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Aflatoxin Contamination in Selected Spice Preparations in the Nyahururu Retail Market, Kenya

William W. Mwangi[1*], Charles M. Nguta[2] and Benson G. Muriuki[3]

[1]Department of Biological Sciences, School of Science and Applied Technology, Laikipia University, P.O. Box 1100-20300, Nyahururu, Kenya.
[2]Chemistry Department, School of Science and Applied Technology, Laikipia University, P.O. Box 1100-20300, Nyahururu, Kenya.
[3]Department of Biological Sciences, Faculty of Science, Egerton University, P.O. Box 536-20115, Egerton, Kenya.

Authors' contributions

This work was carried out in collaboration between all authors. Author WWM designed the study, wrote the protocol and wrote the first draft of the manuscript. Author CMN managed the literature searches and corrected the first draft of the manuscript while author BGM managed the experimental process and supervised data collection. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Commercial spice preparations were analysed for aflatoxins contamination in order to determine the incidence of spice samples containing levels likely to pose a risk to public health. In the months of May and June 2012, spice samples were purchased from different randomly selected premises in the Nyahururu town. Forty-six spice preparations were included in the survey. Individual spices were ground and mixed to uniform consistency using a laboratory mill and analysed for aflatoxins using a commercial ELISA kit. Thirty four (73.9%) samples were positive for aflatoxins while twelve (26.1%) had aflatoxin levels below the detection limit. Over 50% of samples analysed contained aflatoxin <10 µg/kg. The highest quantities of aflatoxins were found in cayenne, paprika and cumin (99.6, 99 and 98 µg/kg, respectively). Relatively high levels of aflatoxins were also found in chilli (31.5 µg/kg) while no mean detectable levels were observed in curry and nutmeg. The average aflatoxins level in positive spice samples was 30.6 µg/kg.

**Corresponding author: E-mail: wachira2007@gmail.com;*

Statistical analysis showed significant difference in aflatoxin levels between spice types ($p < 0.001$). Maximum acceptable aflatoxins levels were exceeded in most of the sampled spices; therefore it is important for regulatory bodies in Kenya to continuously monitor for aflatoxins in retail spices preparations. Additionally, post-harvest methods for the prevention of fungal contamination should be enhanced by all spice handlers.

Keywords: Aflatoxin; commercial spices; ELISA kit; aspergillus; Kenya.

1. INTRODUCTION

Mycotoxins are a group of toxic secondary metabolites produced by certain strains of fungi under favourable conditions on a variety of commodities. These natural food contaminants are found in different kinds of food, spices and beverages, such as cereals, beans, dried fruits, coffee, cocoa, wine, beer, juice, pork, meat, poultry and milk [1,2,3,4]. Most of the mycotoxins are produced by mould species belonging to *Aspergillus*, *Penicillum* and *Fusarium* [5]. Mycotoxigenic fungi can grow easily in unsuitable conditions of growth, harvest, transport and storage [6]. Aflatoxins, which are metabolites of *Aspergillus flavus* and *Aspergillus parasiticus*, are a major group of mycotoxins. The four major types include B1, B2, G1 and G2 while M1 and M2 are metabolites of B1and B2 [1,2].

In some parts of Africa, the mycotoxin hazard is very high because the limited food supply has forced people not to reject any material that can be used as food, even if the organoleptic quality of the food has been changed by moulds [7,8]. In addition, the prevailing malnutrition enhances people's susceptibility to even very low levels of mycotoxins [8,9]. The mycotoxin problem is further aggravated by the warm humid tropical conditions and inadequate drying and storage practices which provide optimal conditions for mould growth and subsequent build-up of mycotoxins within a short span of time [10,11].

