Fungi, Aflatoxins, and Cyclopiazonic Acid Associated with Peanut Retailing in Botswana

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ABSTRACT

Peanuts are important food commodities, but they are susceptible to fungal infestation and mycotoxin contamination. Raw peanuts were purchased from retail outlets in Botswana and examined for fungi and mycotoxin (aflatoxins and cyclopiazonic acid) contamination. Zygomycetes were the most common fungi isolated; they accounted for 41% of all the isolates and were found on 98% of the peanut samples. Among the Zygomycetes, Absidia corymbifera and Rhizopus stolonifer were the most common. Aspergillus spp. accounted for 35% of all the isolates, with Aspergillus niger being the most prevalent (20.4%). Aspergillus flavus/parasiticus were also present and accounted for 8.5% of all the isolates, with A. flavus accounting for the majority of the A. flavus/parasiticus identified. Of the 32 isolates of A. flavus screened for mycotoxin production, 11 did not produce detectable aflatoxins, 8 produced only aflatoxins B1 and B2, and 13 produced all four aflatoxins (B1, B2, G1, and G2) in varying amounts. Only 6 of the A. flavus isolates produced cyclopiazonic acid at concentrations ranging from 1 to 55 µg/kg. The one A. parasiticus isolate screened also produced all the four aflatoxins (1,200 µg/kg) but did not produce cyclopiazonic acid. When the raw peanut samples (n = 120) were analyzed for total aflatoxins, 78% contained aflatoxins at concentrations ranging from 12 to 329 µg/kg. Many of the samples (49%) contained total aflatoxins at concentrations above the 20 µg/kg limit set by the World Health Organization. Only 21% (n = 83) of the samples contained cyclopiazonic acid with concentrations ranging from 1 to 10 µg/kg. The results show that mycotoxins and toxigenic fungi are common contaminants of peanuts sold at retail in Botswana.

Aspergillus, Fusarium, and Penicillium are the main genera of fungi that produce mycotoxins in food (24, 32). Although there are many mycotoxins produced by fungi, the five most important ones are aflatoxins, fumonisins, deoxynivalenol, zearalenone, and ochratoxin A (24). These mycotoxins occur naturally in foods from different parts of Africa (3, 15, 17, 21, 22). Other mycotoxins found in foods include patulin, sterigmatocystin, cyclopiazonic acid, nivalenol, and ergot. In most developing countries, including Botswana, aflatoxins are the most important mycotoxins from the point of view of occurrence, toxicity, and economics. Aflatoxins are produced primarily by strains of Aspergillus flavus and Aspergillus parasiticus (32).

Aflatoxins are potent carcinogens, mutagens, and teratogens (7). Studies in Kenya, Mozambique, Swaziland, and South Africa have shown a positive correlation between aflatoxin levels in the diet and incidence of primary liver cancer (12). Aflatoxin consumption has also been implicated in some infant diseases, such as kwashiorkor, a form of protein energy malnutrition (13), and it is acknowledged that protein-deficient diets can lead to greater toxicity with aflatoxins (14). The aflatoxin problem is not only a health risk, but it can also lead to financial losses to farmers where contaminated produce is rejected at export markets (15, 23).

Cyclopiazonic acid is an indole tetramic acid that is produced by several species of Aspergillus (32). It is associated with peanuts and other oil nuts and may cooccur with aflatoxins (8, 19, 32). Cyclopiazonic acid is toxic and is known to cause fatty degeneration and hepatic cell necrosis in the liver and kidneys of animals. In HeLa cells, cyclopiazonic acid blocks the ribosomal sites involved in peptide bond formation, thereby inhibiting the translation process (34).

Since mycotoxins can have adverse effects on humans who consume the contaminated foods, there is need for constant surveillance of foods for the presence and quantities of mycotoxins. There is also need for government agencies in Africa to develop regulations for acceptable levels in foods meant for human consumption. Despite the widespread occurrence of mycotoxins in Africa, only a few countries have any official limits and regulations on mycotoxins (31). Even in countries such as South Africa, where legislation controlling levels of mycotoxins is very clear and strict, food in many parts of the country is not routinely analyzed (9). Although most of the peanuts retailing in Botswana are imported, there are no official regulations that address acceptable limits of aflatoxins in peanuts. The objectives of this study were to determine the mycotoxins, as well as the presence, of aflatoxins B1, B2, G1, G2, and cyclopiazonic acid in peanuts sold in Botswana.

