Natural Occurrence of Mycotoxins in Staple Foods in India

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The occurrence of mycotoxins in selected staple foods and feeds in India has been studied. A total of 468 samples comprising of 150 samples of sorghum, 102 samples of corn, 58 samples of wheat, 19 samples of whole wheat flour, 37 samples of refined wheat flour and 102 samples of mixed animal feeds were screened for aflatoxins and trichothecenes [deoxynivalenol (DON), nivalenol, 3-acetyldeoxynivalenol, and T-2 toxin]. Occurrence of aflatoxins was observed in 45 samples of corn, 4 samples of sorghum, and 12 samples of mixed animal feeds with concentrations ranging from 0.02 to 7.4 μg/g in corn, from 0.02 to 0.06 μg/g in sorghum, and from 0.04 to 3.0 μg/g in animal feeds. The occurrence of trichothecenes was confined to the rain-affected samples of wheat and wheat products. DON was found to occur in one sample of wheat (0.31 μg/g) and in two samples of whole wheat flour (0.35 and 8.38 μg/g). In refined wheat flour, DON occurred in 11 samples (0.44–4.85 μg/g), nivalenol in 2 samples (0.1 and 0.03 μg/g), and 3-acetyl-DON in 4 samples (0.64–2.49 μg/g). T-2 toxin occurred in three samples of wheat (0.55–4.0 μg/g) and in one sample of refined wheat flour (0.8 μg/g). The study highlights the need for routine surveillance of agricultural commodities to minimize potential hazards to human health.

Mycotoxins are secondary metabolites produced by fungi. Mycotoxin contamination of crops can take place in the field, during harvest and storage, or even at a later point prior to consumption. High moisture, improper storage, and insect damage are some of the conditions leading to fungal growth and elaboration of mycotoxins. Among the mycotoxins, aflatoxins, ergot alkaloids, and trichothecenes are of considerable relevance since they have been found to be responsible for causing disease outbreaks in man (Krishnamachari et al., 1975; Krishnamachari and Bhat, 1976; Bhat et al., 1989). Because of their potentially hazardous nature to man and animals, tolerance limits for selected mycotoxins such as aflatoxins and deoxynivalenol have been established in many countries of the world (van Egmond, 1988). There are several studies on the natural occurrence of these toxins in foodgrains all over the world (Jelinek et al., 1989; Tanaka et al., 1988). In India, although considerable information on the incidence of aflatoxins in foodgrains is available, reports of natural occurrence of trichothecenes are limited (Bhat et al., 1976; Rukmini and Bhat, 1978; Bhat, 1983; Bhavanishankar and Shantha, 1987). Moreover, the simultaneous analysis of samples for aflatoxins and trichothecenes has not been carried out. The present study reports the natural occurrence of aflatoxins and trichothecenes in samples of sorghum, wheat (normal and rain affected), corn, and animal feeds.

MATERIALS AND METHODS

Samples were collected on the basis of maximum production, yield, and consumption for the previous years, adopting a purposive sampling method. One-kilogram samples of corn, wheat, and sorghum (*Sorghum vulgare*) intended for human consumption were obtained from households of agricultural laborers, small farmers, medium farmers, and large farmers as well as from market yards. Sorghum and wheat samples were collected from major sorghum and wheat growing areas in India, namely, the districts of Mahboobnagar and Medak in the State of Andhra Pradesh (sorghum) and the districts of Karnal, Rohtak, Hapur, and Izzatnagar in the States of Uttar Pradesh and Haryana (wheat). In addition, rain-soaked wheat and wheat products were collected from godowns/shops and specific families involved in a major disease outbreak in the Kashmir Valley (Bhat et al., 1989). Corn samples were collected from rural households in Medak as well as from wholesale and retail markets in an around Hyderabad in Andhra Pradesh.

Mixed animal feeds were also collected throughout India since foodgrains unfit for human consumption are normally used for animal feeds. The major ingredients of animal feed were wheat bran, deoiled rice bran, peanut cake, corn grits, wheat grits, and broken rice. The number of samples collected for each commodity is indicated in Table I.

