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A Case for Regular Aflatoxin Monitoring in Peanut Butter in Sub-Saharan Africa: Lessons from a 3-Year Survey in Zambia

SAMUEL M. C. NJOROGE,[1]* LIMBIKANI MATUMBA,[2] KENNEDY KANENGA,[3] MOSES SIAMBI,[4] FARID WALIYAR,[5] JOSEPH MARUWO,[1] AND EMMANUEL S. MONYO[4]

[1]*International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), P.O. Box 1096, Lilongwe, Malawi;* [2]*Lilongwe University of Agriculture and Natural Resources, NRC Campus, Food Technology and Nutrition Group, P.O. Box 143, Lilongwe, Malawi;* [3]*Zambia Agriculture Research Institute, P.O. Box Chipata, Zambia;* [4]*ICRISAT, P.O. Box 39063, Nairobi, Kenya; and* [5]*ICRISAT, Patancheru 502 324, Telangana, India*

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ABSTRACT

A 3-year comprehensive analysis of aflatoxin contamination in peanut butter was conducted in Zambia, sub-Saharan Africa. The study analyzed 954 containers of 24 local and imported peanut butter brands collected from shops in Chipata, Mambwe, Petauke, Katete, and Nyimba districts and also in Lusaka from 2012 to 2014. For analysis, a sample included six containers of a single brand, from the same processing batch number and the same shop. Each container was quantitatively analyzed for aflatoxin B_1 (AFB_1) in six replicates by using competitive enzyme-linked immunosorbent assay; thus, aflatoxin contamination level of a given sample was derived from an average of 36 test values. Results showed that 73% of the brands tested in 2012 were contaminated with AFB_1 levels >20 μg/kg and ranged up to 130 μg/kg. In 2013, 80% of the brands were contaminated with AFB_1 levels >20 μg/kg and ranged up to 10,740 μg/kg. Compared with brand data from 2012 and 2013, fewer brands in 2014, i.e., 53%, had aflatoxin B_1 levels >20 μg/kg and ranged up to 1,000 μg/kg. Of the eight brands tested repeatedly across the 3-year period, none consistently averaged ≤20 μg/kg. Our survey clearly demonstrates the regular occurrence of high levels of AF B_1 in peanut butter in Zambia. Considering that some of the brands tested originated from neighboring countries such as Malawi, Zimbabwe, and South Africa, the current findings provide a sub-Saharan regional perspective regarding the safety of peanut butter.

Key words: Aflatoxin B_1; Food safety; Peanut butter; Standards

Peanut butter, a food paste made primarily from dry roasted peanuts, is a popular food product worldwide *(23, 26)*. It is used mainly as a sandwich spread, and owing to its high lipid and protein contents, it has become a major constituent of ready-to-use therapeutic food in treating malnutrition in children and AIDS patients, particularly in the developing world *(13, 21)*. However, the raw material of peanut butter, groundnuts (peanuts), is prone to aflatoxin contamination via carcinogenic secondary metabolite production by toxigenic fungi *(2, 6, 7, 17, 20)*. Chronic low-level exposure to aflatoxins, particularly aflatoxin B_1 (AFB_1), is associated with increased risk of developing liver cancer, malnutrition, and impaired immune function *(1, 28)*. Furthermore, evidence indicates that aflatoxins increase the rate of progression from human immunodeficiency virus infection to AIDS *(8, 9)*.

To protect consumers from the harmful effects of aflatoxins, most governments have established regulations *(5)*. However, unlike with developed nations, the enforcement of these regulations in developing countries is challenged by several factors, including unavailability of relevant analytical facilities and lack of skilled personnel *(15)*. Consequently foodstuffs such as groundnuts and groundnut-based products sold in these countries may contain high concentrations of aflatoxins, particularly in those countries that lie between latitudes 40°N and 40°S. In such countries, peanut butter may be more contaminated than the groundnut grain because, unlike with the grain, it is nearly impossible to make an informed decision on the quality of peanut butter visually. Buyers of grain, however, can visually discern groundnuts that are broken, shriveled, undersized, insect damaged, or moldy, all of which are proxies for a higher likelihood of the nuts being contaminated with aflatoxin *(29)*. In addition, sellers in such markets try to make efforts to sort and present groundnut grain in ways that would attract buyers; through this sorting, they inadvertently reduce aflatoxin contamination in the grains. Peanut butter does not have telltale signs of mold so one cannot tell whether the grain used to produce it was moldy, insect damaged, or otherwise contaminated. Mitigation efforts are therefore needed and should be guided by data from the markets on current levels of aflatoxin contamination. Unfortunately most aflatoxin–peanut butter surveys conducted in these resource-constrained countries are limited in scope, involving few samples and testing in just

* Author for correspondence. Tel: +265 1 707 067/057; Fax: +265 1 707 298; E-mail: s.njoroge@cgiar.org.