MATERIALS AND METHODS

Sample source and preparation. Raw peanut samples (1 to 2 kg) meant for human consumption were purchased from different retail outlets between July 2001 and January 2002. Samples...
were purchased at 2-week intervals. The samples were shelled and normally packaged in cellophane or plastic bags (with package sizes ranging from 0.1 to 1.0 kg). The samples were immediately analyzed for moisture content and water activity (aw) and then stored in a cold room at 4°C until further analysis. For moisture and aw determination, duplicate samples (30 g) were ground using a mortar and pestle. Ground samples were dried at 105°C overnight to constant weight, and the loss in weight was used to calculate the percentage of moisture content. The aw of the ground samples at 25°C was also measured using a Novasina Thermo-constantar (Pfaffikon, Switzerland).

Fungal isolation. Peanut kernels (40 from each sample) were randomly selected from each lot and were surface sterilized by soaking in 1% sodium hypochlorite for 2 min. This process removes surface contaminants and allows only the fungi actually growing in the peanut kernels to remain viable (25). The kernels were rinsed four times with sterile distilled water to remove all traces of the sodium hypochlorite. The kernels were then plated out on dichloran rose bengal chloramphenicol agar (CM727; Oxoid, Basingstoke, UK) and dichloran 18% glycerol agar (CM729; Oxoid). All plates were incubated at 25°C for 5 days with a lighting regime that consisted of an alternating cycle of 12-h dark and 12-h cool-white fluorescent light.

Subculturing and identification. Spores or hyphae that developed from the surface-sterilized peanuts were subcultured onto Malt Extract Agar (CM59; Oxoid) and Czapek yeast extract agar (25) for identification. Cultures were incubated at 25°C for 7 days with a lighting regime of alternating 12-h dark and 12-h cool-white fluorescent light. Cultures were identified on the basis of macro- and micromorphology and the reverse and surface characteristics of the colonies. Standard texts such as those of Pitt and Hocking and Samson and van Reenen-Hoekstra were used in the identification process.

Colonies suspected of being isolates of A. flavus/parasiticus were distinguished from other Aspergillus spp. using the bright orange-yellow-reverse color on A. flavus and parasiticus agar (27). A. flavus/parasiticus colonies were then processed using the standard methodology for inoculation and the plating regimes outlined by Pitt and Hocking (25) for identification to the species level. No attempt was made to distinguish between A. flavus and Aspergillus nomius.

Inoculation of A. flavus and A. parasiticus isolates on peanuts. The identified isolates of A. flavus (n = 32) and A. parasiticus (n = 1) were further subcultured on malt extract agar, and a spore inoculum of each isolate was prepared in sterile distilled water. Peanut kernels (200 g) were mixed with 30 ml of distilled water and autoclaved at 121°C for 15 min in glass jars. Spore inocula (20 ml containing 2 × 10⁸ spores) were then mixed with the sterile peanut samples. The inocula used were from cultures that had been identified as A. flavus/parasiticus using A. flavus and parasiticus agar and then identified to the species level using standard protocols (25). Inocula were raised by growing positively identified cultures on malt extract agar at 25°C for 7 days with a lighting regime that consisted of an alternating cycle of 12-h dark and 12-h cool-white fluorescent light. Control peanut samples containing 20 ml of sterile water were also prepared. The inoculated samples (and controls) were then incubated at 25°C for 3 weeks in the dark. After the incubation period, the samples were thoroughly mixed, and 25-g portions were removed for aflatoxin and cyclopiazonic acid analysis.