Analysis of Samples. *Ergosterol.* The index of fungal contamination, namely ergosterol, was estimated by the method of Seitz et al. (1979) with slight modifications (Sashidhar et al., 1989). Fifty grams of the sample was placed in a 500-mL Erlenmeyer flask, extracted with 100 mL of methanol, and filtered into a round-bottomed flask. Methanol (50 mL) was used for rinsing. Fifty milliliters of alcoholic potassium hydroxide [20 g of KOH in 50 mL of alcohol (w/v)] was added to the above extract, and the mixture was refluxed for 30 min. On cooling, the contents of the flask were washed with water and transferred into a separatory funnel. Ergosterol was then transferred into 20 mL of petroleum ether. The petroleum ether fraction was evaporated, and the residue was dissolved in 1 mL of benzene–acetonitrile (9:1) for thin-layer chromatography. Solutions of the residue and standard ergosterol were applied to a silica gel coated TLC plate in different amounts. The plate was developed in benzene–acetonitrile (9:1) and viewed under UV light at 360 nm. The quantity of ergosterol was estimated visually by comparing the blue fluorescent spots of the unknown with the standard spots.

Aflatoxins. Aflatoxins were analyzed by the method of Barabalok et al. (1974) using acetone–water (85:15) as the extracting solvent. Initial screening was done by a minicolumn technique (Sashidhar et al., 1988). Positive samples were spotted on TLC plates along with standard aflatoxins and developed with chloroform–acetone (9:1). Quantitation of positive samples was carried out by visual comparison of the fluorescent intensities of sample spots with standard spots in the developed plates under long-range UV light. Animal feeds were analyzed for aflatoxins after the samples were extracted by the method of Romer et al. (1981) using acetonitrile–water (84:16).

Trichothecenes. The trichothecenes, deoxynivalenol, nivalenol, 3-acetyldeoxynivalenol, and T-2 toxin were analyzed by the method of Trucksess et al. (1984). The samples were extracted with acetonitrile–water (84:16), and column cleanup was car-

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Table I. Natural Occurrence of Mycotoxins in Staple Grains in India

commodity	region	no. of samples screened	no. positive for ergosterol	ergosterol, μg/g	no. positive for mycotoxins	mycotoxins, μg/g				
						DON[a]	NV[a]	AC-DON[a]	T-2[a]	aflatoxin B_1
sorghum	Andhra Pradesh	150	40	0.01–2.5	4	ND[b]	ND	ND	ND	0.02–0.06
corn	Andhra Pradesh	102	90	0.10–3.5	45	ND	ND	ND	ND	0.02–7.40
wheat	Andhra Pradesh	10	2	0.02–1.0	nil	ND	ND	ND	ND	ND
	NW India	36	20	0.01–2.0	nil	ND	ND	ND	ND	ND
	NW India[c]	12	8	0.02–5.4	4	0.31 (1)[d]	ND	ND	0.55–4.0 (3)	ND
whole wheat flour	Andhra Pradesh	10	1	0.02	nil	ND	ND	ND	ND	ND
	NW India[c]	9	3	0.20–3.1	2	0.35, 8.38	ND	ND	ND	ND
refined wheat flour	Andhra Pradesh	10	1	0.02	nil	ND	ND	ND	ND	ND
	NW India[c]	27	26	0.02–3.6	18	0.44–4.85 (11)	0.1–0.03 (2)	0.64–2.49 (4)	0.8 (1)	ND
feed samples	all India	102	73	0.01–3.0	12	ND	ND	ND	ND	0.04–3.0

[a] DON, deoxynivalenol; NV, nivalenol; T-2, T-2 toxin; AC-DON, acetyldeoxynivalenol. [b] ND, not detected. [c] Rain-affected wheat/wheat products. [d] Parentheses denote number of samples positive.

ried out with charcoal–Celite–neutral alumina (7:5:3). Separation and detection of trichothecenes was accomplished by thin-layer chromatography using chloroform–acetone–2-propanol (8:1:1) as the developing solvent and aluminum chloride [20% (w/v) in 50% alcohol] as the spray reagent. Confirmation of the analysis was carried out with different spray reagents (Ramakrishna and Bhat, 1987) like sulfuric acid, *p*-anisaldehyde, 4-(*p*-nitrobenzyl)pyridine, chromotropic acid, phloroglucinol, and 20% aluminum chloride. It was found that 20% aluminum chloride was the most suitable because of its sensitivity and stability, and also it acts as a confirmatory reagent since it forms a characteristic blue fluorescent spot with DON and other B-group trichothecenes only at the respective R_f.

