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Analytical Methods Section

TECHNICAL NOTE

Analytical quality assurance for analysis of aflatoxins in agricultural export produce

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Aflatoxin contamination of agricultural commodities has gained global significance as a result of its deleterious effect on health and the importance of aflatoxins to international trade. As a safeguard, many developed and developing countries have laid down stringent regulations on the level of aflatoxins in agricultural commodities they import. This note highlights the importance of analytical quality assurance, based on a check sample programme, among the non-governmental laboratories involved in the analysis of aflatoxins in agricultural commodities intended for export.

INTRODUCTION

Aflatoxins, a group of closely related heterocyclic compounds produced by *Aspergillus flavus* and *Aspergillus parasiticus*, are found as natural contaminants of a variety of agricultural commodities (Anon, 1979*a*; Tulpule *et al.*, 1982). Outbreaks of aflatoxicosis in man and animal have been described in India and other parts of the world (Krishnamachari *et al.*, 1975; Bhat *et al.*, 1978; Ngindu *et al.*, 1982). Aflatoxin contamination of foods has also been linked to high incidence of liver cancer in different parts of the world (Anon, 1979*b*). In view of the hazardous nature of aflatoxin to human and animal health, regulatory measures on its content in foods (ranging from 0–50 ppb†) have been introduced in various countries around the world (van Egmond, 1988). These stringent regulatory measures and their implementation, on aflatoxin content in agricultural commodities and by-products, has resulted in considerable economic losses, with drastic falls in export from developing to developed countries (Bhat, 1988).

It is mandatory, both in India and in most developed countries, that any commodity, known to be at risk for aflatoxin, is analysed and certified before being exported or imported. A permissible limit of 30 ppb for aflatoxin in edible commodities has been fixed in India under the Prevention of Food Adulteration Act (PFA).

To provide quality assurance for analytical laboratories engaged in analysis of aflatoxin, check sample programmes have been organized. The international check sample programmes organized include the Smalley check sample programme of the American Oil Chemists Society (AOCS) and the International Mycotoxin Check Sample Programme of the International Agency for Research on Cancer (IARC) (Egan, 1982; Anon, 1988).

The aflatoxin check sample was first introduced in India and Nepal for the research laboratories participating in the Food Contamination Monitoring Programme of the FAO, Rome, in 1983 and for the research laboratories participating in the Food Contaminants Hazard Project of the Indian Council of Medical Research in 1986 with the National Institute of Nutrition as the coordinating laboratory. A collaborative study among the analysts of government laboratories

† ppb = parts per billion, where billion is 10^9.

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engaged in analysis of aflatoxins in foods under the Prevention of Food Adulteration Act using the newly evolved pressure minicolumn method was successfully conducted (Sashidhar *et al.*, 1989).

Keeping in mind the above considerations, a quality assurance programme exclusively for non-governmental laboratories involved in analysing aflatoxins in agricultural commodities intended for export was organized, for the first time, in India.

METHODOLOGY

A total of 12 non-governmental laboratories in the country which had been involved in the analysis of aflatoxins in agricultural commodities intended for export were approached as to their willingness to participate in the 1988 aflatoxin check sample programme. Although 12 non-governmental laboratories expressed their willingness to participate in the programme and received the check sample, only eight of these laboratories returned the analytical report to the coordinating laboratory.

Naturally contaminated groundnut meal (GNM) and aflatoxin-free maize meal (MM) were used as check samples. Each lot of 2 kg sample was thoroughly blended for homogeneity at 12000 rev min^{-1} in a mechanical blender. Participating laboratories were supplied with 75 g each of GNM and MM, with a report form. The check samples were dried by keeping them in a vacuum oven at 70°C overnight. The samples were sealed in plastic containers and packed with aluminium foil. The analysts were also told to store the samples in the dark and to refrigerate then, until analysis of the samples was carried out. Each participating laboratory was also supplied with authentic, reference standards of aflatoxin B_1 (AFB_1, 5 μg) and aflatoxin B_2 (AFB_2, 2·5 μg) (Roth Chemicals, Germany), along with information on the preparation of standard solutions. The laboratories were asked to analyse the check sample by any standard international method that was routinely used in their laboratories for screening aflatoxins. These methods included Contamination Bureau (CB), Best Food (BF) and Pons's method (Pons, 1969; Egan, 1982). Participating laboratories were identified by a code number, which was only divulged to the concerned laboratory, and were given an analysis period of 45 days.

