Qualitative Evaluation of Aflatoxins in Selected Curry Powders on Ugandan Market in Nakiwogo - Entebbe, Wakiso District. A Cross-sectional study.

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Abstract

Background.

Mycotoxins are biologically active secondary metabolites produced by fungal species in the genera *Aspergillus, Penicillium, and Fusarium* and grow in a variety of foods including cereals, grains, nuts, oil seeds, fruits, vegetables, cocoa and coffee beans, wine, herbs, and spices. Several mycotoxin classes exist but aflatoxins are of great medical importance. Aflatoxins are produced by the fungi *Aspergillus flavus, Aspergillus parasiticus, and Aspergillus nomius.* Aflatoxins exhibit teratogenic, mutagenic, carcinogenic, immunosuppression and growth retardation properties in humans and animals. Although aflatoxins occur in various groups, aflatoxin B, is acutely poisonous. Aflatoxin occurrence in various food matrices has been reported across the globe with tropical countries bearing the most burden. In our setting, there is paucity information on aflatoxin status of food spices. In this study, 36 samples of curry powders from 16 brands in sacks and packed forms were prepared and screened for aflatoxins.

Methods.

The aflatoxins were extracted using 70% methanol and Chloroform for concentration. Aflatoxins were screened using thin layer chromatography and examined under Ultraviolet (UV) light at a wavelength of 366nm.

Results.

Results of this study indicated that 34 samples were contaminated with aflatoxin G (94.4%), followed by 6 aflatoxin B samples (16.7%). Additionally, 5 samples had both types of aflatoxins studied (13.9%).

Conclusion.

This study reports aflatoxin contamination in the commonly sold curry powders that are used in our homes in Uganda.

Recommendations:

The Uganda National Bureau of Standards needs to start periodic aflatoxin screening and quantification in the curry powders and other spices used in the production of curry powders to establish if the contamination is above the acceptable quantities.

Keywords: A qualitative study of aflatoxins, Curry powders, Carcinogenic, Thin layer Chromatography, ChiSquare, Submitted: 27th/10/2022 Accepted: 20th/11/2022

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1. Background:

Mycotoxins are biologically active secondary metabolites produced by fungal species in the genera *Aspergillus, Penicillium,* and *Fusarium* and grow in a variety of foods including cereals, grains, nuts, oilseeds, fruits, dried fruits, vegetables, cocoa and coffee beans, wine, beer, as well as herbs and spices (Lutfullah & Hussain, 2012).

Aflatoxins are mycotoxins produced by the fungi *Aspergillus flavus, Aspergillus parasiticus,* and *Aspergillus nomius.* These mycotoxins are teratogenic, mutagenic, and carcinogenic in humans and animals. The most toxic and carcinogenic member of this family, aflatoxin B, is acutely poisonous, highly mutagenic, and intensely carcinogenic in rodents and other animals. (Tosun & Arslan, 2013). Contamination of crops most commonly occurs during harvest and storage, when damp warm conditions allow the fungi to proliferate (Agol et al., 2017).

Currently, there are two types of spices used: the food spices and the tea spices. However, in this research, food spices have been looked at since they are the commonly used spices. Curry powder is a mixture of spices that take on a yellow colour. One of the main ingredients in curry powder is turmeric and yellow in colour. Other spices that can be incorporated include everything from ginger, cinnamon, garlic, and cloves to mustard seed, black pepper, and fennel seed (Zareshahrabadi et al., 2020).

Curry powders have got strong flavours which make them to be used in small quantities and tend to add few calories to food, even though many herbs and spices, especially those made from seeds, contain high portions of fat, protein, and carbohydrate by weight, while others have got portions of micronutrients to the diet (Kumari et al., 2018). When used in larger quantity, they can contribute a substantial amount of minerals, including iron, magnesium, and calcium to the diet. Most herbs and spices used have substantial antioxidant activity like phenolic compounds, flavonoids, which influence nutrition through many pathways, including affecting the absorption of other nutrients. These antioxidants also can act as natural preservatives, preventing or slowing the spoilage of food, leading to a higher nutritional content in stored food (Ninfali et al., 2005).

2. METHODS AND MATERIALS

2.1. ***Research design***

This was a cross-sectional study in which 36 samples from different brands of curry powders were screened for aflatoxins at Analytical Biosciences Laboratory – COVAB, Makerere University. Dry samples packaged for human consumption were purchased from randomly selected retail shops in Nakiwogo trading Centre, Entebbe municipality – Wakiso district.