Trichothecenes were quantitated by high-pressure liquid chromatography using a Shimadzu C_6 liquid chromatograph with a CR 3A recorder on a reverse-phase Bondapak C_{18} column. Methanol–water (85:15) was used as the mobile phase with a flow rate of 1 mL/min and an absorbance of 224 nm and on attenuation of 0.8 AUFS. The peaks recorded were compared with the standard concentration by using the peak areas and the retention time (Bennett et al., 1981).

RESULTS AND DISCUSSION

Analysis of sorghum samples indicated that 32% were positive for ergosterol with concentrations varying from 0.01 to 2.5 μg/g (Table I). Aflatoxins were present in four samples with concentrations varying from 0.02 to 0.06 μg/g. Trichothecenes were not detected in any of the samples. Among the corn samples analyzed, about 90% were positive for ergosterol with concentrations varying from 0.1 to 3.5 μg/g. Aflatoxins were detected in 45 samples with only 10 samples containing more than 30 ng/g of aflatoxin B_1, which is the tolerable limit in India, while 35 samples contained less than 30 ng/g of aflatoxin. Trichothecenes were not detected in any corn sample.

Analysis of wheat samples showed that over 50% of the normal wheat from the northwestern parts of India contained ergosterol ranging from 0.01 to 2.0 μg/g, while 20% of the wheat from Andhra Pradesh contained ergosterol in the range 0.02–1.0 μg/g. Aflatoxins and trichothecenes were not detected in any of these samples. Although fungal contamination was widely prevalent in most of the samples screened, incidence of DON and other trichothecenes was restricted to the rain-affected wheat and wheat flour samples from the northern parts of India, while aflatoxins were widely prevalent in the corn, sorghum, and animal feed samples. Most of the rain-affected wheat and wheat flour samples were positive for DON and other trichothecenes, but not for aflatoxins. Also, DON was found to co-occur with other trichothecenes in wheat, whole wheat flour, and refined wheat flour (Table II). Of the 12 samples of wheat, 3 were positive for T-2 toxin, ranging in concentration from 0.55 to 4.0 μg/g, and one for DON (0.31 μg/g) (Table I). In whole wheat flour, DON was present in two of the samples having a concentration of 0.35 and 8.38 μg/g. Two samples were positive for nivalenol with concentrations of 0.1 and 0.038 μg/g. Acetyl-DON was present in four of these samples at a concentration of 0.64–2.49 μg/g, while T-2 toxin was present in one of the samples at 0.83 μg/g concentration. Feed samples from all over India indicated that over 70% of the samples were positive for ergosterol in quantities ranging from 0.01 to 3.0 μg/g. Twelve of the feed samples contained aflatoxin in levels ranging from 0.04 to 3.0 μg/g.

Table II. Co-occurrence of Mycotoxins in Rain-Affected Wheat/Wheat Flour Samples from Jammu and Kashmir

mycotoxins	no. of samples positive for mycotoxins		
	wheat	whole wheat flour	refined wheat flour
deoxynivalenol	1	2	11
DON + nivalenol	nil	nil	2
DON + acetyl-DON	nil	nil	4
DON + T-2	nil	nil	1
T-2	3	nil	nil

From the human health angle, the mycotoxin contamination of cereals and millets that are used as staple foods by humans in India are of more importance than contaminations of oilseeds like peanuts. Food consumption surveys in different parts of the world revealed that while the consumption of cereals may be 450 g per person per day, that of groundnut normally varies from 2 to 35 g in India (Bhat, 1989). In fact, all three known mycotoxicoses for which definite cause and effect relationships have been established, namely, the aflatoxic hepatitis (Krishnamachari et al., 1975; Ngindu et al., 1982), enteroergotism (Krishnamachari and Bhat, 1976), and deoxynivalenol mycotoxicoses (Bhat et al., 1989), had occurred because of consumption of staple cereals/millets contaminated with mycotoxins. There was evidence of mold invasion based on ergosterol detection in over 50% of the samples of cereals screened. However, mycotoxin contamination could be observed in only 20% of the samples, indicating it is not the inoculum potential per se but appropriate moistures and temperatures that are essential for the elaboration of the mycotoxins. An earlier study indicated that about 11% of the molds isolated are indeed toxigenic (Ramakrishna et al., 1989). It is significant to note that even in a subtropical country like India natural occurrence of both aflatoxins produced by species of *Aspergillus* and trichoth-

