxin is very nonhomogeneous (Johansson *et al.*, 2000; Whitaker and Wiser, 1969; Whitaker *et al.,* 2010). Much recent research has been devoted to developing simple aflatoxin assay methods suitable for farmers to use on site. However, for several reasons, we believe that these approaches are at best ineffective and at worst counterproductive.

First, the sheer scale of sampling and analysis of all smallholder farms in any country could never be cost effective. Second, such smallholder farmers are unlikely to be willing to donate more than 1 kg to an aflatoxin assay and such a sample, taken in isolation, is of very limited value. As an example, suppose a maize lot contains 100 µg/kg aflatoxin, a 1 kg sample is selected from the lot, the sample is finely ground in a suitable mill, and then a single 50 g test portion is assayed for aflatoxin. Using the FAO Mycotoxin Sampling Tool (FAO, 2013), which was developed to assist the design and evaluation of performance of mycotoxin sampling plans, a 95% confidence limit can be established. The calculations are as follows: total variance =1,635.4; standard deviation (square root of the total variance) = 40.4, 95% confidence limit (standard deviation×1.96) =79.2. In other words, we can state, with a 95% level of confidence, that this lot contains 100±79.2 µg/kg aflatoxin, i.e. between 20 and 180 µg/kg, which is not a high level of assurance. If sample test results are simply gathered from a number of farmers, conducting separate assays, data will always be of questionable accuracy. Statistically valid sampling of individual farms would require large samples which will result in the destruction of an unacceptably large portion of any such crop.

Third, even with modern systems, assaying crops for aflatoxin at or near harvest is not simple. The minute (µg/kg) levels encountered are readily lost by inadequate extraction procedures, by occlusion to unclean or alkaline laboratory glass surfaces or exposure to air at high ambient temperatures (Trucksess *et al.*, 2008). Extractions need to be carried out carefully, as the process concentrates a toxin that can be absorbed through unprotected skin (Riley *et al.*, 1985). Reliable assays require quality control, including the use of positive spiked samples and negative controls. The concept of quality control of assays would be difficult to teach to farmers, and even more difficult to ensure in practice. A series of check sample programmes and proficiency tests in the USA and Europe over a number of years has seen many professional laboratories failing to obtain results within two standard deviations of the mean aflatoxin value even when assaying homogeneous test samples (Horwitz *et al.*, 1993; McKinney and Cavanagh, 1977; Solfrizzo *et al.,* 2013). Analytical methodology has improved but, as pointed out by Whitaker *et al.* (1974), the largest source of error in assaying crops for aflatoxin lies in sampling, not analysis.

Fourth, farmers are rarely in a position to evaluate the significance of aflatoxin results. If the result is even a little above international or local guidelines, farmers (and local communities) may be unnecessarily concerned for family health. Lay people (and media) have a strong tendency to equate maximum permitted levels with maximum levels that will not cause harm, so results from aflatoxin assays need to be interpreted by knowledgeable and responsible, preferably government, officials. In any type of crisis situation, government will be the entity responsible for management, so it is essential that government have access to reliable information.

Fifth, as long as it is economically disadvantageous for a farmer to acknowledge contamination, the incentive remains to move the contaminated material into informal markets rather than to accept its loss.

For the above reasons, we believe that a systematic approach at government level to survey production on small farms collectively would be more beneficial to many countries than advocating aflatoxin assays at the individual farm level. Surveillance system were suggested by Park (1995) and Strosnider *et al.* (2006), in a very broad framework. In this paper we are concerned specifically with the development of a system for detecting highly unacceptable levels of aflatoxin in maize and peanut crops by the time of harvest, in countries where some populations have subsistence economies, i.e. where crops are mostly consumed by farmers or local communities without any aflatoxin monitoring. It is emphasised that this is intended to be relevant to communities rather than to individual farms, and not intended as a regulatory measure, but rather part of a monitoring and surveillance system that allows government to monitor and focus on support interventions.

We believe that effective controls require strong surveillance, which is best served by the setting up of a central aflatoxin laboratory under direct government control, with trained staff and preferably sophisticated equipment, tasked to communicate results directly to government. The laboratory should be serviced by a

system of community people, trained in watching for signs of severe drought stress in developing crops, and trained in sampling crops across a community to provide systematically obtained samples to the central laboratory. Alerted by the laboratory of any serious aflatoxin problem, government authorities would then be in a place to provide feedback to local communities and advise remedial action when deemed necessary. Remedial action might include replacement food supplies, rollout of biocontrol in future years, or even a recommendation that alternative crops be grown in the most susceptible areas.

