Maize (Zea mays) and wheat (Triticum aestivum) collected in the foothills of the Nepal Himalaya Mountains were analyzed for Fusarium species and mycotoxins: fumonisins, nivalenol (NIV), and deoxynivalenol (DON). Predominant species were Gibberella fujikuroi mating population A (F. moniliforme) in maize and F. graminearum in maize and wheat; G. fujikuroi mating population D (F. proliferatum), F. acuminatum, F. avenaceum, F. chlamydosporum, F. equiseti, F. oxysporum, F. semitectum, and F. torulosum were also present. Strains of G. fujikuroi mating population A produced fumonisins, and strains of F. graminearum produced NIV or DON. By immunoassay or high-performance liquid chromatography, fumonisins were >1000 ng/g in 22% of 74 maize samples. By immunoassay or fluorometry, NIV and DON were >1000 ng/g in 16% of maize samples but were not detected in wheat. Fumonisins and DON were not eliminated by traditional fermentation for producing maize beer, but Nepalese rural and urban women were able to detoxify contaminated maize by hand-sorting visibly diseased kernels.

**Keywords:** Fusarium; maize; wheat; mycotoxins; fumonisins; nivalenol; deoxynivalenol; HPLC; immunoassay

**INTRODUCTION**

Both maize (Zea mays) and wheat (Triticum aestivum) are important food crops in the foothills of the Nepal Himalaya Mountains (Anonymous, 1997a,b). In the foothills, most maize is grown under rain-fed conditions in marginal lands along the steep hillside slopes. At elevations of 1000–2000 m, maize is a summer crop planted from February through April. At lower elevations, maize is also grown as a winter crop. Summer maize matures during the monsoon season and, after the plants are harvested, the fields are replanted with rice, millet, wheat, and other crops. For long-term storage, maize is traditionally left in the husk on a platform or rack in the farmyard. Maize is a nontraditional crop in the foothills of Nepal but was apparently accepted in traditional farming systems relatively soon after its introduction to India from the Americas in the 1600s. By 1800, maize was the major food grain of populations throughout the Nepal foothills and the particular food of the poor (Hamilton, 1819; Lohani, 1980). Maize remains today the staple food of most foothill populations, who use it to produce a variety of porridges, breads, snack foods, and fermented beverages.

In the foothills, wheat is a winter crop planted from September through November after the maize and rice harvests. Wheat is threshed by traditional methods to separate the grain from stems and husks, sun-dried to decrease its moisture content, and stored in baskets or bins in a well-ventilated area of the farmhouse. Wheat cultivation has a long history in the foothills of Nepal (Hamilton, 1819; Lohani, 1980). In the 1960s, improved semidwarf varieties were introduced and are now extensively grown throughout the foothills region, especially in the agriculturally developed Kathmandu valley and adjoining regions (Morris et al., 1994).

Surveys of maize ear rot in Nepal have found that Fusarium species predominate among many other ear-rotting fungal pathogens (Anonymous, 1997a; Macdonald and Chapman, 1997). Overall, the most frequently isolated species is Gibberella fujikuroi mating population A (MP-A) (anamorph F. moniliforme, synnym F. verticillioides). Fusarium graminearum (teleomorph Gibberella zeae) also is prevalent in the foothills region. Both G. fujikuroi MP-A and F. graminearum are common pathogens of maize worldwide (Marasas et al., 1984). F. graminearum is also a causal agent of wheat head scab (Fusarium head blight) (Parry et al., 1995), a disease that has not yet been reported in Nepal (C. Karki and S. Sharma, personal communication).

The occurrence of G. fujikuroi MP-A and F. graminearum in Nepalese maize is cause for concern because these species produce mycotoxins that can impair human and animal health. G. fujikuroi MP-A produces fumonisins (Figure 1), sphingolipid analogues that can cause equine leukoencephalomalacia, porcine pulmonary edema, and experimental liver cancer in rats. Furthermore, epidemiological studies have associated consumption of maize containing high levels of G. fujikuroi MP-A and fumonisins with the occurrence of high rates of human esophageal cancer in certain regions of South Asia.