ecenes produced by species of *Fusarium* was recorded in different commodities in various regions of the country. It has been earlier observed by Japanese investigators that the extent of natural occurrence of deoxynivalenol is greater in the northern region of Japan than in the southern region (Yoshizawa, 1983), and they have attributed it to high incidence of DON producers in the northern region compared to the southern region (Ichinoe et al., 1981). However, in the present study in India, trichothecenes were detected in the rain-damaged wheat originating in northwestern India, while normal wheat samples collected from the same region and southern India did not contain trichothecenes, which indicates that environmental factors and microecology at the time of harvest and not geography alone are important.

Tanaka et al. (1988) has recently observed that in 500 samples collected from 19 countries 42% were found to be contaminated with trichothecenes. It is pertinent that the number of samples positive for trichothecenes depends on the sampling design. In the present study, the majority of samples were collected at the time of a suspected disease outbreak in humans. A correct picture on the extent of trichothecene contamination would emerge only if a statistically designed sampling methodology is followed. The levels of DON and other trichothecenes as well as their co-occurrence found in the rain-damaged wheat and wheat flour samples are in agreement with the earlier studies from other parts of the world (Pathre and Mirocha, 1979; Vesonder and Hesseltine, 1981; Tanaka et al., 1988). DON and T-2 toxin do not normally occur together. However, one of the samples of refined wheat flour contained both DON and T-2 toxin, which can be explained from the fact that the wheat used for milling was from different sources and consisted of a mixture of sound, clean and rain-affected wheat. The present study emphasizes the need for surveillance of high-risk grain products and feeds for mycotoxins like aflatoxins and deoxynivalenol in staple foods in developing tropical and subtropical countries of the world.

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LITERATURE CITED

Barabalok, R.; Colburn, C. R.; Smitt, R. J. Rapid screening method for examining corn and corn-derived products for possible aflatoxin contamination. *J. Assoc. Off. Anal. Chem.* **1974**, *57*, 764–766.

Bennett, G. A.; Peterson, R. E.; Plattner, R. D.; Shotwell, O. L. Isolation and purification of deoxynivalenol and a new trichothecene by HPLC. *J. Am. Oil Chem. Soc.* **1981**, *58*, 1002A–1005A.

Bhat, R. V. Mycotoxin health hazards in India. *Proc. Jpn. Assoc. Mycotoxicol.* **1983**, *17*, 7–12.

Bhat, R. V. Risk to human health associated with consumption of groundnuts contaminated with aflatoxins. *Aflatoxin contamination of groundnut*; Proceedings, International Workshop, Oct 6–9, 1987, ICRISAT Centre; ICRISAT (International Crops Research Institute for the Semi-Arid Tropics): Andhra Pradesh, India, 1989; pp 19–29.

Bhat, R. V.; Roy, D. N.; Tulpule, P. G. The Nature of alkaloids of ergoty pearl millet or bajra and its comparison with alkaloids of ergoty rye and ergoty wheat. *Toxicol. Appl. Pharmacol.* **1976**, *36*, 11–17.

Bhat, R. V.; Sashidhar Rao, B.; Ramakrishna, Y.; Munshi, K. L. Outbreak of trichothecene mycotoxicosis associated with consumption of mould damaged wheat products in Kashmir Valley, India. *Lancet* **1989**, *1*, 35–37.

Bhavanishankar, T. W.; Shanta, T. Natural occurrence of *Fusarium* toxins in peanut, sorghum and maize in Mysore, India. *J. Sci. Food Agric.* **1987**, *40*, 327–332.

Ichinoe, M.; Amano, R.; Morooka, N.; Yoshizawa, T.; Suzuki, T.; Kurisu, M. *Geographic* difference of toxigenic fungi of *Fusarium* spp. *Proc. Jpn. Assoc. Mycotoxicol.* **1981**, *11*, 20–21.