Aflatoxin determination. Aflatoxins were determined according to the AOAC International (2) method with modifications. High-pressure liquid chromatography (HPLC) was used for quantification. Peanut samples (50 g for the retail samples and 25 g for the inoculated samples) were blended with 200 ml of methanol-water (85 + 15 [vol/vol]). The mixture was filtered, and the filtrate was defatted with 25 ml of n-hexane before taking up the aflatoxins in two 25-ml portions of chloroform. The chloroform was evaporated to dryness, and the residue was transferred to glass vials with chloroform. The chloroform was then evaporated off under a gentle stream of nitrogen at 40°C. The residue was redissolved in 250 μl of chloroform and stored at −20°C until analysis.

The chloroform from each sample extract was evaporated off under nitrogen at 40°C, and the residue was redissolved in an equivalent amount of methanol for HPLC analysis. Samples (10 μl) were analyzed on a Waters HPLC System, model 610G pump, a model 486 Tunable Wavelength Detector, and a model 474 Scanning Fluorescence Detector (Waters, Milford, Mass.). The detectors were connected in series starting with the UV detector. The UV detector was set at 360 nm, while the fluorescence detector was set at excitation (λex) and emission (λem) wavelengths of 360 and 465 nm, respectively. Separations were done on a Hypersil ODS (3 μm, 100 by 4.6 mm) column (Sigma Chemical Co., St. Louis, Mo.) at room temperature (25 to 28°C). Isocratic elution with methanol-water-glacial acetic acid (45:55:2 [vol/vol/vol]) at a flow rate of 1.5 ml/min was used. Aflatoxin quantification was by comparison of peak areas with aflatoxin standards (working solution of 1 ng/μl) (Sigma) using the Apex Chromatography Workstation (Autochrom Inc., Milford, Mass.).

Cyclopiazonic acid determination. The method was based on protocol outlined by Lansden (18), with thin-layer chromatography quantification. Only 83 retail samples were analyzed for cyclopiazonic acid. Ground peanut samples (50 g) were first defatted with 300 ml of petroleum ether and dried overnight in a fume hood. Dried samples were then extracted with 250 ml of methanol-chloroform (20 + 80 [vol/vol]) containing 1 ml of phosphoric acid (85%). After filtering, 50 ml of the filtrate was shaken with 50 ml of sodium bicarbonate (0.5 N). The aqueous top layer was then acidified with 7 ml of concentrated hydrochloric acid, and the cyclopiazonic acid was taken up in two 25-ml portions of chloroform. The chloroform was evaporated to dryness, and the residue was transferred to a glass vial using a small portion of chloroform. The chloroform was evaporated off under a gentle stream of nitrogen, and the residue was taken up in 250 μl of chloroform for thin-layer chromatography.

Sample extracts (5 μl) were spotted on precoated thin-layer chromatography plates (Whatman, 0.2 cm thick) along with various concentrations of standard cyclopiazonic acid (working solution of 20 ng/μl) (Sigma). The plates were developed in ethyl-lactate-propan-2-ol-25% ammonium hydroxide (50:15:10 [vol/vol/vol]). After development, the plates were air dried and then sprayed with Ehrlich’s reagent (dimethylaminobenzaldehyde, 1 g dissolved in a mixture of 75 ml of ethanol and 25 ml of concentrated hydrochloric acid) until the first appearance of purple/blue spots corresponding to cyclopiazonic acid standards were observed. The color was left to develop for 10 min at room temperature. Quantification was by visual comparison with standards.

RESULTS

The aw of all the samples collected (120) was measured, and 119 (99%) samples had aw values ranging from 0.30 to 0.69. In fact, 111 (93%) samples had an aw below 0.61. One sample had an aw of 0.894. When the moisture content was determined, 98 (82%) of the samples had a moisture content below 7%, 20 (17%) of the samples had
TABLE 1. Fungi isolated from 120 peanut samples collected between July 2001 and January 2002 in Gaborone, Botswana