Although this paper is concerned specifically with monitoring excessive aflatoxin levels preharvest, the proposed central aflatoxin laboratory could also have important basic functions. By assaying harvested, dried or stored maize and peanut crops over the whole country, such a laboratory would also be able to provide government with an overview of the specific local regions where aflatoxin is most likely to be a problem. Where countries establish national grain reserves, it would also be important that aflatoxin levels be monitored by that laboratory, both at intake and before outturn. In time, when a country has established and implemented risk based standards, the central laboratory could of course develop a regulatory function, with obvious benefits for both internal and external trade.

## 2. A systematic approach to monitoring aflatoxin at farm level

A systematic approach to monitoring aflatoxin in peanuts or maize crops will require three basic components: a central aflatoxin laboratory, a central government coordinator and a network of community officials. These are considered in turn below.

### A central aflatoxin laboratory

The primary function of a central aflatoxin laboratory would be to conduct aflatoxin assays on samples submitted as the result of implementation of an early warning system (see below). This would enable effective management of situations arising from the occurrence of unacceptable aflatoxin levels in commodities at risk at harvest time.

In periods when assays of such samples is not necessary, the laboratory's function would be to carry out a systematic survey of susceptible commodities harvested from all districts where these commodities are grown. Over two or three seasons, this would provide invaluable information about the aflatoxin risk throughout the country, enabling selection of areas where early warning surveillance is needed, and where control measures such as biocontrol should be implemented. Equally importantly, an overview of the aflatoxin risk in all growing areas would provide information on areas where rainfall is normally adequate to produce crops low in aflatoxin. In such areas remedial measures, such as biocontrol, would not make economic sense.

In time, the capability of this laboratory should be expanded to obtain information on levels of other mycotoxins, especially fumonisins and trichothecenes, in maize and ochratoxin A in, for example, coffee or cocoa.

The central laboratory could commence operation on a quite modest scale, utilising available aflatoxin test kits that have been tested for performance, for example by AOAC International. Requirements would include a coffee grinder or similar machine for finely grinding samples, a fume cupboard for solvent extractions, a refrigerator for storage of assay kits and untested samples and a locked storage for extraction chemicals. The laboratory should be staffed by a graduate in analytical chemistry or preferably with a PhD, plus two technicians, one to assist in analyses, the other to accession, catalogue and grind samples. In due course, more sophisticated instrumentation, perhaps an HPLC instrument with post-column derivatisation could be a very cost effective upgrade. It is also important that the laboratory take part in a proficiency testing programme such as FAPAS (2017) so that performance can be monitored long term.

It is essential that sufficient money be allocated over the long term to pay salaries, purchase essential chemical supplies and to provide for instrument maintenance. It is also important that travel money be allocated so the chemist can attend international meetings on mycotoxins at least once a year.

To improve precision with available resources, it is recommended that, from each of 10 farms in a community, a 1 kg maize sample should be selected, weighed accurately (±5 g), then ground in a suitable mill. These should then be combined and mixed thoroughly before a 50 g test portion is taken for analysis. The sampling precision associated with a 10 kg sample is 10 times better than for a single sample of 1 kg. For peanuts, 2 kg of shelled nuts taken from 10 farms should be weighed out, milled, combined and mixed before a 100 g test portion is taken from the comminuted sample for analysis.

### Central coordinator

The central coordinator would be a government official, who would operate in consultation with a body responsible for scientific advice and a separate management body responsible for decision making (risk management body), with all three fitting into the context of a national food control system (Figure 1). The central coordinator's job would be to supervise the laboratory, to coordinate the

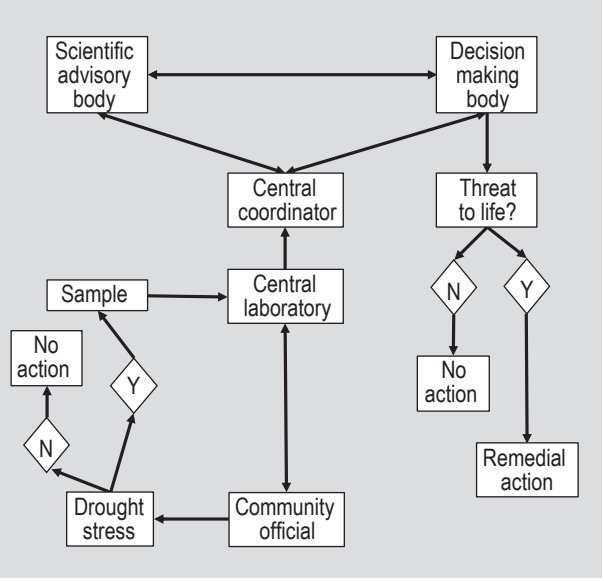


**Figure 1. A diagrammatic representation of the interactions of the parties involved in the proposed early warning system.**