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of 27 wheat grain samples were collected from smallholder farmers in the Lamjung district. From May to July 1997, a total of 195 maize grain samples, including yellow and white varieties, and maize-based foods were collected from farms and were representative of the diversity of maize and wheat used for human consumption, in maize and wheat grain used for human consumption, in the foothills of the Nepal Himalaya Mountains.

**Figure 1.** Chemical structures of fumonisins B<sub>1</sub>, fumonisins B<sub>2</sub>, fumonisins B<sub>3</sub>, nivalenol, and deoxynivalenol.

Fumonisins have been detected in maize, and trichothecenes have been detected in both maize and wheat, in North America, South America, Europe, Asia, and Africa (Beardall and Miller, 1994; Marasas et al., 1993; Shepard et al., 1996). However, there is no information available on the occurrence of trichothecenes in maize in Nepal. Only one study on the occurrence of fumonisins in maize from a single location (Ueno et al., 1993). The high reported incidence of maize ear rot, in particular, suggests a potential for contamination of maize with fumonisins and trichothecenes. Objective 1 of the present study was to determine the incidence of Fusarium species in maize and wheat from smallholder farms and from markets in the foothills of the Nepal Himalaya Mountains.

**Objective 2** was to determine the natural occurrence, in maize and wheat grain used for human consumption, of the mycotoxin fumonisins, NIV, and DON, which are most likely to be important in human mycotoxicoses. Objective 3 was to determine the effect of traditional Nepalese methods of postharvest processing on mycotoxin levels in contaminated maize.

**Materials and Methods**

**Maize and Wheat Sample Collection.** In March 1993, three visibly moldy yellow maize grain samples were collected from smallholder farms in Lamjung district, and a yellow maize grain sample from a Kathmandu market was provided by T. Karki at the Central Food Research Laboratory, HMG Ministry of Agriculture, Kathmandu. From February to July 1997, 78 samples of maize grain, including yellow and white varieties, and maize-based foods were collected from farms and markets in 10 districts of central to eastern Nepal (Figure 2). The smallholder farms in the Lamjung district were selected for sample collection to maximize diversity of ethnic groups and socioeconomic levels, as determined in interviews with household members. At least 0.5 kg was collected per maize sample, except for samples from Chitwan, Dhanakuta, and Morang districts, and other occasional samples, which were approximately half that weight. From May to July 1997, a total of 27 wheat grain samples were collected from smallholder farms in four districts of central Nepal (Figure 2). Twenty-four samples were improved wheat cultivars; three samples from the Lamjung district were a small-seeded local variety of wheat. At least 0.5 kg was collected per wheat sample.

**Isolation and Identification of Fusarium Strains.** For isolations in 1993, maize kernels were placed in ~0.1% aqueous iodine for 5 min, rinsed five to six times in spring water, and placed on selective medium containing penta-chloronitrobenzene (Nelson et al., 1983). For isolations in 1997, maize and wheat kernels were surface-disinfested in 1% NaOCl for 1 min. Maize kernels were placed immediately on selective medium, but wheat kernels were first rinsed twice in sterile water. Kernels were incubated for 5–7 days, and then one colony per kernel was reisolated from a single spore and identified by morphology as described (Nelson et al., 1983). For the 1993 maize samples and the wheat samples, every Fusarium colony obtained was identified to species. For the 1997 maize samples, colonies were grouped by morphological criteria, and representative colonies were identified to species. Strains identified by morphology as F. torulosum were confirmed by species-specific primers (Yoder and Christianson, 1998).

Strains identified by morphology as F. graminearum were tested for production of the G. zeae sexual stage on carrot agar as described (Klittich and Leslie, 1988). To determine mating population and mating type, strains identified by morphology as F. moniliforme or F. proliferatum were crossed on carrot agar (Klittich and Leslie, 1988) as males with tester strains of G. fujikuroi MP-A, MP-D, and mating population F (MP-F) (Kerenyi et al., 1999). Strains that were nonfertile in the first test were retested one or more times. Female fertile strains of MP-A isolated from Nepalese maize in 1993 were used as testers for strains collected in 1997.

