J. Mycopathol. Res. 41(1) : 21-26 (2003)

Aflatoxin problem in wheat grains at Kathmandu, Nepal

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A total of 126 wheat samples were collected from different agricultural farms, store-houses and grocers' shops located in and around Kathmandu valley, Nepal. *Aspergillus flavus* and species of *Alternaria, Fusarium, Penicillium, Helminthosporium, Curvularia, Chaetomium, Trichothecium, Mucor* and *Rhizopus* were found to be associated with wheat grains. Out of 65 isolates of *A. flavus* screened for aflatoxin production in liquid SMKY medium, 25 isolates (38.46%) were found to be toxigenic and these produced aflatoxin B_1 in the range of 200 to 1800 µg/1. Only 66 out of 126 samples were positive to BGYF test but aflatoxin could be detected only in 15 samples and level of aflatoxin was also in the range of 20 to 130 µg/kg.

Key words : Aflatoxin, wheatgrains, Nepal

INTRODUCTION

Wheat is the third important crop after rice and maize in Nepal. It represents 22 per cent of total cultivated area (0.66 million hectares) and its production in 1999/2000 was 1.18 million Metric tonnes (Anonymous, 2000). It is utilized in bakery industries and for preparation of many indigenous products.

Wheat grains/seeds have been shown to harbour a large number of storage fungi including *Aspergillus flavus* and *A. parasiticus*. Reports of aflatoxin contamination in wheat are available from majority of the wheat growing countries of the world, such as in India (Prasad *et al.* 1982, 1987 ; Agarwal *et al.*, 1983 ; Jeswel, 1986 ; Sinha and Sinha, 1990 ; Sinha, 1991 ; Prasad *et al.*, 1997), Brazil (Furlong *et al.*, 1990, 1995), Egypt (Rabie, 1997), Romania (Curtui *et al.*, 1998) etc. The severity and magnitude of the problem, however, varied according to the geographical locations.

In Nepal, wheat is cultivated in Terai as well as hilly areas and is usually sown in winter and harvested in rainy seasons. Local farmers mostly store the grains for their own consumption but some of the seeds are also stored for the propagation in the following year. Earlier reports on wheat grains harbouring toxigenic strains of *A. flavus* and subsequent aflatoxin elaboration in them are almost lacking in Nepal. Moreover, aflatoxins have also not been detected in wheat samples of Nepal in earlier works (Karki *et al.*, 1979 ; Karmacharya, 1988 ; Shrestha and Amatya, 1997 ; 1998). Present report aims to record the problem of aflatoxin contamination as well as the incidence of toxigenic strain of *A. flavus* in wheat grains collected from various parts of Nepal particularly of Kathmandu Valley.

The Kathmandu valley (alt. 1231 m) is located between latitudes 27.25°N and 27.50°N and longitudes 85.11°E and 85.32°E. Kathmandu valley covers an area of 899 square kilometers and includes three culturally rich districts of Nepal viz, Kathmandu, Lalitpur and Bhaktapur.

MATERIALS AND METHODS

Sample collection

Wheat samples stored from one month to one year were collected during four different seasons for one year from June 2000 to July 2001. The stored samples were collected from storage centres of farmers and markets situated in various parts of Kathmandu valley.

In Kathmandu district, the wheat samples were obtained from Kirtipur, Machhegaon, Pharping,

Taudaha, Thankot, Satungal, Naikap, Ramkot, Manmaiju, Jitpurphedi, Tokha, Budanilkantha, Gokarna, Sundarijal, Sankhu and many parts of Kathmandu Metropolitan area.

In Lalitpur district, wheat samples were collected from Khumaltar, Siddhipur, Lubhu, Lamatar, Godawari, Bedegaon, Harisidhi, Sunakothi, Thecho, Chapagaon, Khokana, Bungamati, Lele, Nallu, Tikabhairab and some parts of Lalitpur Sub-metropolitan area.

In Bhaktpaur district, the wheat grain samples were obtained from Sanothimi, Nayathimi, Bode, Changunarayan, Duwakot, Chhaling, Nagarkot, Nankhel, Nalinchowk, Tathali, Suryabinayak, Dadhikot, Gundu and some parts of Bhaktapur municipality area.