2.2. ***Study area***

The samples were collected from the selected shops and supermarket within Nakiwogo trading Centre – Entebbe municipality, Wakiso district – Uganda which has a concentrated population of shops selling curry powders and the samples were purchased between 8^{th} – 12^{th} August 2022.

2.3. ***Sample size determination***

This was qualitative research and a total of 36 samples from 16 brands were obtained and screened for presence of aflatoxins. A random sampling method was used. These number of samples were selected based on the central limit theorem. When the sample size is large, the distribution of the sample mean is approximated to normal distribution. Therefore, statisticians and researchers have accepted the criterion of the sample size to be greater or equal to 30. (Chang et al., 2008).

2.4. ***Sampling criteria***

The samples were selected based on the big number of shops selling curry powders and the many restaurants buying them for food spicing and only commonly used curry powder samples were sampled and tested.

2.5. Aflatoxin extraction and screening

This was done according to the procedures developed by Analytical Biosciences Laboratory (Protocol No. ABL292018).

Briefly, aflatoxins were extracted using 70% methanol and absolute Chloroform for concentration. Aflatoxins were screened using thin layer chromatography (TLC) which uses adsorption chromatography.

Detection of aflatoxin B and G was done by viewing the developed and dried TLC plate under UV lamp at 366nm.

2.6. Procedure for extraction of Aflatoxins

1. Weighed 25g of the sample, added 2g of NaCl to defat, and 75mls of 70% methanol.
2. Vortexed for 5 minutes and filtered to obtain the filtrate
3. To the filtrate, added 5mls of distilled water and 10mls of Chloroform
4. Vortexed for 5 minutes
5. Transferred to the separating funnel and left to stand to obtain the chloroform layer
6. Collected the lower layer into a clean test tube

2.7. Procedure for Screening of Aflatoxins

- Made a loading plan on the TLC plate
- Pipetted and spotted 8μl on the labelled TLC plate and allowed to air dry
- Transferred the plate to the chromatographic tank having an Acetone-chloroform mixture and allowed it to move to about $\frac{3}{4}$ of the plate.
- Allowed to air dry and viewed the plate under the UV light in the spectrophotometer for aflatoxin B and G respectively at a wavelength of 366nm.

2.8. Data management and analysis

Data were analyzed based on the results from the UV light emission using the Chi-Square biostatistics method.

3. RESULTS INTERPRETATION AND ANALYSIS

Results Interpretation

A total of 36 samples obtained were successfully analyzed in the analytical Biosciences laboratory (ABL) specifically in the physiology section and the results obtained as below.

4. Results Interpretation

4.1. Storage of samples

Most of the samples tested positive and a few negative for both AFB and AFG regardless of whether they were collected from the metallic container shops, market, or shops in buildings. The samples from the market were found together on the same stall with other items like onions, cinnamon leaves, mush rooms among many other items as shown in table 3 below.

4.2. Statistical analysis

The research involved obtaining categorical data about the contamination of curry powder by aflatoxin B and G. A relationship between the presence of both AFB and AFG was analyzed using the Chi Square test (Table 4).

Using a critical point of 0.05 (9.488) that corresponds to the calculated value (1.6), it is lower than the critical value thus the null hypothesis is accepted. Therefore, there is acceptance that the curry powders consumed in the households contain the aflatoxins which may pose health risks to humans like live cancer if consumed in large amounts.

5. DISCUSSION:

Curry powders make an important part of the human diet due to their high nutritive value. Curry powder is a mixture of spices which are exposed to a wide range of microbial contamination due to poor collection conditions, poor production process, extended drying times and improper storage. Spices can be contaminated through dust,

Table 1: Shows Aflatoxin screening results

SAMPLE ID	AFLATOXIN B	AFLATOXIN G
01	-	+++
02	-	++
03	-	+++
04	-	+++
05	-	-
06	-	+++
07	-	+++
08	-	+++
09	-	+++
10	-	-
11	-	++
12	-	+++
13	-	+++
14	-	+++
15	-	+++
16	-	+++
17	-	+++
18	-	+++
19	++	+++
20	-	+++
21	-	+++
22	-	+++
23	-	+++
24	-	+++
25	-	+++
26	-	+++
27	++	+++
28	++	-
29	++	+++
30	++	+++
31	-	+++
32	-	+++
33	+++	+++
34	-	+++
35	-	+++
36	-	+++