Ingestion of mycotoxins can result in the illness or death of animals and humans, and indeed a number of mycotoxins and their acute and chronic effects on human and livestock health have been identified [4,12,13]. Among these mycotoxins, aflatoxins are the most dangerous to human health because they are highly toxic, carcinogenic, teratogenic, hepatotoxic and mutagenic [14,15]. Indeed aflatoxin B1 (AFB1) has been classified as a group 1 human carcinogen by the International Agency for Research on Cancer [16]. Several mycotoxicoses have been reported due to the consumption of mycotoxin-contaminated food and feed [17,18,19,20,21].

Spices and other condiments are increasingly being used in a range of meals to enhance flavour and aroma and create variety in the kitchen [5]. However, spices, such as chillies, turmeric, black pepper, coriander and dry ginger, may become contaminated with aflatoxins pre-harvest, post-harvest and/or during storage and transport [22]. In Kenya, monitoring and enforcement of standards regarding mycotoxins is rare; therefore, there is likelihood that people are being exposed to unsafe levels of various mycotoxins (e.g. aflatoxins) in spices. The resulting public health burden has also been overlooked as information regarding levels and occurrence of aflatoxins in spices, sold in retail outlets, in Kenya is lacking. In this study, retail spice preparations sold in the Nyahururu retail market in Kenya were analysed in order to determine aflatoxins levels present and incidence of spices containing quantities likely to pose risk to public health.

2. MATERIALS AND METHODS

2.1 Sample Collection and Storage

Forty-six spice preparations of chilli, curry powder, cayenne, paprika, cinnamon, pepper, ginger, cloves, garlic, nutmeg, turmeric, cumin and mixed spices, were in the months of May and June of 2012 purchased from different, randomly-selected retail outlets in Nyahururu, including supermarkets, fresh provision shops and market stalls. While the samples collected were taken to reflect market share, a wide range of brands was collected, in order to ensure that the survey was as comprehensive as possible and representative of the commercial spice products available to consumers. Food items sold loose or pre-packed were included in the study. Collection of samples was done from the top to the bottom of the sample package so as to obtain composite samples. Samples hence collected were sealed in polyethylene plastic bags, coded for easier identification, sub-divided into three batches, and stored at 4ºC. The first batch was kept aside as a backup, the second for fungal flora composition and the third for aflatoxin analyses.

2.2 Analysis of Aflatoxins from Retail Spice Preparations

Spice preparations were ground and mixed to uniform consistency using a laboratory mill. The samples were analysed for aflatoxins using a commercial ELISA (Boratest®, Bora Biotech, Kenya) kit. Briefly, the samples were extracted in methanol water (50:50). Defatting was done in hexane. A direct competitive ELISA kit was used for the detection of the total aflatoxins. In the test, free aflatoxins in the sample compete with added enzyme-labeled aflatoxin (conjugate) for antibody binding site. The intensity of colour, produced by reaction of the substrate with the enzyme attached to the toxin, in both standards and sample extract wells was determined by reading the absorbance at 450nm using an ELISA plate reader, 10 minutes after addition of the stop solution. The detection limit was 2 parts per billion (ppb).

2.3 Statistical Analysis

The data were analysed using Graph-Pad Prism 5 statistical software [23]. Analysis of variance (ANOVA) was applied to test for significant difference between mean aflatoxin concentrations and were considered significantly different at P-values <0.001.

3. RESULTS AND DISCUSSION

Spices are used daily throughout the world for flavouring foods as well as for medication due to their antimicrobial properties [24]. This study clearly demonstrates the presence of aflatoxins in spices sold in retail outlets in Nyahururu town. Results of aflatoxin occurrence in spice samples are shown in Table 1. Overall, out of forty-six spice samples analysed, thirty four (73.9%) samples were positive for aflatoxins while twelve (26.1%) had aflatoxin levels below the detection limit. Over 50% of samples analysed contained aflatoxin <10 µg/kg (ppb). The highest quantities of aflatoxins were found in cayenne, paprika and cumin (99.6, 99 and 98 µg/kg, respectively). Relatively high levels of aflatoxins were also found in chilli (31.5 µg/kg) while no mean detectable levels were observed in curry and nutmeg. The mean aflatoxins concentration across all positive spice samples was 30.6µg/kg. Statistical analysis showed significant difference in aflatoxin levels between spices types (P<0.001).