**Immunassays.** The initial mycotoxin analysis of maize and wheat was conducted by immunosay in Nepal. One hundred grams of a sample was ground to a coarse meal consistency in a coffee mill or on a grinding stone. Two 50 g
Fumonisins and Trichothecenes in Grain from Nepal


Table 1. Fusarium Species from Maize and Wheat Grain

<table>
<thead>
<tr>
<th>fungal species</th>
<th>no. of samples infected with the indicated speciesa</th>
<th>from maize</th>
<th>from wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. fujikuroi, all strains</td>
<td>66</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>mating population A</td>
<td>52</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>mating population D</td>
<td>3</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>not fertile</td>
<td>12</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>fertility not tested</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>F. graminearum</td>
<td>16</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>F. acuminatum</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>F. avenaceum</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>F. chlamydosporum</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>F. equiseti</td>
<td>3</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>F. oxyssporum</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>F. semitectum</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>F. torulosum</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>none (clean samples)</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>68</td>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

a Samples tested were 4 maize grain samples collected in 1993 (12 to 35 seeds per sample were tested), 64 maize grain samples collected in 1997 (50 seeds per sample were tested), and 27 wheat grain samples collected in 1997 (100 seeds per sample were tested).

Processing Contaminated Maize by Fermentation. Nepalese women were recruited to determine whether mycotoxins in maize grain could be decreased to acceptable levels by hand-sorting visibly diseased kernels. In April and May 1997, 12 participants each received 300–800 g of maize sample purchased in a Kathmandu market. The maize sample selected for the study contained a high proportion of visibly diseased kernels, and immunoassay indicated that the fumonisin and DON levels were each above the detection limit of 1000 ng/g. The 12 participants were 4 trained plant pathologists, 3 urban women, and 5 women from smallholder farms in the Lamjung district. Participants were informed that the goal of the study was to efficiently clean the sample for human consumption by (1) removing visibly diseased kernels and (2) maximizing the recovery of cleaned sample. Fumonisins were determined by immunoassay or HPLC, and 8-ketotrichothecenes were determined by fluorometric quantitation as described above.

RESULTS

Occurrence of Fusarium Species in Maize and Wheat Grain. Fusarium strains were recovered from 99% of the 68 maize grain samples tested and from a mean of 32 ±21% of the 3300 maize kernels tested (Table 1). Ninety-seven percent of the maize samples contained strains identified by morphological traits as species of the G. fujikuroi complex. Efforts were made to assign 103 strains from this morphological group to mating populations by fertility with tester strains of G. fujikuroi MP-A, MP-D, and MP-F (anamorph F. thapsi-
Eighty-two strains were G. fujikuroi MP-A, with 35% of the fertile strains mating type MATA-1 and 65% MATA-2. Both MATA-1 and MATA-2 were widely distributed among the samples. Five strains were G. fujikuroi MP-D; two were MATA-1, and three were MATA-2. None of the strains were G. fujikuroi MP-F. F. graminearum was recovered from 24% of the maize samples, but other Fusarium species were rare. Twenty of 25 strains of F. graminearum tested were self-fertile and produced perithecia of the sexual stage G. zeae in culture.

Fusarium strains were recovered from 24 of the 27 wheat samples, but the average incidence of recovery was only 4% for the 2700 kernels tested (Table 1). Species recovered, in order of frequency from the wheat samples, were F. graminearum (56%), F. equiseti (52%), G. fujikuroi MP-D (37%), F. oxysporum (30%), F.avenaceum (22%), F. semitectum (19%), F. tolcusulom (7%), F. acuminatum (7%), and F. chlamydosporum (4%). Seven strains of G. fujikuroi MP-D were mating type MATD-1, and three were MATD-2.