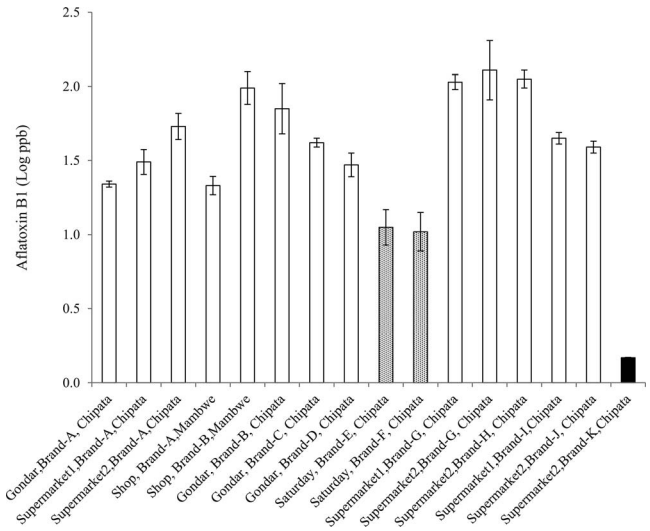

FIGURE 1. *Mean aflatoxin B_1 contamination (log micrograms per kilogram) in peanut butter samples from Chipata and Mambwe districts in 2012. Each bar represents a mean of 30 values and error bars represent 1 standard error of the mean. Open, dotted, and solid bars represent aflatoxin levels >20 (>1.32 log), >10 ≤ 20 (>1 ≤ 1.3 log), >4 ≤ 10 (>6 ≤ 1 log), and ≤4 (≤0.6 log) μg/kg, respectively. Total containers analyzed were 96.*

1 year *(16, 19, 22, 25)*; therefore, these samples may not be representative because aflatoxin contamination is highly heterogeneous and varies over time.

Thus, the objective of our study was to conduct a comprehensive multiyear analysis of aflatoxin contamination in peanut butter in sub-Saharan Africa, with Zambia as a case study. The findings of the study will influence policy direction on management of such high-risk food products.

MATERIALS AND METHODS

Peanut butter sample collection. In 2012, 16 samples of 11 peanut butter brands were collected from 25 October to 1 November from Chipata and Mambwe districts. In 2013, 42 samples of 15 peanut butter brands were collected from 28 February to 2 March from Chipata, Petauke, and Katete districts and Lusaka. In 2014, 101 samples of 19 brands of peanut butter were collected from 7 to 12 December from Lusaka and Chipata, Nyimba, and Katete districts. In all years, a sample consisted of six 250- or 500-g containers of a single brand, with the same processing batch number and from the same supermarket or shop. Therefore, the total number of containers collected in 2012, 2013, and 2014 was 96, 252, and 606, respectively (i.e. 42 samples were collected in 2013; therefore, the total number of containers was 42 × 6 [containers per sample] = 252 containers). Samples were taken to laboratories at the International Crops Research Institute for the Semi-Arid Tropics in Lilongwe, Malawi, where they were kept in a cold room at 5°C until analysis.