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>No. of isolates</th>
<th>% of total isolates</th>
<th>No. of samples</th>
<th>% of total samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zygomycetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absidia corymbifera</td>
<td>823</td>
<td>15.3</td>
<td>76</td>
<td>63</td>
</tr>
<tr>
<td>Mucor racemosus</td>
<td>19</td>
<td>0.4</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Rhizopus microsporum</td>
<td>567</td>
<td>10.5</td>
<td>44</td>
<td>37</td>
</tr>
<tr>
<td>R. stolonifer</td>
<td>788</td>
<td>14.6</td>
<td>63</td>
<td>53</td>
</tr>
<tr>
<td>Syncephalastrum racemosus</td>
<td>13</td>
<td>0.2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Aspergillus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. clavatus</td>
<td>170</td>
<td>3.2</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>A. flavus&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(32)</td>
<td>(0.6)</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>A. parasiticus&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(1)</td>
<td>(0.0)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>A. niger</td>
<td>1,099</td>
<td>20.4</td>
<td>98</td>
<td>82</td>
</tr>
<tr>
<td>A. tamarii</td>
<td>60</td>
<td>1.1</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>A. terreus</td>
<td>60</td>
<td>1.1</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>A. ustus</td>
<td>13</td>
<td>0.2</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Emericella nidulans</td>
<td>8</td>
<td>0.1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Eurotium amstelodami</td>
<td>22</td>
<td>0.4</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>E. chevalieri</td>
<td>5</td>
<td>0.1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Neosartorya</td>
<td>3</td>
<td>0.1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Penicillium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. aurantiogriseum</td>
<td>72</td>
<td>1.3</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>P. italicum</td>
<td>1</td>
<td>0.0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P. janthinellum</td>
<td>26</td>
<td>0.5</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>P. nalgiovense</td>
<td>41</td>
<td>0.8</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>P. viridicatum</td>
<td>32</td>
<td>0.6</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Penicillium spp.&lt;sup&gt;b&lt;/sup&gt;</td>
<td>866</td>
<td>16.1</td>
<td>108</td>
<td>90</td>
</tr>
<tr>
<td>Talaromyces bacillisporus</td>
<td>7</td>
<td>0.1</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Fusarium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. pallidoroseum</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alternaria alternata</td>
<td>4</td>
<td>0.1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>A. infectoria</td>
<td>8</td>
<td>0.1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Chrysosporium sitophila</td>
<td>1</td>
<td>0.0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ulocladium botrytis</td>
<td>4</td>
<td>0.1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Others</td>
<td>219</td>
<td>4.1</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>5,383</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> These isolates (of the 457 A. flavus/parasiticus isolates) were positively identified as A. flavus or A. parasiticus and used in mycotoxin production experiments.

<sup>b</sup> Most Penicillium species were not identified.

A moisture content between 7 and 8%, and only 2 (1%) of the samples had a moisture content above 8%.

A total of 5,383 fungal isolates were obtained from the 120 peanut samples collected between July 2001 and January 2002 (Table 1). Zygomycetes were the most predominant taxa, accounting for 41% of all the isolates. These were followed by Aspergillus spp. (35%) and Penicillium spp. (19%). Only two Fusarium isolates were obtained. The species that did not fall under the above four groups were referred to as miscellaneous fungal species and accounted for 4% of the total isolates. Zygomycetes were also the most widespread, being found on 118 (98%) of all the samples analyzed. Aspergillus spp. were detected in 113 (94%) samples, while Penicillium spp. were found in 108 (90%) samples.

Among the Zygomycetes, Absidia corymbifera was the most prevalent, accounting for 15.3% of all the isolates (Table 1). Other Zygomycetes isolated included Rhizopus stolonifer (14.6%) and Rhizopus microsporum (10.5%).

Aspergillus niger was the most common among the Aspergillus species isolated and accounted for 20.4% of all the isolates. In all, 457 (8.5%) isolates of A. flavus/parasiticus were obtained. Subsequently, A. flavus species were distinguished from A. parasiticus on the basis of morphological differences in the vesicle size and the conidial shape and ornamentation (25). A. flavus produced conidia that...
were variable in shape and size, with relatively thin and smooth walls and vesicles of up to 50 μm that usually bear metulae, whereas *A. parasiticus* produced conidia that were spherical, with thick, rough walls and smaller vesicles (20 to 35 μm), mostly lacking metulae. A total of 33 isolates were positively identified as *A. flavus* or *A. parasiticus* for use in the mycotoxic analysis. Of the 33 isolates, 32 were *A. flavus*, and 1 was *A. parasiticus*. *A. nomius* resembles *A. flavus*, and the two are difficult to distinguish using only morphological features, which was the only procedure used in this study. Hence, no attempt was made to further differentiate isolates believed to be *A. flavus*.