### Occurrence of Fumonisins in Maize

In an initial survey conducted in Nepal, fumonisin levels in 36 grain samples and 9 flour samples were determined by immunoassay (Table 2). Seventeen of the 45 samples contained fumonisin above the detection limit of 1000 ng/g, and the mean fumonisin level of the positive samples was 2300 ng/g. To confirm the immunoassay, further subsamples of 39 of the 45 samples from the initial survey were analyzed for fumonisins by HPLC (Table 3). Twenty-seven additional samples of grain and two samples of cornflake cereal also were analyzed for fumonisins by HPLC (Table 3). Fifty-eight of the 68 samples contained fumonisin above the level of 100 ng/g, and the mean fumonisin level of the positive samples was 380 ng/g. Overall, by immunoassay or by HPLC analysis, fumonisins were >1000 ng/g in 22% of the 74 samples of maize and were >2000 ng/g in 9% of the samples. Fumonisin B₁ was the predominant homologue in all samples tested; fumonisins B₂ and B₃ were usually present in only trace amounts.

Comparison of data from the two methods indicated that immunoassay generally gave higher levels of fumonisins than did HPLC of the same maize sample. To investigate this discrepancy, both techniques were applied to extracts from one flour sample and three grain samples. The four samples contained fumonisins at 240, 1900, 2300, and 9700 ng/g by HPLC analyses and 1500, 2200, 5600, and 16900 ng/g, respectively, by immunoassay. Thus, fumonisin levels were 1.2–6.2-fold higher by immunoassay than by HPLC. Similar results have been reported in other studies, which have concluded that these differences are due in part to the presence of compounds that are structurally related to fumonisins but are not detected by the HPLC method (Sydenham et al., 1996).

In a previous survey (Nelson et al., 1991), strains of G. fujikuroi MP-A isolated from maize from a Kathmandu market produced little or no fumonisin in culture. To determine whether fumonisin-non-producing strains are widespread in Nepalese maize, we analyzed fumonisin production by strains of G. fujikuroi MP-A isolated in 1993 from maize, 26 strains from Lamjung farms and 2 strains from a Kathmandu district market. All 28 strains tested produced high levels of fumonisins in culture; the means + standard deviations were 4680 ± 2420 μg/g fumonisin B₁, 2210 ± 2710 μg/g fumonisin B₂, and 3470 ± 3250 μg/g fumonisin B₃.

### Occurrence of NIV and DON in Maize and Wheat

In an initial survey conducted in Nepal, DON levels in 36 maize grain and 9 flour samples were analyzed by immunoassay using an antibody specific for DON (Table 2). Seven of the 45 samples contained DON above the detection limit of 1000 ng/g, and the mean DON level of the positive samples was 2500 ng/g. To confirm the immunoassay, further subsamples of 39 of

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### Table 2. Immunodermassay of Fumonisins and Deoxynivalenol in Maize

<table>
<thead>
<tr>
<th>district</th>
<th>sample type</th>
<th>occurrence</th>
<th>range (ng/g)</th>
<th>mean (ng/g)</th>
<th>occurrence</th>
<th>range (ng/g)</th>
<th>mean (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamjung</td>
<td>grain</td>
<td>8/24</td>
<td>1200–6500</td>
<td>2600</td>
<td>4/24</td>
<td>1300–2100</td>
<td>1800</td>
</tr>
<tr>
<td>Kathmandu</td>
<td>grain</td>
<td>2/6</td>
<td>2300–3500</td>
<td>2900</td>
<td>3/6</td>
<td>1200–6500</td>
<td>3100</td>
</tr>
<tr>
<td>Lalitpur</td>
<td>flour</td>
<td>3/6</td>
<td>1100–2600</td>
<td>2100</td>
<td>0/6</td>
<td>1200–6500</td>
<td>3100</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>17/45</td>
<td>1100–6500</td>
<td>2300</td>
<td>7/45</td>
<td>1300–3500</td>
<td>2500</td>
</tr>
</tbody>
</table>

* Detection limit was 1000 ng/g.