aflatoxin data evaluation and to ensure collaboration with the body responsible for decision making in the country. Initial responsibilities would be to set up the aflatoxin laboratory and hire staff, to set up the surveillance system and coordinate the gathering of samples from communities. This official would also have responsibility for calling on an established group of advisors to assess and evaluate the aflatoxin results from the central laboratory and to connect with the risk management body to enable them to recommend action as necessary. For regions where excessive aflatoxin occurs, this would include plans to limit health effects. Possible scenarios of information to the government decision making body could be that: (1) aflatoxin levels are above recommended levels, but not high enough to cause harm to humans or animals; (2) levels are high enough that some remedial action is desirable; or (3) catastrophically high, when food replacement becomes a necessity.

The central coordinator would also be responsible for establishing a systematic surveillance programme for aflatoxin levels occurring in maize and peanut crops in all areas of the country. After acquisition and evaluation of such information, the government would be in a position to decide where which additional actions to limit aflatoxin formation might be beneficial, for example, by biocontrol or where such action would not be of economic benefit.

## Community officials

For the proposed system to be effective, it would be essential to appoint a network of community officials, with primary responsibility to survey local maize and peanut crops before harvest, looking for signs of severe drought stress. Although a number of useful surveillance approaches have been proposed, drought stress is the simplest, most readily observed early warning signal for both crops for the potential for formation of unacceptable levels of aflatoxin by harvest time. In each community, or group of local communities, an existing agricultural extension agent, or community councillor or whomever is appropriate, should be selected as surveillance officer. This community official would be trained in: (1) observation of severe drought stress, leading him to communicate with the central coordinator; (2) standard procedures for sampling maize and peanuts on farms in his community; and (3) forwarding samples to the central aflatoxin laboratory, after drying them if necessary. It would be essential that funds be provided for such officials to facilitate the travel needed to sample and for transmitting samples. This official would also have the role of conveying information from government to farm community and farm community to government.

## Sample selection protocol at the farm level

A variety of protocols have been put forward for sampling maize crops in the field. A simple and effective sampling procedure is to establish two approximate diagonals across a field and then sample at 10 more or less equidistant points along each diagonal. Adequate samples can be obtained by selecting at random two cobs from different stalks at each of the 20 sampling points. The samples should immediately be shelled out and weighed, using kitchen scales or a small spring balance, such as those used by fishermen, and composited to provide a 1 kg sample representing that specific farm. The sample size should be at least 1 kg: the precise weight is unimportant provided it exceeds 1 kg, as it will be weighed accurately at the central laboratory.

For peanuts, a similar protocol should be used, but the wide variation in both peanut size and numbers of kernels per bush is such that the precise number of bushes to be pulled for a particular sample should be assessed in the field. Nuts should be stripped immediately from the pulled bushes and shelled, then dried. The sample size for the shelled peanuts should be 2 kg or more.

If samples are dry, they should be individually packed in robust plastic bags, labelled with a unique identifier code and forwarded to the central analytical laboratory. Samples that are not fully dry should be dried immediately either in the sun or in an oven at set at low temperature (50-70 °C) before packing and dispatch. A practised operator will have little difficulty in determining the state of dryness of samples, however it is recommended that all operators be provided with 'DryCard' cards (UC Davis, 2016). The DryCard measures relative humidity. If a card is placed with a sample in a closed plastic bag overnight, the sample may be considered to be dry enough for dispatch if the DryCard then indicates 70% or lower relative humidity.

No hard and fast rule can be established for the number of samples that should be taken at one time in a single community. However, in a community of perhaps 100 farms, one sample taken from each of 10 farms should be sufficient to provide a good indication of the status of the crop in that particular community. If one or more samples indicate high levels, then further samples should be taken to provide a statistical basis for the assessment. Farmers should be recompensed for providing samples.

## 3. Discussion

Essentially all developed countries have either a central government laboratory for assaying mycotoxins, or a set of private sector laboratories overseen by some type of registration system that ensures accurate reporting of mycotoxin levels in foods. Compelling reasons exist why less developed economies should follow their example. Unquestionably, a place exists for industry based laboratories to provide mycotoxin analyses for company use. However, in our opinion, a well-equipped and regulated central laboratory is essential to provide the government of a developing country with information on the aflatoxin status of maize and peanuts in defined geographical areas. Aflatoxin is such a major problem that adequate information on levels near harvest time is the only sure way to enable avoidance of catastrophic situations. That information would also provide a solid basis to show where – and whether – aflatoxin reduction strategies like biocontrol would be beneficial and economically sensible.