Key:
Negative (-)
Trace (+)
Moderately Positive (++)
Strongly Positive (+++)

Table 2: Summary of Aflatoxin screening results

	Negative	**Trace**	**Moderately Positive**	**Strongly Positive**	
AFB	30	0	5	1	36
AFG	3	0	2	31	36

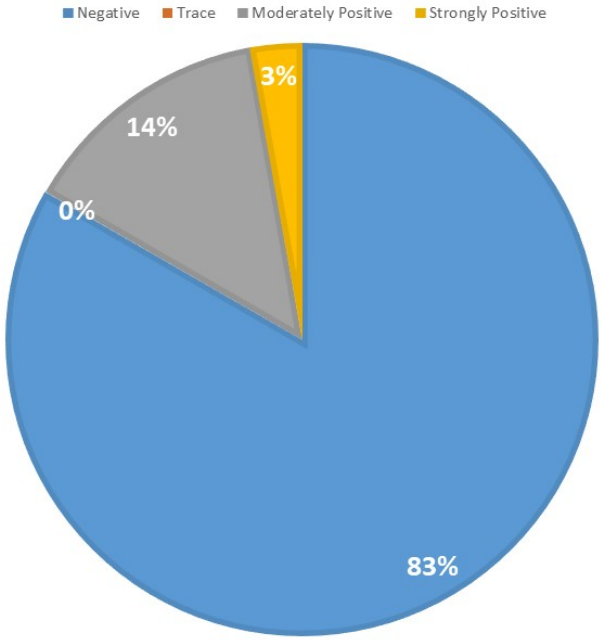

Figure 1: Percentage of samples with Aflatoxin B

Table 3: Storage of Curry powders in shops

	Metallic container shops	**Supermarket**	**Market**
Positive	27 **(96.4%)**	2 **(66.7%)**	5 **(100%)**
Negative	1	1	0
Total	**28**	**3**	**5**

Table 4: Observed values of the test

		Aflatoxin G			
		Negative	Moderately Positive	Strongly Positive	Total
Aflatoxin B	Negative	02	02	26	30
	Moderately Positive	01	00	04	05
	Strongly Positive	00	00	01	01
	Total	03	02	31	36