Aflatoxin analysis: ELISA. AFB_1 quantification was done following methods of Monyo et al. *(17),* with modifications on the number of subsamples analyzed per peanut butter container and on the number of containers constituting a sample. In brief, from each peanut butter container, we weighed six subsamples of 20 g. Extraction of aflatoxin from each of the 20-g samples proceeded by adding and blending 100 ml of 70% methanol (vol/vol) containing 0.5% KCl. The mixture was then transferred into a 250-ml conical flask and shaken (Gallenkamp orbital shaker, Loughborough, UK) at 300 rpm for 30 min. Next, the mixture was filtered through a Whatman No. 4 filter paper (Whatman, Maidstone, UK) and diluted 1:10 in phosphate-buffered saline with Tween (PBST; Sigma-Aldrich, Taufkirchen, Germany). The PBST was prepared by mixing in 2 liters of distilled water, 2.38 g of Na_2HPO_4, 0.4 g of KH_2PO_4, 0.4 g of KCl, 16.0 g of NaCl, and 1 ml of Tween 20. Enzyme-linked immunosorbent assay (ELISA) microtiter plates (Nunc MaxiSorp, Roskilde, Denmark) sensitized with AFB_1-bovine serum albumin (BSA) conjugate (Sigma-Aldrich) were incubated at 37°C for 1.5 h, and each well was then washed twice with 150 μl of PBST. Next, 0.2% blocking solution of BSA was added to the plates and they were incubated for 30 min at 37°C; thereafter, each well was washed with 150 μl of PBST. AFB_1 standards (Sigma-Aldrich) at concentrations between 25 and 0.097 ng/ml were prepared in PBST-BSA with 7% methanol; 100 μl per well of AFB_1 standards was added into two rows of the ELISA plates. Similarly, 100 μl of diluted sample extract (1:10 in PBST) was added to the other rows of wells in the ELISA plate. Next, 50 μl of diluted polyclonal antibody (in-house product, 1:6,000 in PBST-BSA; International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India), and the plates were incubated for 1 h at 37°C. Finally, 150 μl of diluted anti-rabbit–immunoglobulin G–alkaline phosphatase (1:4,000 in PBST-BSA) was added to all the wells, and the plates were incubated for 1 h. Thereafter, each well was washed with 150 μl of PBST. *p*-Nitrophenyl phosphate, prepared in 10% diethanolamine, pH 9.8, was added to each well. Color developed in 20 to 30 min, and the plates were read in a BioTek ELX800 UV reader (Romer Labs, Tullun, Austria) at 405 nm. Mean ELISA reading values for each standard and sample were determined. Standard curves were plotted by placing AFB_1 standard concentration values on the *y* axis and optical density values on the *x* axis. Regression curves were used to estimate the aflatoxin value in each sample. The limit of detection is 1 μg/kg AFB_1. The analytical method used was validated with naturally contaminated corn reference materials (4.2 and 23.0 μg/kg AFB_1, product no. TR-A100, batch no. A-C-268 and A-C 271; R-Biopharm AG, Darmstadt, Germany).

Data analysis. Aflatoxin contamination values were not normally distributed and were log transformed, i.e., log(X + 1). AFB_1 sample means were then calculated by averaging 30 log-transformed values (five containers, each subsampled six times) obtained from ELISA analysis. To determine variation within samples, standard error of the mean was calculated.

RESULTS

We documented aflatoxin contamination in 24 peanut butter brands sold in Zambia from 2012 to 2014. However, not all brands were consistently available during the sampling period; therefore, 11, 15, and 19 brands were sampled in 2012, 2013, and 2014, respectively. In 2012, only 3 (27%) of 11 brands tested had AFB_1 levels ≤20 μg/kg (Fig. 1). The rest of the brands had AFB_1 levels >20 μg/kg, up to a maximum of 130 μg/kg. In 2013, results indicated that only 2 (13%) of 15 brands tested had consistent AFB_1 contamination levels of ≤20 μg/kg, whereas 1 brand had variable AFB_1 contamination ranging from ≤4 to 100 μg/kg; the rest of the brands consistently had AFB_1 levels >20 μg/kg and ranged up to 10,000 μg/kg (Fig. 2). In 2014, nine brands, i.e., 47% of brands tested that year, consistently had AFB_1 contamination ≤20 μg/kg, whereas

