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Cover: Research worker in IITA's maize field collecting tassels for hand pollination.
Photo by C. Ono-Raphael.

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TOOL BOX

Afla-ELISA: A simple and low-cost quantitative test for the estimation of aflatoxins

Lava Kumar (L.kumar@cgiar.org) and R. Bandyopadhyay

Aflatoxin testing using Afla-ELISA.
Source: L. Kumar

Aflatoxins threaten human and animal health

Aflatoxins are the hepatotoxic and carcinogenic secondary metabolites produced by *Aspergillus flavus* and *A. prasiticus*. They are common contaminants in several staple crops, such as maize and groundnut, produced in the tropics and subtropics. Aflatoxins are a group of four toxins: aflatoxin B1 (AFB1), AFB2, AFG1, and AFG2. A metabolite of aflatoxins, namely AFM1, is detected in milk. Aflatoxin contamination in foods is considered to be unavoidable, as the causative fungi are ubiquitous in the tropical parts of the world. However, fungal infestation and toxin contamination are unpredictable and depend on certain environmental conditions. Aflatoxin exposure in humans and animals results from the consumption of aflatoxin-contaminated foods and feeds.

Regulations check aflatoxin contamination

Stringent food safety regulations are enforced in most countries to prevent use of aflatoxin-contaminated foods and feeds. These programs are executed through a monitoring process by testing all commodities for aflatoxins and rejection of those with toxin levels exceeding the tolerable limits [ranges

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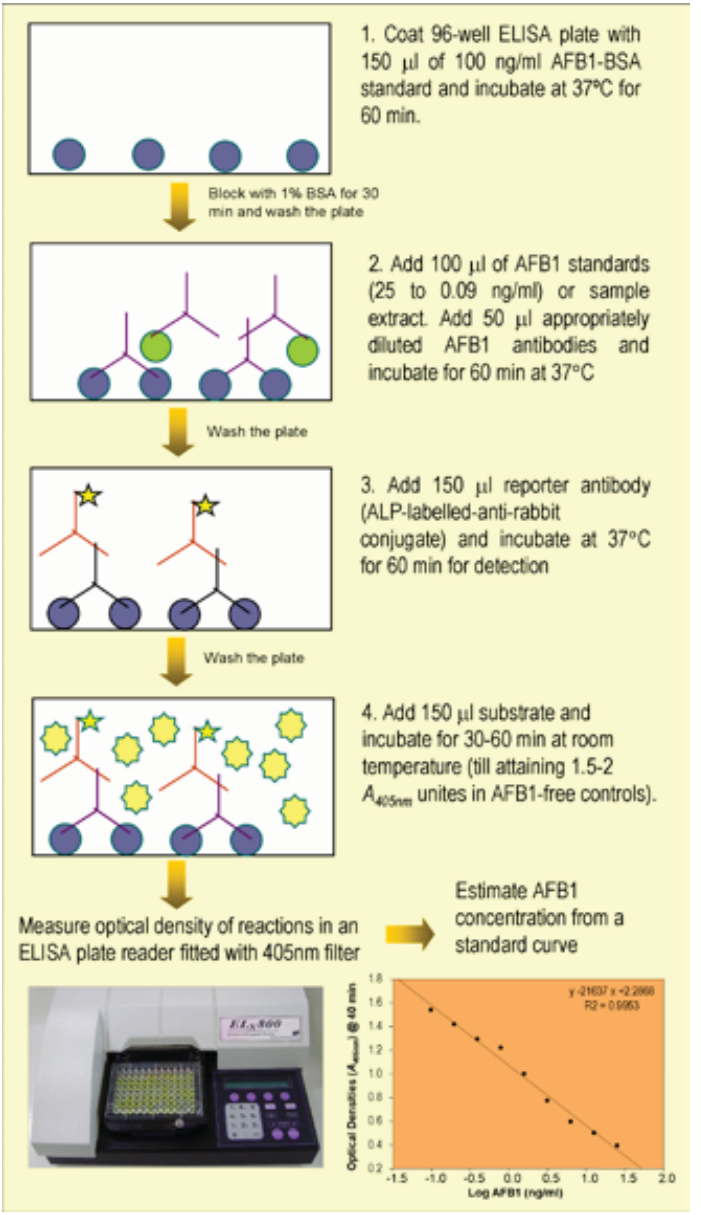

Outline of Afla-ELISA testing scheme. Source: L Kumar.

between 2–20 parts per billion (ppb), depending on the type of toxin and country[1]]. Heavy infestation of fungi results in moldy products which can be physically sorted. However, aflatoxins per se are invisible and leave no visual clues of their presence in the contaminated products. Aflatoxins can be found even in commodities that show no apparent signs of fungal infestation. This situation poses a serious challenge to monitoring aflatoxin contamination, which depends on aflatoxin-monitoring tools.

Aflatoxin control relies on monitoring tools

Monitoring for aflatoxins has become integral to effective measures to control aflatoxins in foods and feeds. A variety of aflatoxin monitoring tools are available to detect and quantify aflatoxin levels[2]. Quantitative estimation is most critical as decisions are based on aflatoxin levels in the commodity. Products with aflatoxin levels within the permissible range are allowed in trade and those with exceeding levels are rejected[1].

Despite the availability of a wide variety of diagnostic tools for monitoring aflatoxins, their use in most of the developing countries is limited by high cost, difficulties with importation, and lack of appropriate laboratory facilities and well-trained staff. Among the many types of aflatoxin-monitoring tools, antibody-based

methods were proven to be relatively easy for developing countries to adopt.

Convenient option

At IITA, we developed an enzyme-linked immunosorbent assay (ELISA) named Afla-ELISA, for quantitative estimation of aflatoxins. Very high titered rabbit polyclonal antibodies for AFB1 were produced. These antibodies have an end-point titer of 1:512,000 (v/v) against 100 ng/mL AFB1-BSA standard; they are highly specific to AFB1 and also react with ABF2, AFG1, and AFG2. They were used to develop Afla-ELISA based on the principle of indirect competitive ELISA for quantitative estimation of aflatoxins. This assay has a lowest detection limit of 0.09 ng/mL, and a recovery of 98±10% in maize.

Afla-ELISA is simple to perform, offers sensitive detection, and is convenient for adoption in sub-Saharan Africa. This test is suitable for routine aflatoxin surveillance in crops and commodities, and offers a low-cost alternative to official monitoring methods. It offers a sustainable solution to the problem of ever-increasing demand for monitoring programs related to food safety and trade, and has the potential to enhance aflatoxin monitoring capacity in sub-Saharan Africa. To contribute to capacity development, training workshops have been organized on monitoring for mycotoxins and application of Afla-ELISA.

References

[1]FAO. 2003. Worldwide regulations for mycotoxins in food and feed. FAO Food and Nutrition Paper #81. FAO, Rome, Italy.

[2]Reiter, E. et al. 2009. Review on sample preparation strategies and methods used for the analysis of aflatoxins in food and feed. Molecular Nutrition & Food Research 53: 508–524.

Prototype Afla-ELISA kit—a quantitative serological assay for the estimation of total aflatoxins in maize and other commodities, using 96-well microtiter plates. Up to 20 samples can be tested in each 96-well plate at a cost of US$4 per sample. Source: L Kumar.

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