The storage samples were kept in metallic bins, gunny bags and earthern pots for future consumption. Each sample consisted of at least 500 g of stored grains and was kept in polythene bags. The samples were brought to University Department of Botany, Bhagalpur University, Bhagalpur and stored in a freezer until examined.

Isolations of mycoflora

For the isolation of mycoflora associated with wheat samples, standard rules as recommended by the International Seed Testing Association (ISTA, 1966, 1985) were followed. Each sample of 100 kernels was surface disinfected with 2% sodium hypocholorite (NaOCI) solution for 10 minutes. Subsequently, the seeds were aseptically plated on moist blotting paper and on Czapeks dox agar medium in petridishes in order to isolate the associated mycoflora. The plates were incubated in BOD incubator for seven days at 28±2°C under the alternating cycles of 12 h of darkness of fluoresent exposure. The seeds were regularly examined under Nikon Steriobinocular microscope (SMZ-10) from third day and developing fungal colonies particularly *Aspergillus flavus* group were isolated and maintained on PDA and Czapeks' dox agar media. Percentage incidence of these fungi was calculated on the basis of the occurrence of a particular fungus per 100 samples.

Screening of A. flavus isolates for aflatoxin production

All the *A. flavus* group isolates were tested in SMKY medium (Diener and Davis, 1966) for their ability to elaborate aflatoxins. The culture filtrate was extracted with chloroform and concentrated chloroform extract was used for the qualitative and quantitative estimation of aflatoxin.

Aflatoxin analysis of wheat samples

A portion of each wheat sample was ground coarsely and observed under long wave UV - light for the characteristic BGY (Bright Greenish Yellow) fluorescence test (Fennell *et al.*, 1973 ; Shotwell, 1983). The BGYF positive samples (200 g) were further ground and mixed to obtain 50 g sub samples for aflatoxin analysis.

Qualitative and quantitative estimation of aflatoxins

Initial detection of aflatoxin was carried out by thin layer chromatography (TLC) using toluene - isoamyl alcohol-methanol (90:32:2, v/v/v) solvent system (Reddy *et al.*, 1970) under long-wave UV light (360 nm).

Chemical confirmation of aflatoxin B was performed with trifluoroacetic acid (Stack and Pohland, 1975) and by spraying with 25% sulphuric acid. Quantity of aflatoxin B_1 was determined spectrophotometrically (AOAC, 1984).

RESULTS AND DISCUSSION

As shown in Table 1, the climatic conditions of Kathmandu were fluctuating in the year 2000 and 2001. In the year 2000, the temperature ranged from 2.4°C to 29.1°C whereas in 2001 in ranged from 2.1°C to 29.9°C. In the year 2000, the total rainfall during preharvesting and harvesting periods of wheat (i.e. from March-June) was 559.8 mm whereas it was only 473.3 mm during the same period in 2001. In the year 2000, the average monthly temperature during March-June was 20.5°C whereas it was 20.9°C during the same months in 2001. In 2000, the relative humidity ranged from

47-99% and average relative humidity from March to June was 79.2%.

Table 1 : Meteorological records of Kathmandu of the Year 2000 & 2001 (average temperature, relative humidity and rainfall)

Year	Months	Temperture range(°C)	Average temperature °C Max	Min	Average	Average Relative Humidity(%)	Total Rainfall in mm
2000	Jan-Feb	2.4-20.7	20.90	2.7	11.8	76.00	6.60
	Mar-Apr	6.8-29.0	27.20	9.45	18.32	65.00	83.40
	May-Jun	17.2-29.0	28.85	18.50	23.67	81.00	476.40
	Jul-Aug	20.0-29.1	29.05	20.05	24.55	85.50	721.00
	Sep-Oct	13.5-28.1	28.00	15.80	21.90	82.00	120.10
	Nov-Dec	3.4-24.5	22.80	6.40	14.60	80.50	0.40
	Mean		26.10	12.10	19.10	78.30	
	Total Monsoon						1107.00
	Total Annual Rainfall						1407.90
2001	Jan-Feb	2.1-24.6	22.5	3.45	13.05	NA	22.5
	Mar-Apr	7.4-29.6	43.0	9.30	18.72	NA	43.0
	May-June	16.4-28.5	430.0	17.80	23.07	NA	430.3
	July-Aug	19.9-29.9	959.1	20.00	24.72	NA	959.1
	Sept-Oct	14.3-28.5	166.0	16.40	22.25	NA	166.0
	Nov-Dec	3.8-25.2	0.0	6.15	14.52	NA	0.0
	Total Monsoon						1120.40
	Total Annual Rainfall						1620.90

Source : *Department of Hydrology and Meteorology, HMG, Kathmandu, Nepal.*
NA = Not Available.