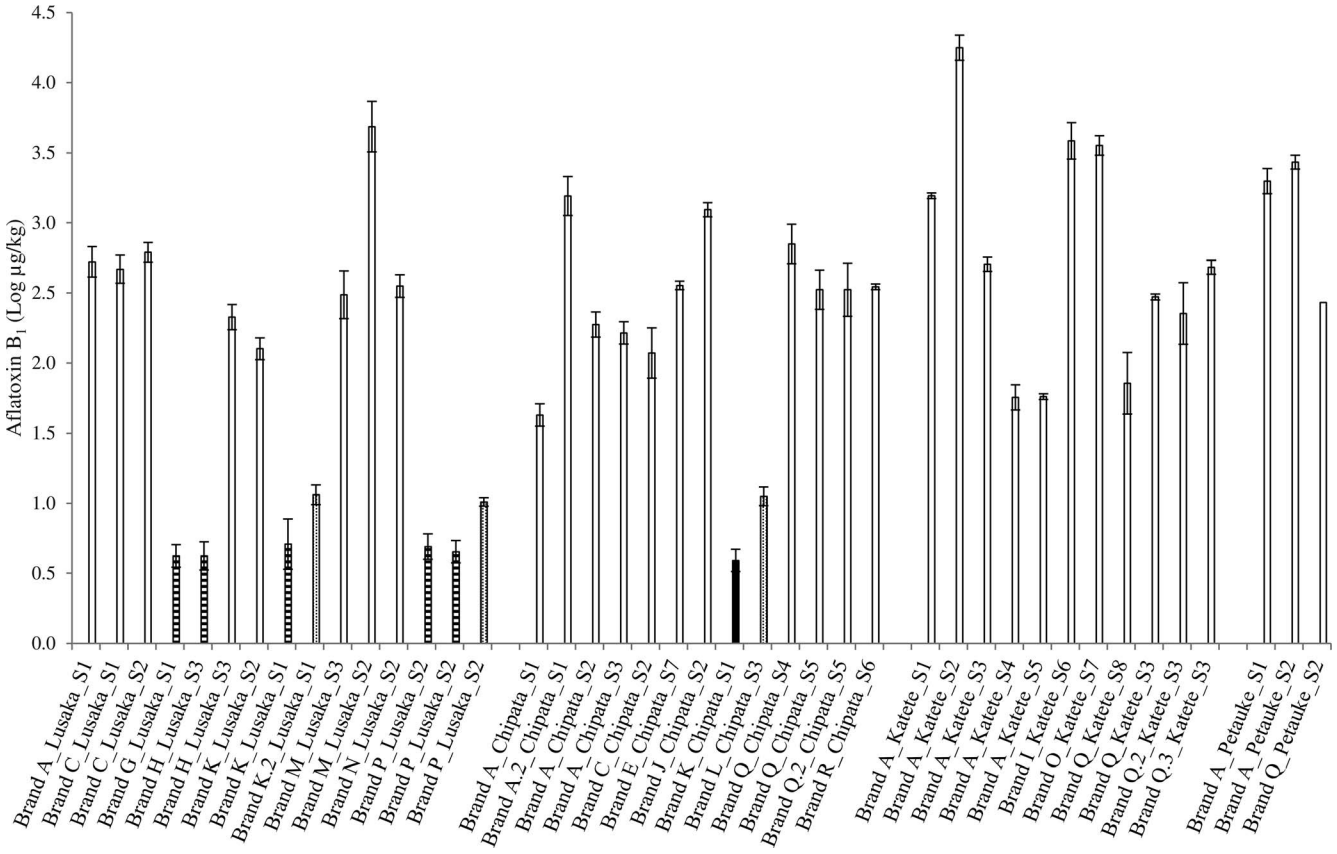

FIGURE 2. *Mean aflatoxin B_1 contamination (log micrograms per kilogram) in peanut butter samples from Lusaka City and Chipata, Katete, and Petauke districts in 2013. Each bar represents a mean of 30 values, and error bars represent 1 standard error of the mean. Open, dotted, horizontal, and solid bars represent aflatoxin levels >20 (>1.32 log), >10 ≤ 20 (>1 ≤ 1.3 log), >4 ≤ 10 (>6 ≤ 1 log), and ≤4 (≤0.6 log) µg/kg, respectively. Total containers analyzed were 252.*

the rest of the brands consistently had AFB_1 levels >20 µg/kg and ranged up to 1,000 ppb (Figs. 3 and 4). Aflatoxin contamination also varied within brands and across years (Figs. 1 through 4). Of the eight brands tested in all 3 years, none had a mean of ≤20 µg/kg in all years. Comparatively, 12 brands were tested repeatedly over 2 years and only one brand (brand P), i.e., 8% of tested brands, had AFB_1 mean values ≤20 µg/kg. In addition, nine brands were tested only in 1 year, and four of these brands, i.e., 44%, had AFB_1 values ≤20 µg/kg.