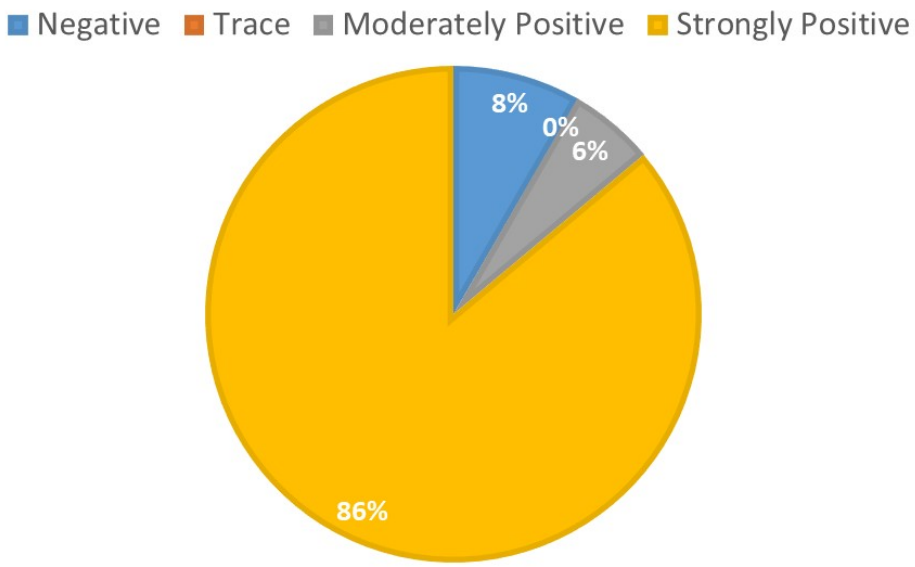

Figure 2: Percentage of samples with Aflatoxin G

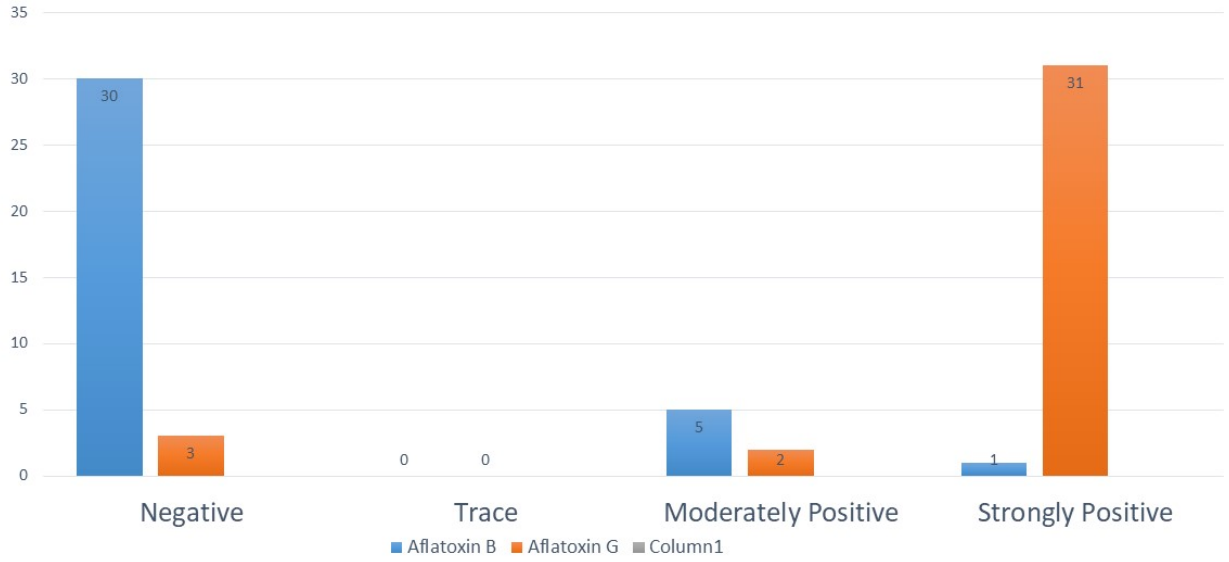

Figure 3: Number of samples with Aflatoxin B and G

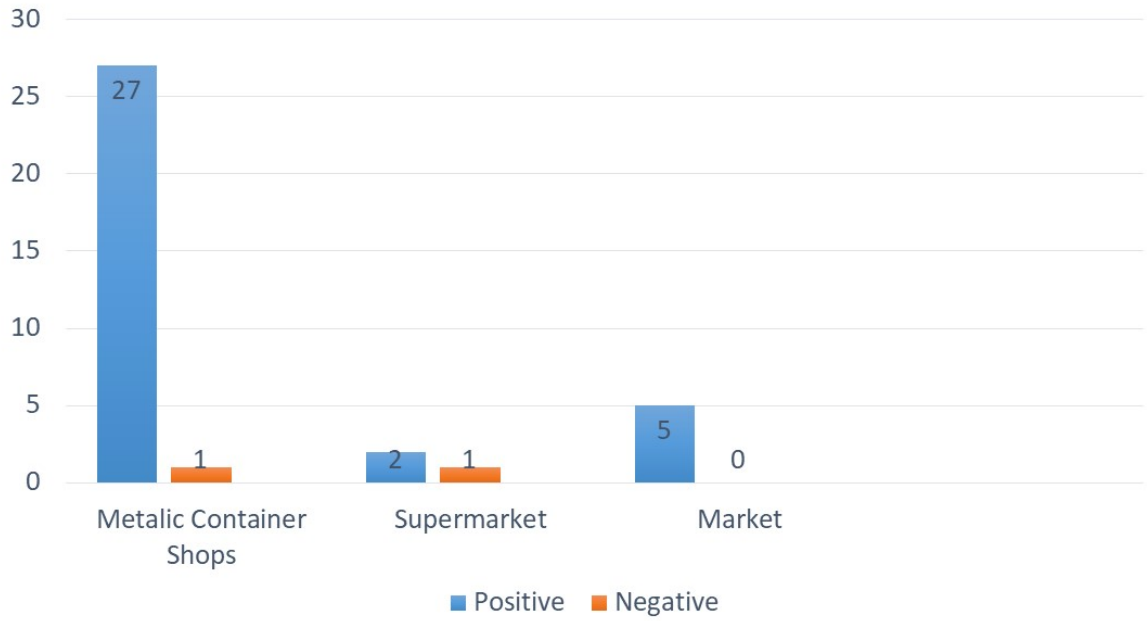

Figure 4: Aflatoxin positive samples by shop

Table 5: Expected values of the test

		Aflatoxin G			
		Negative	Moderately Positive	Strongly Positive	Total
Aflatoxin B	Negative	2.5	1.7	25.8	30
	Moderately Positive	0.4	0.3	4.3	5
	Strongly Positive	0.1	0.1	0.9	1
	Total	3	2	31	36
Chi =1.6					

wastewater and poor handling practices of unpacked spices, which are sold in markets (Rani et al., 2022).