Jelinek, C. F.; Pohland, A. E.; Wood, G. E. Worldwide occurrence of mycotoxins in foods and feeds-An update. *J. Assoc. Off. Anal. Chem.* **1989**, *72*, 223–230.

Krishnamachari, K. A. V. R.; Bhat, R. V. Poisoning by ergoty bajra (Pearl millet) in man. *Indian J. Med. Res.* **1976**, *64*, 1624–1628.

Krishnamachari, K. A. V. R.; Bhat, R. V.; Nagarajan, V.; Tilak, T. B. G. Hepatitis due to aflatoxicosis: An outbreak in Western India. *Lancet* **1975**, *1*, 1061.

Ngindu, A.; Johnson, B. K.; Kenya, P. R.; Ngira, J. A.; Ocheng, D. M.; Nandwa, H.; Omondi, T. N.; Jensen, A. J.; Ngara, W.; Kaviti, J. N.; Gate, D.; Splongok, T. A. Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. *Lancet* **1982**, *1*, 1346–1348.

Pathre, S. V.; Mirocha, C. J. Trichothecenes, natural occurrence and potential hazards. *J. Am. Oil Chem. Soc.* **1979**, *56*, 820–823.

Ramakrishna, Y.; Bhat, R. V. Comparison of different spray reagents for identification of trichothecenes. *Curr. Sci.* **1987**, *56*, 524–526.

Ramakrishna, Y.; Bhat, R. V.; Ravindranath, V. Production of deoxynivalenol by *Fusarium* isolates from sorghum cultivars associated with specific plant diseases and samples of wheat associated with human mycotoxicosis outbreak. *Appl. Environ. Microbiol.* **1989**, *55*, 2619–2620.

Romer, T.; Greaves, D.; Gibson, G. 6th Spring Workshop of the AOAC, Ottawa, Ontario, 1981.

Rukmini, C.; Bhat, R. V. Occurrence of T-2 toxin in *Fusarium* infested sorghum from India. *J. Agric. Food Chem.* **1978**, *26*, 647–649.

Sashidhar, R. B.; Ramakrishna, Y.; Bhat, R. V. Rapid detection of aflatoxins by pressure minicolumn technique. *Anal. Lett.* **1988**, *21*, 507–518.

Sashidhar, R. B.; Sudershan Rao, V.; Ramakrishna, Y.; Bhat, R. V. Rapid and specific method for screening ergosterol as an index of fungal contamination in cereal grains. *Food Chem.* **1989**, *31*, 51–56.

Seitz, L. M.; Sauer, D. B.; Burroughs, R.; Mohr, H. E.; Hubbard, J. D. Ergosterol as a measure of fungal growth. *Phytopathology* **1979**, *69*, 1202.

Tanaka, T.; Hasegawa, A.; Yamamoto, S.; Lee, U. S.; Sugiura, Y.; Ueno, Y. Worldwide contamination of cereals by the *Fusarium* mycotoxins, nivalenol, deoxynivalenol and zearalenone. 1. Survey of 19 countries. *J. Agric. Food Chem.* **1988**, *36*, 979–983.

Trucksess, M. W.; Nesheim, S.; Eppley, R. M. Thin layer chromatographic determination of DON in wheat and corn. *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 40–43.

van Egmond, H. P. Current situation on regulations for mycotoxins. Overview of tolerance and status of standard methods of sampling and analysis. *Food Add. Contam.* **1988**, *6*, 139–188.

Vesonder, R. F.; Hesseltine, C. W. Vomitoxin: Natural occurrence on cereal grains and significance as a refusal and emetic factor to swine. *Process. Biochem.* **1981**, *16*, 12–15.

Yoshizawa, T. Red mold diseases and natural occurrence in Japan. *Trichothecenes: Chemical, biological and toxicological aspects*; Ueno, Y., Ed.; Kodansha: Tokyo, 1983; pp 196–209.

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Registry No. DON, 51481-10-8; ergosterol, 57-87-4; nivalenol, 23282-20-4; 3-acetylnivalenol, 115889-63-9; T-2 toxin, 21259-20-1; aflatoxin B_1, 1162-65-8.