The identified *A. flavus* and *A. parasiticus* species isolated from peanuts were further analyzed for their ability to produce aflatoxins and cyclopiazonic acid. Of the 32 isolates of *A. flavus* analyzed, 21 (66%) produced aflatoxins, and the rest (11 isolates) did not produce detectable amounts of aflatoxins. The *A. flavus* isolates that produced aflatoxins fell into two groups depending on the types of aflatoxins produced (Table 2). Thirteen (62%) of the isolates produced all four aflatoxins, with total aflatoxin concentrations ranging from 44 to 1,200 μg/kg. The other eight (38%) isolates produced only aflatoxin B1 and B2, with total concentrations ranging from 20 to 600 μg/kg. The *A. parasiticus* isolate produced all four aflatoxins (B1, B2, G1, and G2), with a total aflatoxin concentration of 1,200 μg/kg.

Of the 32 isolates of *A. flavus* tested for cyclopiazonic acid production, only 6 (19%) produced cyclopiazonic acid, with concentrations ranging from 1 to 55 μg/kg and a mean of 33 μg/kg (Table 2). The *A. parasiticus* isolate did not produce cyclopiazonic acid.

Most of the *Penicillium* spp. were not identified to the species level, as they were prolific and variable and not the subject of interest in this study. Of the *Penicillium* spp. identified, *Penicillium aurantiogriseum* was the most common (1.3%). Only one species of *Fusarium*, *Fusarium palidoroseum*, was isolated from the peanut samples in this study.

The raw peanut samples collected from retail outlets were also analyzed for aflatoxins and cyclopiazonic acid. Aflatoxins were detected in 93 (78%) of the 120 samples tested. The total aflatoxin (B1 + B2 + G1 + G2) concentration ranged from 12 to 329 μg/kg, with a mean of 118 μg/kg (Table 3). All the major aflatoxins (B1, B2, G1, and G2) were detected in the samples. Aflatoxin B1 was the most dominant, being found in 78 (65%) of the samples. Aflatoxin G2 was detected in only 45 (38%) of the samples. When the samples were divided into groups according to level of total aflatoxin concentration, 34 (28%) had concentrations below 20 μg/kg, 29 (24%) had concentrations between 20.1 and 100 μg/kg, and 30 (25%) had concentrations above 100 μg/kg (Table 4). Cyclopiazonic acid, on the other hand, was detected in only 18 (21%) of the 83 peanut samples tested, and the concentrations ranged from 1 to 10 μg/kg (Table 3).

### DISCUSSION

The a aw of most samples used in this study was below 0.7. Fungal growth can be prevented if agricultural products are dried in such a way that the a aw is below 0.65, the minimum required to support fungal growth (11). Most of the samples (93%) analyzed in this study had a aw values below 0.61. The moisture content of most samples was also below 7%, and such moisture contents cannot support fungal proliferation (11). Hence, the samples analyzed were well...
TABLE 4. Number of peanut samples containing the various concentrations of aflatoxins B$_1$, B$_2$, G$_1$, and G$_2$ and total aflatoxins (B$_1$ + B$_2$ + G$_1$ + G$_2$)

<table>
<thead>
<tr>
<th>Aflatoxin</th>
<th>0.1–20</th>
<th>20.1–100</th>
<th>&gt;100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B$_1$</td>
<td>62 (52)</td>
<td>13 (11)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Aflatoxin B$_2$</td>
<td>26 (22)</td>
<td>17 (14)</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Aflatoxin G$_1$</td>
<td>17 (14)</td>
<td>19 (16)</td>
<td>15 (13)</td>
</tr>
<tr>
<td>Aflatoxin G$_2$</td>
<td>31 (26)</td>
<td>9 (8)</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Total aflatoxins$^b$</td>
<td>34 (28)</td>
<td>29 (24)</td>
<td>30 (25)</td>
</tr>
</tbody>
</table>

$^a$ The numbers in parenthesis are the percentages of 120 samples.
$^b$ Total aflatoxins (B$_1$ + B$_2$ + G$_1$ + G$_2$).