Given such a central laboratory, the early warning system described in this paper can be set up quite simply, as it relies almost entirely on community based surveillance, sampling and communication. The cost, involving community salaries, training, infrastructure and transportation, is low in comparison with the assurance provided to government about the status of maize and peanut crops.

Apart from the inherent difficulties in obtaining farm based aflatoxin assays with any degree of reliability, a major issue is that assays from single farms are of little value to government. However, a coordinated system, where several farms in any community are sampled and then analysed by a central laboratory, will provide an effective data set for government action and mycotoxin control.

As shown above in the introduction, single 1 kg samples analysed by farmers provide analytical results of very limited value due to the large variability among 1 kg sample test results. However, if a community is treated as a single lot, 1 kg samples are taken from several farms and all samples are analysed by a single analyst, a considerable improvement in precision can result due to the larger sample size. To continue the earlier example, if 1 kg samples of maize are collected from each of 10 farms and composited into a single pooled sample of 10 kg, the 10 kg sample is ground and a 50 g test portion is analysed, the following calculations can be made using FAO Mycotoxin Sampling Tool (FAO, 2013): total variance = 581.8; standard deviation = 24.1; 95% confidence limit = 47.2. The level of aflatoxin in the maize crop from that community can now be stated, with 95% confidence, to be lie between 50 and 147 µg/kg (FAO, 2013). This figure is sufficiently accurate to be of value to a government.

Can maize or groundnuts from community farms be treated as a single lot in the current context? Subsistence communities are almost always quite small geographical units, with farms likely to have similar rainfall, temperature and soil characteristics, so this assumption is not unreasonable for the gathering of surveillance data. Regulatory levels, such as 15 µg/kg, are not of relevance here, as we are interested in high levels, in excess of 100 µg/kg. Again, we do not seek high precision, but only a good indication that a community may be affected by excessively high aflatoxin levels.

In tropical and subtropical countries where maize or peanuts are staple foods, aflatoxin is the major mycotoxin of concern. This surveillance system has been designed to provide warning of hazardous aflatoxin levels. However, in countries where maize is the staple, it needs to be stressed that fumonisins are also produced wherever maize is grown. Once aflatoxin is monitored and can be controlled at a national level, fumonisins must become the next mycotoxin of concern. In countries where maize is a staple food, removal of aflatoxin is not sufficient to guarantee a safe food supply (Pitt *et al.*, 2012b). However, fumonisins are not an issue in peanuts, as peanut plants do not support the growth of *Fusarium verticillioides,* the major producer of fumonisins in maize. Other mycotoxins important in tropical countries, i.e. trichothecenes in maize or ochratoxin A in coffee or cocoa, are normally present only at the low levels relevant to international trade, rather than levels with significance to human health.

The early warning system described here is an important element of an integrated approach to aflatoxin mitigation. In countries where systems for food control are weak and the culture of surveillance not yet established, a number of potential challenges pose a risk to the success of such a system.

At the local level, difficulties often arise in ensuring political commitment and agreement between involved government agencies towards a coordinated approach leading to collaboration and data sharing. This is often related to a lack of a functioning system of communication between regional and central authorities. Both political commitment and communication between agencies are needed to ensure that basic issues such as sampling, transport of samples

to the central laboratory, sample preparation and sample size for analysis are standardised. The development of an intelligent quality management system able to incorporate lessons learned is a necessity.

Increased funding for laboratory infrastructure is often put on the table without concern for operational sustainability or a clear understanding of who is to bear the costs of analyses. Programmes focusing on infrastructure development need to describe how laboratories will operate beyond project end.

It needs to be understood at government level that data raised in support of food safety are both a public good and a public responsibility. The opinion is often heard that surveillance of analytical systems that ensure public health and enhance trade should be revenue generating. In our estimation, this is both worrying and wrong.

While we welcome the renewed awareness of the global aflatoxin problem, we are concerned that the many ongoing activities are taking place without good governance in place. Donors often promote single approaches as a total solution to the problem, with time lines too short for careful programme development, unreasonable expectations of results and lack of consultation with major stakeholders. This situation has led to confusion, especially among the smallholder farmers who need help most. Improved coordination and communication about programmes addressing aflatoxin risk are urgently needed, as well as a commitment to improved transparency by donors, authorities, development agencies and research institutions alike.

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