We compared AFB_1 contamination in imported brands with that in local brands. In 2012, aflatoxin contamination in imported brands (arithmetic mean [AM] 10 µg/kg, $n = 26$, range 1 to 74 µg/kg) was significantly lower ($P = 0.0253$) than that of local brands (24 µg/kg, $n = 70$, range 1 to 263 µg/kg). In 2013, contamination in imported brands (55 µg/kg, $n = 82$, range 1 to 10,740 µg/kg) was not significantly different ($P = 0.388$) from local brands (130 µg/kg, $n = 170$, range 1 to 4,375 µg/kg). In 2014, imported brands also had significantly lower ($P = 0.0435$) aflatoxin (6 µg/kg, $n = 200$, range 1 to 600 µg/kg) compared with local brands (35 µg/kg, $n = 406$, range 1 to 3,000 µg/kg).

DISCUSSION

To best of our knowledge, this is the first published report on aflatoxin contamination in peanut butter in Zambia and probably the first study carried out worldwide with such significantly high numbers of peanut butter samples tested. Market and trade samples provide information on the risk of exposure from various foods in the diet, especially when local food processors undertake operations such as milling without quality control *(28)*. From these results, it is clear that aflatoxin contamination in peanut butter is pervasive. The brands tested originated from Zambia and also from southern Africa, i.e., Malawi, Zimbabwe, and South Africa, indicating that the problem of aflatoxin contamination may also be pervasive in these countries. Our findings corroborate data by Mupunga et al. *(19)* who detected aflatoxins in 10 (91%) of 11 peanut butter samples from Zimbabwe, with a mean contamination of 75.6 µg/kg. Interestingly, these authors found no statistically significant mean differences between factory-processed and cottage industry–processed peanut butters, revealing that quality control among manufacturers as required by law either was not being done or was compromised. In contrast, in Malawi locally manufactured peanut butter was found to contain significantly higher aflatoxin levels (34 to 116 µg/kg, $n = 14$) than the imported peanut butter (<0.2 to 4.3 µg/kg, $n = 11$) *(14)*.

About 100 countries worldwide have set standards for the maximum amount of aflatoxin allowable in foodstuffs *(29)*. As mentioned, peanut butter that was tested in this survey came from Zambia and also from Malawi, Zimbabwe, and South Africa. The country phytosanitary standards for maximum allowable limits for aflatoxin in