Table 2 : Percentage frequency of important fungi associated with different wheat samples collected from Kathmandu Valley.

Fungi	A (TS=21)		B (TS=58)		C (TS=10)		D (TS=16)		Total no. of all samples studied=105	
	IS	PI	IS	PI	IS	PI	IS	PI	Total sample	% PI
Aspergillus flavus	18	85.71	20	34.4	5	50	14	87.5	57	54.2
A. niger	3	14.28	4	6.8	-	-	8	50	15	14.2
Alternaria sp.	10	47.6	46	79.3	7	70	11	68.7	74	70.4
Fusarium sp.	11	52.3	17	29.3	1	10	1	6.2	30	28.5
Penicillium sp.	3	14.2	13	22.4	5	50	2	12.5	23	21.9
Chaetomium sp.	8	38.0	6	10.3	4	40	5	31.2	23	21.9
Helminthosporium sp.	–	–	31	53.4	3	30	1	6.2	35	33.3
Curvularia sp.	–	–	3	5.1					3	2.8
Trichothecium sp.	–	–	2	3.4					2	1.9
Rhizopus sp.	4	19.0	16	27.5					20	19
Mucor sp.	3	14.0	3	5.1					6	5.7

TS = Total no. of samples studied.
IS = Total no. of infected samples.
PI = Percentage of infected samples.
A = Wheat samples collected during June - Aug., 2000
B = Wheat samples collected during Oct. - Nov., 2000
C = Wheat samples collected during March - 2001
D = Wheat samples collected during June - 2001

Table 2 shows list of important fungi found to be associated with wheat samples along with their percentage incidence. *Alternaria* sp. had the higher incidence, in almost all the categories of wheat samples, followed by *Aspergillus flavus, Fusarium* sp. *Helminthosporium* sp., *Penicillium* sp. and *Chaetomium* sp.

Table 3 : Aflatoxin producing potentials of *A. flavus* isolates

Nature of aflatoxin production	No. of isolates	Percentage
Screened for aflatoxin production	65	
Positive to aflatoxin production	25	38.46
Producing only aflatoxin B_1	20	80.00
Producing both aflatoxin B_1 and B_2	5	20.00
Grouped as :		
High toxin producers (501-2000 µg/1)	9	36.00
Moderate toxin producers (301-500 µg/1)	7	28.00
Low toxin producers (below 300 µg/1)	9	36.00

Table 4 : Aflatoxin incidence in wheat samples collected from Kathmandu Valley.

Range of aflatoxin concentration (µg/kg)	No. of contaminated samples	Percentage of contaminated samples
1-20	1	7
21-40	3	20
40-60	6	40
61-80	2	13
81-120	2	13
121-140	1	7

Total number of samples analysed for aflatoxin contamination = 66
Total number aflatoxin positive samples = 15 (23%)
Total number of samples in which aflatoxins were not detected = 51 (77%)

Table 3 shows aflatoxin producing ability of *Aspergillus flavus.* Out of 65 isolates screened, 25 elaborated aflatoxin in liquid SMKY medium. Majority of toxigenic strains produced aflatoxin B_1 only. Only 5 toxigenic strains produced aflatoxin B_1 and B_2. The range of aflatoxin B_1 produced by the toxigenic isolates were grouped into three categories : high (501-2000 µg/1) ; moderate (301-500 µg/1) and low (below 300 µg/1) toxin producers.

Table 5 : District wise incidence of aflatoxin contamination in wheat samples collected from Kathmandu Valley and Bhairahawa, Rupandehi.