### Table 3. Chemical Analysis of Fumonisins and 8-Ketotrichothecenes in Maize

<table>
<thead>
<tr>
<th>district</th>
<th>occurrence</th>
<th>range (ng/g)</th>
<th>mean (ng/g)</th>
<th>occurrence</th>
<th>range (ng/g)</th>
<th>mean (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>grain samples</td>
<td>Chitwan</td>
<td>5/5</td>
<td>200–520</td>
<td>380</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dhankuta</td>
<td>4/4</td>
<td>110–280</td>
<td>200</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dhanusa</td>
<td>2/2</td>
<td>400–520</td>
<td>460</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kathmandu</td>
<td>4/6</td>
<td>240–2300</td>
<td>1000</td>
<td>2/6</td>
<td>2300–6500</td>
</tr>
<tr>
<td></td>
<td>Lalitpur</td>
<td>4/5</td>
<td>120–520</td>
<td>250</td>
<td>1/5</td>
<td>1400</td>
</tr>
<tr>
<td></td>
<td>Lamjung</td>
<td>26/29</td>
<td>120–8400</td>
<td>730</td>
<td>6/29</td>
<td>1260–11000</td>
</tr>
<tr>
<td></td>
<td>Morang</td>
<td>1/1</td>
<td>220</td>
<td>1/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nuwakot</td>
<td>5/5</td>
<td>110–210</td>
<td>140</td>
<td>1/5</td>
<td>3300</td>
</tr>
<tr>
<td></td>
<td>Sarlahi</td>
<td>1/1</td>
<td>250</td>
<td>0/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>subtotal</td>
<td>52/58</td>
<td>110–8400</td>
<td>550</td>
<td>10/58</td>
<td>1260–11000</td>
</tr>
<tr>
<td>flour samples</td>
<td>Kathmandu and Lalitpur</td>
<td>6/8</td>
<td>150–2400</td>
<td>800</td>
<td>1/8</td>
<td>3000</td>
</tr>
<tr>
<td>cornflakes sample</td>
<td>Kathmandu</td>
<td>0/2</td>
<td></td>
<td>0/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>52/58</td>
<td>110–8400</td>
<td>580</td>
<td>11/68</td>
<td>1260–11000</td>
</tr>
</tbody>
</table>

* Total fumonisins (fumonisin B₁, fumonisin B₂, and fumonisin B₃) are reported. Detection limit was 100 ng/g for fumonisins determined by HPLC and 1000 ng/g for 8-ketotrichothecenes determined by quantitative fluorometry.
the 45 samples from the initial survey were analyzed for NIV, DON, and other 8-ketotrichothecenes by fluorometric quantitation (Table 3). Twenty-seven additional samples of grain and two samples of cornflakes were analyzed for 8-ketotrichothecenes by fluorometric quantitation (Table 3). Eleven of the 68 samples contained 8-ketotrichothecenes above the detection level of 1000 ng/g, and the mean 8-ketotrichothecene level of the positive samples was 3200 ng/g. The highest trichothecene levels of 6500 and 11000 ng/g were found in two samples of grain from ears selected for visible symptoms of red ear rot.

Comparison of the data from the two methods indicated that fluorometric quantification sometimes gave higher levels of 8-ketotrichothecenes than did immunnoassay of the same maize sample. To investigate this discrepancy, seven samples were analyzed by liquid chromatography–mass spectrometry (LC-MS) for the presence of NIV and other 8-ketotrichothecenes that are detected by fluorometric analysis but do not cross-react with the antibody to DON (Casale et al., 1988; Malone et al., 1998). Some maize grain samples from the Lamjung, Kathmandu, and Nuwakot districts contained both NIV and DON; NIV levels as high as 10000 ng/g were found. By immunoassay or by fluorometric quantitation, NIV and DON were > 1000 ng/g in 16% of the 76 samples of maize and were > 2000 ng/g in 12% of the samples. Both 8-ketotrichothecenes and fumonisins were > 1000 ng/g in four grain samples from Lamjung farms and in one flour sample and one grain sample from the Kathmandu market.