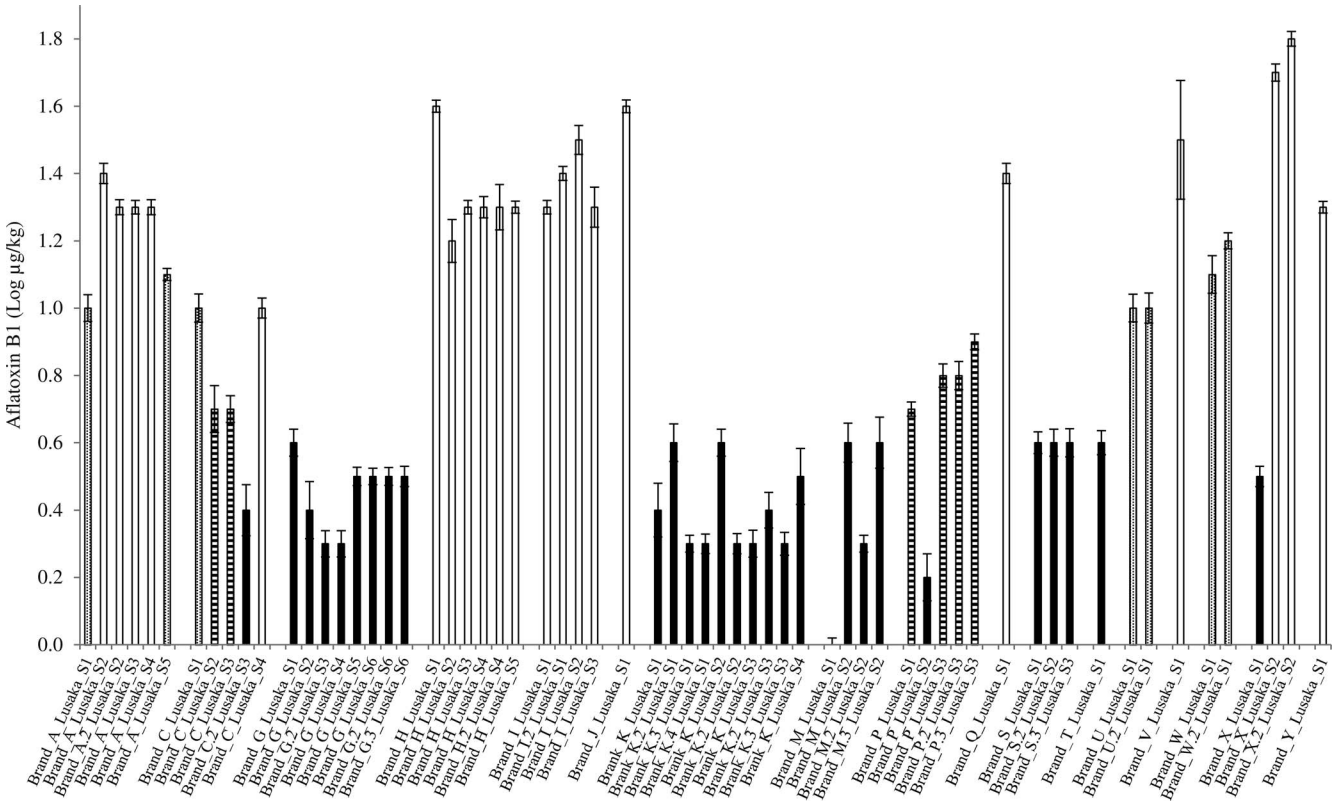

FIGURE 3. *Mean aflatoxin B_1 contamination (log micrograms per kilogram) in peanut butter samples from Lusaka in 2014. Each bar represents a mean of 30 values, and error bars represent 1 standard error of the mean. Open, dotted, horizontal, and solid bars represent aflatoxin levels >20 (>1.32 log), >10 ≤ 20 (>1 ≤ 1.3 log), >4 ≤ 10 (>6 ≤ 1 log), and ≤4 (≤0.6 log) µg/kg, respectively. Total containers analyzed were 378.*

groundnuts in Zambia is currently under review and the proposal is to set limits for AFB_1 and total aflatoxin to 5 and 10 µg/kg, respectively (K.K., personal communication). For Malawi, Zimbabwe, and South Africa, the limits for total aflatoxin allowable are 3, 15, and 10 µg/kg, respectively *(12, 19)*. Setting of standards does not ensure a safe food supply, especially in low-income countries where food rarely undergoes formal safety inspection *(23, 29)*. The median level in food-established legislations worldwide is 10 µg/kg. The levels of aflatoxin contamination from the survey are of concern, and regulatory measures need to be enforced to reduce aflatoxin contamination and ensure compliance.

Groundnuts are exclusively produced in the tropics and subtropics, which means that groundnuts consumed in the temperate region are all imported. Ironically, peanut butter tested in developed, temperate climate–based countries such as the United Kingdom and Japan contain comparatively lower aflatoxins levels than peanut butter from the groundnut-producing countries such as Zambia *(10, 18)*. These results are a clear manifestation of robust regulatory systems in the developed countries. However, the production of a clean "aflatoxin-free" lot for export often involves sorting the groundnuts *(4, 29)*; unfortunately, such sorting may lead to concentrating aflatoxins on the local market *(16)*. Therefore, for sorting to be a viable route for reducing aflatoxins, local solutions or practical detoxification methods have to be offered for the sorted out groundnuts, especially for small-scale processors in less formal settings.

Filbert and Brown *(4)* suggested that contaminated groundnuts can be transformed into cooking briquettes in low-efficiency stoves in Haiti. This option may not work for Zambia, considering that groundnuts have a high value compared with firewood, an alternative cooking fuel. Extracting oil from contaminated groundnuts seems to be a viable option, because only a small fraction of aflatoxins is sequestered into vegetable oils due to their lipophobicity *(11)*. Moreover, research has indicated the possibility of removal of up to 90% of aflatoxins from oil by using ethanol-water (50:50, vol/vol) *(24)*.

Ideally, a more holistic approach to managing aflatoxin in food should be adopted, covering the whole value chain from farm to fork *(3)*. Critical areas to be monitored are (i) the crop during production, making sure that good agricultural practices are implemented for reducing aflatoxin contamination *(3, 27)*; (ii) suppliers of raw materials to the processors need to understand regulatory requirements and customer food standards so that they can monitor for quality, store correctly, and supply products within specification *(3)*; and (iii) the factory or processor should carry out tests on batches being received and also on finished products, representing the last opportunity for forward control *(3)*. These processes are easier to implement on peanut butter compared to groundnut grain sold in the markets, since the majority of peanut butter sold goes through formal traceability systems. Interventions in formal trading systems would then hopefully cascade into informal systems,