Type of Samples	Samples from Kathmandu TS	CS	PI	Lalitpur TS	CS	PI	Bhaktapur TS	CS	PI	Bhairahawa (Rupandehi) TS	CS	PI	Total of all districts No. of samples	No. of contaminated samples	% of contamination
A	6	2		4	2		1	1		2	1		13	6	46.15
B	17	4	19.35	6	2	19.04	9	3	33.33	—	—	50.0	32	9	28.12
C	1	—		6	—		—	—		—	—		7	—	0
D	7	—		5	—		2	—		—	—		14	—	0
Total	31	6		21	4		12	4		2	1		66	15	22.72

A = Wheat samples collected during June-Aug., 2000
B = Wheat samples collected during Oct.-Nov., 2000
C = Wheat samples collected during March, 2001
D = Wheat samples collected during June, 2001
TS = Total no. of samples analysed.
CS = No. of samples contaminated with aflatoxin.
PI = Average percentage of contaminated samples.

Nine isolates were high toxin produces while 7 were moderate toxin producers and the rest were low toxin producers.

Table 4 shows that out of 66 samples of wheat grains analysed 15 (23%) samples were contaminated with aflatoxin B_1 in the level, ranging from 20 to 130 µg/kg. The data revealed that 46% of wheat samples collected during June-August, 2000 and 28% of wheat samples collected during October to November, 2000 were contaminated with aflatoxins, whereas aflatoxins were not detected at all in any samples collected during the year 2001 (Table 5).

The result also showed that aflatoxin contamination was found in 45% of samples collected from grocers' shops, followed by 19% and 15% of the samples collected from farmers' storage centers and agricultural farms respectively (Table 6). The comparative study of aflatoxin contamination in different storage structures shows that metallic drums/bins seem to be most appropriate storage structures for storing wheat grains for future consumption (Table 7).

Table 6 : Aflatoxin contamination in wheat grain samples of Kathmandu valley on the basis of sources of collection (i.e. farmer's storage centers. grocers' shops and agricultural research centers)

Sources of collected samples	A	B	C	D	Total
Farmer's storage centers					
TS	9	23	1	9	42
CS	3	5	—	—	8
% of CS					(19%)
Markets (Grocer's Shop)					
TS	2	6	2	1	11
CS	2	3	—	—	5
% of CS					(45%)
Agricultural Res. Centres					
TS	2	3	4	4	13
CS	1	1	—	—	2
% of CS					(15%)

CS = No. of Contaminated samples ; TS = Total No. of samples ; A, B, C, D = As mentioned in Table 5.

Table 7 : Percentage of aflatoxin contamination in wheat samples from Kathmandu Valley collected from various storage structures.

Storage	A TS	CS	B TS	CS	C TS	CS	D TS	CS	Total No. of analysed samples	No. of contaminated samples	% of contamination
Gunny bags	9	3	14	5	2	—	5	—	30	8	26.66
Metallic drum/bin	3	2	9	1	4	—	7	—	23	3	13.04
Earthern Pots	—	—	8	2	—	—	1	—	9	2	22.22
Jute sacks	1	1	1	1	1	—	1	—	4	2	50.00
Total	13	6	32	9	7	—	14	—	66	15	22.72

TS = Total No. of analysed samples CS = No. of contaminated samples A, B, C, D = As mentioned in Table 5.

Production of aflatoxin in wheat grains samples, collected in the year 2000 may be due to appropriate environmental conditions of Kathmandu Valley for the elaboration of aflatoxins during the harvesting period of wheat. There was unseasonal continuous and heavier rainfall in Kathmandu valley in the preharvesting and harvesting periods of wheat (March-June, 2000) during that year as compared to the following year 2001 (Table 1).

Besides, temperature and relative humidity may be contributing factors for the elaboration of aflatoxins in those wheat grain samples. Wheat grain samples collected during that period might have been improperly and insufficiently dried due to uninterrupted rainfall at the time of the harvesting period in the year 2000 as the farmers of Kathmandu valley depend entirely on sunlight for drying their freshly harvested cereals.

ACKNOWLEDGEMENTS

The authors are highly grateful to Prof, Dr. J. V. V. Dogra, Head, University Department of Botany, T. M. Bhagalpur University, Bhagalpur for providing the laboratory ficilities to carry out the present work. The first author is highly grateful to Faculty of Education, Tribhuvan University, Nepal for providing the fellowship and giving the study leave.

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(Accepted for publication September 16 2002)