Table 1. Mean total aflatoxin content in commercial spice samples

Spice	Mean total aflatoxin (µg/kg)
Pepper	12.25
Chilli	31.5
Curry	ND*
Cayenne	99.6
Paprika	99
Cinnamon	15.1
Turmeric	6
Cumin	98
Cloves	7
Ginger	9.64
Garlic	2
Nutmeg	ND*
Mixed Spices	18.09

**ND=no detectable levels*

Previous investigations have reported varied levels of aflatoxins in spices including pepper, chilli, ginger, turmeric, nutmeg, curry, cayenne among others [2,5,25,26,27,28]. The present study also reports different concentrations of aflatoxins in various spice preparations. It is important to note that the variations reported in these previous studies, and those of the present paper, are probably due to region and environmental factors [29,30] which may affect fungal growth and thus mycotoxin contamination. Indeed, it has been reported that most of the countries in tropical and sub-tropical areas experience a higher level of aflatoxin contamination than countries found in the temperate regions [28,30]. The low concentration of aflatoxins in curry, nutmeg, turmeric, cloves and garlic may be attributed to the inhibitory effects of the spice oils and other secondary metabolites contained in these spices. It has been reported elsewhere that spices have inhibitory activity towards the growth, respiration and production of lipases and mycotoxins by fungi [31,32,33,34]. Additionally, the combination of essential oils (EOs) from spices has synergistic effects on fungal growth inhibition [34]. Although not all essential oils present in spices have the ability to inhibit fungal growth and aflatoxinogenesis [32]. For example, it was shown that curcumin and ginger EOs were unable to totally inhibit the growth of *Aspergillus parasiticus* at 1% concentration [32].

Compared to the limit established by the European Commission (10 µg/kg), over 50% of all positive samples of spices exceeded the maximum permitted levels. [35]. The majority of commercial spice samples from Nyahururu markets analysed in this study posed a risk to public health since consumption of the contaminated spices could lead to acute and/ chronic health conditions [36,37]. It is noteworthy some experiments showed aflatoxin levels in spice are not reduced by domestic cooking with either microwave or conventional gas oven [38]. Therefore, it is important for public health agencies in Kenya to continuously monitor the extent of aflatoxin contamination in retail spice preparations.

The high incidence and elevated levels of aflatoxin contamination in spices sold in Nyahururu town could partly be attributed to warm and humid climatic conditions, which may favour proliferation of toxigenic fungi [39,40]. This environment provides optimal conditions for mould growth and subsequent accumulation of mycotoxins in spices within a short stretch of time since spices constitute a natural medium for the growth of moulds [41,42]. Additionally, agricultural practices and postharvest handling of the spices could also

contribute to contamination by toxigenic fungi. It has been reported that improper storage conditions promote the growth of moulds and aflatoxin contamination [2].

4. CONCLUSION

The results of this study indicated high levels of aflatoxins in some commercial spices sold in Nyahururu retail markets. The maximum levels of aflatoxins in spices set by the European Commission were exceeded in most spices sampled. It is therefore important for regulatory bodies in Kenya to continuously monitor for aflatoxins in spices preparations available in the consumer market since it is a food safety concern. In addition, post-harvest procedures such as drying techniques and storage should be carefully controlled to minimize fungal growth and thus prevent mycotoxin contamination. This is because prevention of mycotoxin production at farm level is the best way to control mycotoxin contamination in agricultural products. Also employed should be other methods that inactivate aflatoxins, or reduce their levels, in postharvest foodstuffs; particularly while they are in storage. More comprehensive surveys for aflatoxin contamination in different towns in Kenya are recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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