RESULTS AND DISCUSSIONS

The results of the analysis of the check sample by each of the participating laboratories are presented in Fig. 1. The levels of AFB_1 and AFB_2 in GNM ranged from 100 to 160 μg kg^{-1} and 35–60 μg kg^{-1}, respectively. One participating laboratory (Code AL 17) reported

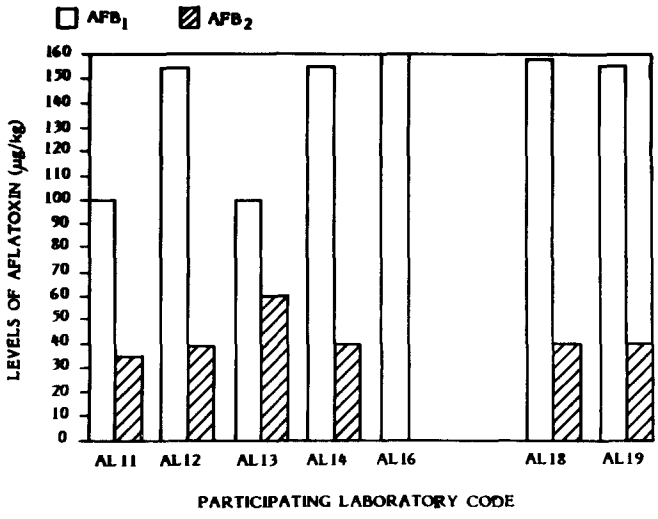

Fig. 1. Aflatoxin levels in check samples—groundnut meal. Aflatoxin B_2 analysis for AL16 was not carried out.

that the GNM sample was negative for aflatoxins. In the case of the MM sample—except one laboratory (AL 11), which reported a value of 5 μg/kg for AFB_1 and 4 μg kg^{-1} for AFB_2—all laboratories reported the sample was negative for aflatoxins. The mean of the reported values for AFB_1 and AFB_2 in the contaminated GNM were 140·42 μg kg^{-1} and 42·3 μg kg^{-1}, respectively. The coefficient of variation (CV) for AFB_1 and AFB_2 in GNM ranged from 11–29% and 5–42%, respectively. Results from one participating laboratory (AL 17) was considered as an outlier. Earlier, the IARC aflatoxin B_1 check sample programme, conducted on a worldwide basis for the years 1985–87, with 200 participating laboratories, indicated that the CVs for maize meal and peanut meal ranged from 43–55% and from 45–63%, respectively (Anon, 1988).

A naturally contaminated sample was preferred over a spiked sample to reduce the error due to homogeneity in a spiked sample. The variations observed in the present study could be attributed to either methodological variation or analytical errors at the analyst level. In the present study it was observed that of the eight participating laboratories only four used the CB method, two of them used BF, and the remaining two used Pons's method for aflatoxin analysis.

Participation in the check sample programme affords an opportunity for laboratories to have an analytical quality assurance. Such an assurance is essential, especially for laboratories engaged in analysing samples intended for export in view of the fact that export consignments are also analysed at the port of import. Detection of aflatoxin, above permissible limits, in consignments imported has resulted in levying a penalty, litigation and arbitrations or total rejection of the lot, leading to heavy economic losses to the exporters (Bhat, 1988). Participation in analytical check sampling is an assurance for the participating laboratory of their

analysis. If any discrepancy is observed by way of variation with other laboratories, the erring laboratory gets an opportunity to improve the analytical techniques.

This quality assurance programme, perhaps the first to be conducted in any developing country, should help reassure the Food Law implementing authorities, especially those of the European Economic Community, of the increasing analytical quality-consciousness in India.

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