Fifteen of the 27 wheat grain samples were infected with F. graminearum, but the highest level of seed contamination was only 2%. Furthermore, in these 15 samples, levels of NIV and DON as determined by fluorometric quantification were below the detection limit of 1000 ng/g. To determine the prevalence of NIV-producing and DON-producing strains, we analyzed production of 8-ketotrichothecenes by 13 strains of F. graminearum isolated in 1997 from Nepalese maize and wheat. Eight strains were NIV producers and four strains were DON producers, with production levels ranging from 10 to 100 μg/g of culture material. One strain produced low levels (0.5 μg/g) of both toxins. DON-producing strains produced no detectable NIV, but NIV-producing strains produced trace levels (~1%) of DON. Nivalenol-producing strains and DON-producing strains were isolated from both maize and wheat and from samples from several districts.

Detoxification of Maize by Fermentation. In Nepal, maize and other grains are fermented to produce traditional beers that are the basis for various distilled beverages. To determine the effect of fermentation on mycotoxin contamination, experimentally contaminated maize that contained fumonisin B₁ at 54 μg/g and DON at 11 μg/g was cooked and inoculated with a traditional Nepalese starter culture. After 24 h of fermentation, both the contaminated maize and clean maize produced the characteristic sour and ethanolic odor. After a total of 5 days of fermentation, the fumonisin B₁ level showed no change, and the DON level decreased by 50%. Untreated clean maize and fermented clean maize contained no detectable fumonisin or DON.

Detoxification of Maize by Hand-Sorting. Because visibly diseased maize kernels contain most of the fumonisin and DON (Reid et al., 1996; Desjardins et al., 1998), their removal should reduce residual contamina-

Figure 3. Efficiency of 12 Nepalese women in detoxifying a naturally contaminated maize grain sample by hand-sorting visibly diseased kernels. Efficiency is the weight percent of the original maize sample that each participant discarded to obtain what she considered to be a visibly clean product that would be acceptable for human consumption. Each open circle represents an urban participant trained in plant pathology, each solid circle an untrained, urban participant, and each open square an untrained, rural participant. Open symbols indicate the combined levels of fumonisins, determined by HPLC, and 8-ketotrichothecenes, determined by fluorometric quantitation; solid circles indicate the level of fumonisins only, determined by immunnoassay. Because trends for the fumonisin and trichothecene data were the same, the data were combined to simplify the figure. Fumonisin levels in 10 subsamples of the starting maize sample ranged from 100 to 2600 ng/g with a mean of 1700 ng/g, determined by HPLC, and 8-ketotrichothecene levels ranged from 1400 to 2300 ng/g with a mean of 1900 ng/g, determined by fluorometric quantitation. Levels of fumonisin and 8-ketotrichothecines in the cleaned product were < 1000 ng/g for all 12 participants.

DISCUSSION

In the present study, G. fujikuroi MP-A was the predominant Fusarium species in maize grain collected in central and eastern Nepal. F. graminearum was also frequently isolated from both maize and wheat. These results confirm previous reports of these two Fusarium species in Nepalese maize (Anonymous, 1997a; MacDonald and Chapman, 1997). G. fujikuroi MP-A from maize produced the high levels of fumonisins that are typical of this species (Nelson et al., 1993). F. graminearum strains from Nepalese maize and wheat could be divided into two approximately equal groups of NIV producers and DON producers. Nivalenol-producing strains of G. zeae have previously been isolated from wheat and barley in east Asia (Marasas et al., 1984).
Other species found in both maize and wheat include F. semitectum and F. equiseti, which are generally weak pathogens common to subtropical areas (Holiday, 1980). Both of these species were previously isolated from wheat, but not from maize, in Nepal (Shrestha, 1977). The species G. fujikuroi MP-D, F. acuminatum, F. avenaceum, F. chlamydosporum, and F. oxysporum were present in maize and/or wheat; all of these species have been reported from cereals in Asia (Parry et al., 1995; De Nijjs et al., 1996; Desjardins et al., 1997). Wheat samples from the Lamjung and Kavre districts yielded F. torulorum, which has been isolated from wheat in Australia and Europe (Yoder and Christiansen, 1998). The last six species were identified for the first time in maize and wheat from Nepal.