In this study, a total of 36 samples belonging to 16 brands were collected from the shops in Nakiwogo, Entebbe and screened for aflatoxin B and G. 34 (94.4%) samples tested positive and 2 samples negative for aflatoxin G, 6 (16.7%) samples positive and 30 samples negative for aflatoxin B, 5 (13.9%) samples tested positive, and 2 samples negative for both aflatoxin B and G. This study shows different results from the study done in Iran on the spices used in making curry powder which indicated AFB at 27.5% and AFG at 4.17% (Khazaeli et al., 2017) while another study in Punjab, Pakistan by Nazir et al., 2019 showed aflatoxin presence by 30% (21).

However, the study is similar to the one done in Korea for total aflatoxins which indicated 68.1% - 103.9% for AFG (Cho et al., 2008). A study

in Pakistan indicated 66.7% (12/18) presence of total aflatoxins in the food spices used to make curry powder (Hussain et al., 2012).

There was a relationship between the presence of AFB and AFG in the curry powders and accept that curry powders have a lot of aflatoxin which might be carried from the ingredients used. The presence of aflatoxins in curry powder had relationship to the storage since all samples obtained from the metallic container shops, food market and shops in buildings all tested positive. This showed that the samples got from the market were more contaminated than others despite the few samples collected and this might be attributed to the poor storage and containers used which expose them to environmental conditions, followed by those in the metallic container shops which heat up and accumulate moisture in the containers used in packaging and those collected in the supermarket were less contaminated since they were located in the building that attract less heat.

The high positivity of the samples with aflatoxins puts a risk to the consumers of these products to cancer especially liver cancer although the amounts in each product need to be established.

Therefore, with the different rates of contamination in the food spices in the different studies, improper harvest, drying, packaging practices, post-harvest and storage conditions also cause for wide spectrum of aflatoxins (Motloung et al., 2018). It shows that aflatoxins are predominant contaminants of spices used in the production of the curry powders.

6. CONCLUSION AND RECOMMENDATIONS

7. Conclusion

This study reports aflatoxin contamination of the commonly sold curry powders and used in our homes in Uganda. These results give baseline information and alert on the potential aflatoxin contamination in the curry powders.

The curry powders sold in metallic container shops and buildings were already packed in opaque packets by the manufacturers. However, despite the different packaging both from the factory and locally, all samples had aflatoxins.

Proper handling and storage conditions of curry powders should be observed to minimize aflatoxin contamination. If fungal contamination could be kept at minimal levels, it would consequently lower aflatoxin levels hence help maintain health standards of curry powder consumers. There is need to advocate for proper storage and handling of curry powders that will minimize risks of aflatoxin contamination.

In Uganda, most of the literature on aflatoxins has been so much in maize, ground nuts, sorghum, millet among others and this has been hard in getting comparable data with the spices that are used in production of curry powder.

8. Recommendations

This was a qualitative test for aflatoxins in curry powders and therefore I would urge the public health specialists to carry out a quantitative evaluation of the aflatoxins to establish the values whether they are within the recommended amount of consumption that cannot cause harm to the human health.

Therefore, this report could guide UNBS to start the aflatoxin screening and quantification in the curry powders and other spices used in the production of curry powder to establish if the contamination is above the acceptable quantities.

9. Source of funding

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11. LIST OF ABBREVIATIONS

ABL - Analytical Biosciences Laboratory
AFB - Aflatoxin B
AFG - Aflatoxin G
COVAB - College of Veterinary Medicine, Animal Resources and Biosecurity
ELISA - Enzyme-Linked Immunosorbent Assay
FAO - Food and Agricultural Organization
HPLC - High Performance Liquid Chromatography
NaCl - Sodium Chloride
TLC - Thin Layer Chromatography
UNBS - Uganda National Bureau of Standards
UV - Ultraviolet

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