A Research Note

Field Level Evaluation of Aflatoxin Detection Kit

R. V. SUDERSHAN, G. S. PRASAD, T. PRASANNA KRISHNA, and RAMESH V. BHAT*

National Institute of Nutrition, Indian Council of Medical Research, Hyderabad-500 007, India

(Received for publication June 12, 1991)

ABSTRACT

An aflatoxin detection kit was evaluated at the field level for groundnut and groundnut meal. A total of 88 groundnut samples and 20 groundnut meal were analyzed with the help of the kit in the field. Aliquots of same samples were analyzed in the laboratory by the conventional BF-thin layer chromatographic method. In 89% of the groundnut samples and 100% groundnut meal, results of both methods were comparable. Aflatoxins were overestimated in 5% of the groundnut samples, and in 6% of the samples, aflatoxins were underestimated.

Aflatoxins are toxic secondary metabolites produced by certain strains of Aspergillus flavus and A. parasiticus. They are often found as contaminants in various agricultural commodities posing potential human and animal health hazards (3). There is considerable evidence for their carcinogenicity (8). Statutory tolerance levels for these mycotoxins in foodstuffs have been imposed in many countries (1/2). The Government of India has fixed 30 ng/g tolerance limits for aflatoxins in foods.

Various analytical methods like thin layer chromatography (TLC), high performance liquid chromatography (HPLC), radio immunoassay and enzyme-linked immunosorbent assay (ELISA) have been developed for the detection of aflatoxins in foods and feeds. Most of these methods require sophisticated equipment and elaborate cleanup procedures and are not useful for routine screening of samples. Minicolumn chromatography has been suggested for routine screening and since 1968 many improvements have been suggested (6). The U.S. Department of Agriculture Federal Grain Inspection Service (USDA FGIS) has adopted the Holaday-Velasco (5) method for routine analysis. The major disadvantage of this method has been lack of specificity in corn samples. To overcome this problem, USDA FGIS has been evaluating commercially available aflatoxin detection kits. Many of these kits are based on immunological techniques which require refrigeration facilities, have limited half-life, and are expensive. A pressure minicolumn method has earlier been developed (9) and cooperatively studied (10). An aflatoxin field detection kit has been fabricated which contains the requisite chemicals, glassware and ultraviolet (UV) lamp and pressure minicolumns (4). The present communication reports the evaluation of the efficacy of using this aflatoxin detection (AD) kit to detect aflatoxins in groundnut and groundnut meal.

MATERIALS AND METHODS

Samples

Eighty-eight groundnut samples were collected from the farmer’s lot intended for sale at the primary collection centers of Andhra Pradesh Oilseed Growers Federation in Kurnool District. The groundnut meal was collected from the Central Processing Unit of Andhra Pradesh Oilseed Growers Federation, Beechpally, Andhra Pradesh.

Aflatoxin detection kit

The AD kit is a box of 45 x 48 x 48 cm size made of galvanized iron, containing activated pressure minicolumns, a simple balance to weigh 100 g of sample, hand-operated grinder, UV viewing chamber and aflatoxin standard columns corresponding to 30 ng, 60 ng, and a blank for comparison, besides extraction solvents and glassware necessary for analysis (4). Aflatoxin B1 was standard was procured from Roth Chemicals, Germany. All the chemicals and solvents used for laboratory analysis were of analytical grade.

Sample processing and analysis

One kg of groundnut samples was taken and was decorticated; 200 g of kernels was powdered with hand-operated grinder. The powdered samples were made in to two subsamples of 100 g each. One lot was analyzed immediately in the field by the AD kit and the other subsamples were brought to laboratory in polythene bags. The transit time was 5 h. The storage temperature was 37°C (RT). Moisture percentage of the samples varied from 3 to 7. A similar procedure was followed for groundnut meal.

Aflatoxin analysis using kit in the field

The aflatoxins were extracted by the BF method with minor modification of adding 20% lead acetate solution to methanol:water extract (7) and the semi-quantitation was done by comparing the
intensity of aflatoxin band with standard columns under UV light. The samples were then graded as samples having aflatoxin levels above 30 ng/g and below 30 ng/g level. The samples graded as below 30 ng/g include aflatoxin negative samples also. The 30 ng/g cut-off point was taken in view of the Indian tolerance limit of 30 ng/g.