Despite the presence of F. graminearum strains that produce NIV and DON, Nepalese wheat showed no contamination with these mycotoxins above the level of 1000 ng/g. The low contamination level of wheat from smallholder farms is a reassuring finding and is probably due in part to dry weather during the wheat harvest, which provides poor conditions for fungal infection and mycotoxin production. In addition, traditional postharvest practices include sun-drying to lower moisture content and winnowing to remove seeds that are lighter in weight due to poor grain fill or disease. Both sun-drying and winnowing were found to reduce levels of rice seed infection with the blast fungus (Manandhar et al., 1998) and are likely to similarly reduce levels of wheat seed infection with F. graminearum.

Among maize samples collected from farms and markets in 10 districts of Nepal in 1997, a 76% incidence of G. fujikuroi MP-A was associated with an 83% incidence of fumonisins (detected limit = 100 ng/g). A 1990–1991 survey of 24 maize grain samples from markets in the Kathmandu district found a 50% incidence of fumonisins (detected limit = 50 ng/g), with a maximum of 4600 ng/g fumonisin B₁ and 5500 ng/g fumonisin B₂ in one sample (Ueno et al., 1993). Among 13 maize grain and flour purchases in markets in the Kathmandu and Lalitpur districts in 1997, the present survey found a higher (70%) incidence but a lower (2600 ng/g) maximum of fumonisin B₁ and only trace amounts of fumonisin B₂. Thus, both surveys indicate significant Fusarium mycotoxin contamination of maize grain and flour in Kathmandu area markets. The occurrence of fumonisins and trichothecenes in maize flour is of particular concern because mold is not usually visible in this product. Thus, urban consumers cannot readily reduce their risk by detecting and discarding contaminated product.

The contamination of maize with fumonisins, NIV, and DON probably is increased by maturation of the crop during the summer monsoon season, when abundant warm rains provide ideal conditions for fungal infection and for mycotoxin production in infected ears. Furthermore, the demand for increased food grain production is changing farming practices in the foothills from the traditional rice and fallow rotation to intensive cropping of rice and maize within the same year. Farmers interviewed in the Lamjung district stated that, to transplant rice, they sometimes harvested their maize crop while the ears had a high moisture content. The continuing rains of the monsoon season further hinder the rapid and thorough drying of maize ears that is necessary to prevent mycotoxin production in storage (Warfield and Gilchrist, 1999). Although some maize samples from Lamjung farms did contain relatively high levels of fumonisins, none contained fumonisins at the very high levels (>100000 ng/g) that have been associated with human esophageal cancer in southern Africa (Rheeder et al., 1992).

An integrated approach to control mycotoxins in food grains should include efforts both to prevent contamination and to detoxify contaminated grain, especially where food resources are limited. Biological detoxification methods such as fermentation can eliminate some classes of mycotoxins, but yeast ethanolic fermentation was not an effective decontamination method for fumonisins in maize (Bothast et al., 1992). Our study also found that a traditional Nepalese fermentation method for producing maize beer did not affect the fumonisin level and only partially decreased the DON level of contaminated maize. Because visibly diseased maize kernels contain most of the fumonisins and DON (Desjardins et al., 1998; Reid et al., 1996), physical separation of diseased kernels can be an effective decontamination method. Our field study demonstrated that Nepalese urban and rural women were able to detoxify contaminated maize grain by hand-sorting visibly diseased kernels. Residual contamination in the cleaned grain was at acceptable levels (<1000 ng/g). Half of the study participants, however, were inefficient in discriminating and removing only diseased kernels. Hand-sorting is economically viable for populations with limited food resources only if most of the starting material is recovered in the cleaned product. Thus, initiatives to reduce the risks of fumonisins and trichothecenes in Nepalese maize should inform consumers about the occurrence of mycotoxins, and educate them to recognize and discard visibly diseased kernels.

ABBREVIATIONS USED

DON, deoxynivalenol; ELISA, enzyme-linked immunosorbent assay; GC, gas chromatography; HPLC, high-performance liquid chromatography; HMG, His Majesty's Government; MP, mating population; MS, mass spectrometry; NIV, nivalenol.

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