Dried, and the possibility of fungal growth in storage was minimal as long as the samples were kept in a dry environment.

Stored commodities are usually contaminated with fungal spores because fungal spores are ubiquitous and impossible to eliminate in the storage environment. Careful handling and treatment of stored products cannot exclude fungal spores but will prevent the germination of spores and infestation of the food products by fungi. However, because of the microscopic nature of fungal mycelia and spores, contamination may not be evident. The peanut samples used in the study were visibly free from fungal contamination, but as mentioned, the absence of visible fungi does not mean the peanuts were free from fungal spores, infestation, or mycotoxin contamination.

Three major fungal groups were isolated and identified from the peanuts in this study: Zygomycetes, Penicillium, and Aspergillus spp., which is consistent with previous studies (20, 29). Of the predominant Zygomycetes group, Rhizopus spp. were most abundant, particularly R. stolonifer, which agrees with the observation that R. stolonifer is one of the most common Zygomycetes isolated from dried, stored products, including peanuts (25). In the present study, 63 (53%) peanut samples contained R. stolonifer. However, Rhizopus species do not appear to produce any mycotoxins, although one isolate has been reported to be toxic to ducklings (28). The most prevalent Zygomycetes species isolated was A. corymbifera, which has previously been isolated from peanuts but for which no mycotoxin production has been reported (25).

Aspergillus isolates made up 35% of all the fungal isolates and were found on most of the peanut samples. A. niger was the most common fungal isolate found in peanut samples in this study. In several studies carried out on peanuts previously (16, 26), A. niger was a common species, and it has been suggested that this species competes with A. flavus and affects the relative abundance of the two species (25). In the present study, this was not very evident, with total infection rates of 66 and 82%, respectively, for A. flavus/parasiticus and A. niger.

The A. flavus/parasiticus spp. made up 8.5% of the fungal isolates detected and were found on 66% of the peanut samples. A. flavus has been found to be the most common contaminant of peanuts in studies in the wet tropics (26) and is also important in other areas. A. flavus is more common than A. parasiticus and is found in food, water, soil, and air (33). This makes it easy for this species to contaminate stored products. Records of A. parasiticus in foods are rare because it is a less common contaminant in foods and also because most investigators do not differentiate between A. flavus and A. parasiticus (33). A. parasiticus is also not common in some parts of the world, although it is endemic in peanuts (25, 33). In this study, only 1 isolate of A. parasiticus was identified compared to 32 A. flavus isolates. Therefore, A. parasiticus may not be a common contaminant of peanuts retailing in Botswana.

The 32 isolates of A. flavus and 1 isolate of A. parasiticus were tested for their ability to produce aflatoxins and cyclopiazonic acid. The A. parasiticus isolate produced all four aflatoxins (B$_1$, B$_2$, G$_1$, and G$_2$). Of the 32 isolates of A. flavus tested, 21 (66%) produced aflatoxins. Abarca et al. (1) reported aflatoxin production by 100% of the A. flavus isolates from mixed feeds, but only 8% of A. parasiticus isolates produced aflatoxins. Among the isolates that produced aflatoxins in this study, some isolates produced only aflatoxins B$_1$ and B$_2$, but the majority of the isolates produced all four aflatoxins (B$_1$, B$_2$, G$_1$, and G$_2$). Reports indicate that all toxigenic A. parasiticus isolates produce all four aflatoxins (B$_1$, B$_2$, G$_1$, and G$_2$) but that most A. flavus isolates produce only aflatoxins B$_1$ and B$_2$ (33). However, in terms of aflatoxin production, A. flavus is actually the most important (1, 6, 33).