Laboratory analysis

The samples brought from the field were analyzed for aflatoxins in the laboratory. Aflatoxins were extracted by the BF method (7), and quantitation was done by thin layer chromatography with visual comparison method.

Statistical analysis

Statistical analysis was done according to Armitage (2) and Swets (11).

RESULTS AND DISCUSSION

A total of eighty-eight groundnut samples and 20 samples of groundnut meal were analyzed by both the methods. Total aflatoxins were estimated by AD kit, whereas only aflatoxin B1 was measured by TLC. At the field level analysis using AD kits, 60 groundnut samples were found to have aflatoxin levels below 30 ng/g and 28 samples above 30 ng/g. Eighteen samples of groundnut meal had aflatoxin levels above 30 ng/g and only two samples had levels below 30 ng/g. The laboratory analysis by TLC showed that the range of aflatoxin B1 varied from 0.0 to 200 ppb in groundnut samples and 12.5 to 125 ppb in samples of groundnut meal. Two groundnut samples and 4 groundnut meal samples had aflatoxin B2 levels ranging from traces to 5 ppb. Aflatoxins G1 and G2 were not detected. Of the 88 groundnut samples analyzed by TLC, 59 were below 30 ng/g and 29 samples had aflatoxin B1 above 30 ng/g. Eighteen samples of groundnut meal had aflatoxin B1 levels above 30 ng/g and only 2 had aflatoxin B1 levels below 30 ng/g. When AD kit analyses were compared with the TLC method, 79 (90%) groundnut sample values of the AD kit method were comparable with TLC method, whereas 5 (6%) groundnut samples were underestimated and 4 (5%) groundnut samples were overestimated (Table 1). In the case of groundnut meal all the AD kit values agreed with the TLC method (Table 2).

To see the agreement between the two methods, Youden’s index (2) was calculated and it was 0.76 in groundnut samples and 1.0 in the case of groundnut meal on a 0.0 to 1.0 scale, indicating that the AD kit analyses are comparable with the TLC method. The specificity and sensitivity of the kit were 0.93 and 0.83 in groundnut samples and 1.0 in groundnut meal, i.e., the kit is specific enough to screen groundnut samples having aflatoxin B1 levels below or equivalent to 30 ng/g with four false positives out of 59 samples. However, the AD kit is less sensitive in samples having aflatoxin B1 levels above 30 ng/g, with five false negatives out of 29 samples. In the case of groundnut meal there were no false positives or negatives.

A comparative evaluation of commercial kits based on ELISA and a minicolumn method indicated that they are comparable (5). Although such commercial ELISA kits have been found to be useful in developed countries, their use in developing countries is limited in view of shorter half-life, requirement of refrigeration, and higher cost. The present kit has the advantage of being less expensive and operable even in summer conditions at higher temperatures, sometimes above 40°C. The added advantage is that the kit could be assembled locally with the need of importation of few essential items such as the UV lamp and aflatoxin standards. Moreover, recurring expenditure of consumables is also minimal. The National Peanut Council of the U.S.A., after in-depth review of alternatives, had concluded that screening of farmer’s stock of groundnut offered more promise for reducing aflatoxin contamination. Under the peanut quality enhancement project, farmers’ groundnut lots were screened for aflatoxin by HPLC. However, a direct analytical method for the detection of aflatoxins at the field level has been suggested as ideal to reduce aflatoxin contamination in groundnuts (1). In countries where the segregation approach is being tried, the aflatoxin detection kit may be ideal for adoption. In order to validate the method, there is need for collaborative trials to be carried out at field levels in other developing countries.

ACKNOWLEDGMENTS

We wish to thank Dr. Vinodini Reddy, Director, National Institute of Nutrition, for her keen interest in the work, and Mr. Nadamuni Naidu for the statistical analysis. Thanks are due to the authorities of Andhra Pradesh Oilseed Growers Federation for permitting us to carry out the study at their primary collection centers and Central Processing Unit, Beechpally, Andhra Pradesh, India. The cooperation received from Mr. Subba Rao (Divisional Officer, Gadwal), Mr. Nageshwar Rao, and Mrs. Nirmala (Chemists) is gratefully acknowledged.

REFERENCES