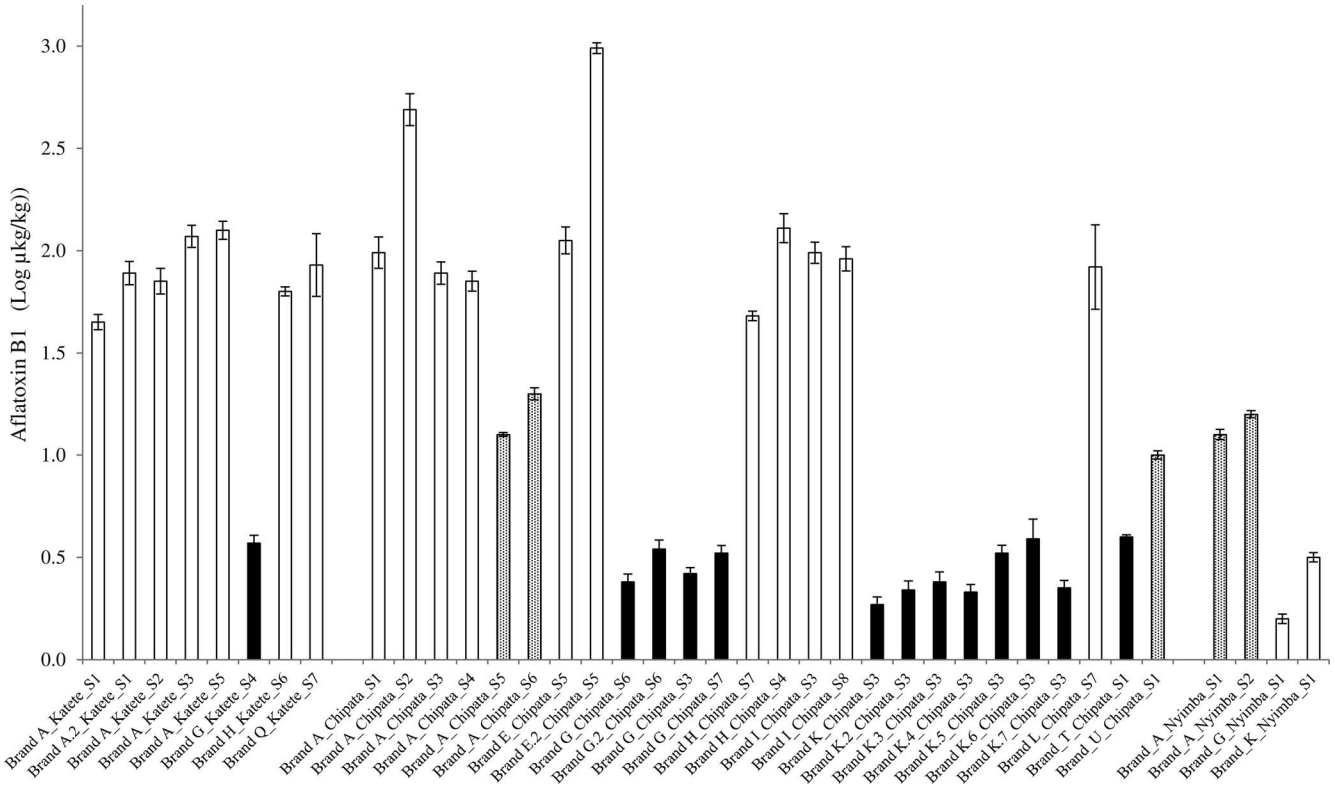

FIGURE 4. *Mean aflatoxin B_1 contamination (log microgram per kilogram) in peanut butter samples from Katete, Chipata, and Nyimba districts in 2014. Each bar represents a mean of 30 values, and error bars represent 1 standard error of the mean. Open, dotted, and solid bars represent aflatoxin levels >20 (>1.32 log), >10 ≤ 20 (>1 ≤ 1.3 log), >4 ≤ 10 (>6 ≤ 1 log), and ≤4 (≤0.6 log) µg/kg, respectively. Total containers analyzed were 228.*

reducing the risks of aflatoxin exposure from consuming peanut butter.

In conclusion, the levels of AFB_1 in peanut butter reported herein are of concern, and regulatory measures need to be enforced to reduce aflatoxin contamination levels. Interventions are needed to enforce compliance, and follow-up surveys are required to confirm that levels of contamination are within safety limits.

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