A. flavus can be divided into two strains on the basis of the dimensions of the sclerotia produced; the S strain is known to produce high levels of aflatoxins, while the L strain produces lower levels of aflatoxin (6, 10). Within the S strains, some isolates produce only the B toxins (termed S$_B$), while others produce both the B and G toxins (termed S$_{BG}$). Studies of North American and West African S strains of A. flavus indicate that most North American strains are the S$_B$ types, whereas most of the West African strains are the S$_{BG}$ types (6). The majority of our isolates produced all four aflatoxins, with only a few of the isolates producing only aflatoxins B$_1$ and B$_2$. The presence of different varieties of A. flavus isolates may be due to genetic and ecological variations in the isolates. Another possibility is that some of the isolates identified were not actually A. flavus but rather, the closely related A. nomius species. A. nomius strains are known to produce all four aflatoxins (25). When the 33 strains of A. flavus/parasiticus were checked after 7 days of growth, as recommended by Pitt and Hocking (25), only one had produced sclerotia and could be determined as an S strain in terms of the size of the sclerotia (<400 μm).

All the peanut samples collected from retail outlets were analyzed for aflatoxins. Most of the samples (78%) analyzed in this study contained aflatoxins at concentrations ranging from 12 to 329 μg/kg. Approximately 28% of the samples had total aflatoxin concentrations falling below the 20-μg/kg limit set in many countries (31), but 49% of the samples had concentrations above this limit. In fact, all pos-
itive samples contained aflatoxins above the maximum (4 μg/kg total aflatoxins) set by the European Union for peanuts intended for direct human consumption (23).

In work carried out on peanuts from different geographical locations of India, 45% of the samples contained aflatoxin B₁ at concentrations ranging from 5 to 833 μg/kg (4). In another survey carried out by the U.S. Food and Drug Administration for 8 years, 2.5 to 22.2% of total roasted peanut samples contained aflatoxins (28). Recently, between 9.4 and 31.3% of the peanut samples were contaminated with aflatoxins in Mozambique (5). Aflatoxins have previously been reported in peanut samples from Botswana (30). However, in the present study, up to 78% of the samples had aflatoxins compared to only 52% in the earlier report. The levels were also much higher: total aflatoxin concentrations ranged from 12 to 329 μg/kg compared to the concentrations ranging from 3.2 to 48 μg/kg previously reported. Most of the peanuts retailing in Botswana are imported and are therefore affected by environmental factors prevailing in the originating country. Since there are no official limits regarding the levels aflatoxins in peanuts, it is possible that some importers are bringing low-quality peanuts into the country.

Aflatoxin B₁ was the most commonly isolated aflatoxin among the four analyzed. Aflatoxin B₁ is one of the most carcinogenic compounds known, and any amounts, especially those above 20 μg/kg, should be of concern to consumers (11). High concentrations of aflatoxins in peanuts also negatively affect international trade in peanuts (23).

The production of cyclopiazonic acid among the A. flavus and A. parasiticus isolates was not as widespread as that of aflatoxins. The concentration of cyclopiazonic acid produced was also low. Lansden and Davidson (19) also showed the production of cyclopiazonic acid by A. flavus isolates but not by A. parasiticus isolates. The peanut samples from retail outlets also contained cyclopiazonic acid but to a lesser extent. Others have also reported the presence of cyclopiazonic acid in peanuts (19). Cyclopiazonic acid can also cooccur with aflatoxins (32), and there appears to be an interaction in the toxicities of the two toxins (34).

Penicillium spp. were isolated from 90% of the peanut samples tested in this study, which is higher than in previous studies (26). Penicillium spp. are mostly storage fungi, and they can proliferate on improperly stored products. The presence of toxigenic fungi and mycotoxins such as aflatoxins and cyclopiazonic acid in the peanut samples analyzed should be of concern to consumers. This is especially true for aflatoxins, since the levels detected were mostly above the limits set by the World Health Organization and other countries such as those in the European Union (23). The presence of fungi and especially A. flavus and A. parasiticus means that the potential for aflatoxin production is always present, and increases in the a₀ (above 0.8) may lead to fungal growth and aflatoxin production (11). Although maximum limits for aflatoxins in food have been set in different countries, there are no official limits in Botswana. Hence, there is a need for studies such as these to collect baseline data on which regulations can be based.

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REFERENCES


