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ASSESSMENT OF AFLATOXINS B_1, B_2, G_1 AND G_2 STATUS OF HOME GROWN MAIZE IN SWAZILAND

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ABSTRACT

Aflatoxins belong to a group of naturally occurring toxins which contaminate foodstuffs under favourable conditions. *Aspergillus flavus* and *Aspergillus parasiticus* produce aflatoxins; the four major aflatoxins which contaminate foodstuffs are B_1, B_2, G_1 and G_2. Maize, groundnuts, rice, wheat and other foodstuffs are susceptible to aflatoxin contamination. B_1 in the majority of cases is the most abundant, most toxic and the most potent natural carcinogen and is classified as a Group 1 human carcinogen. The extent of contamination varies with geographical location, agricultural practices of farmers and susceptibility of crops to fungal activity during pre- and/ or post-harvesting. Research on groundnuts in Swaziland showed contamination with aflatoxins; however, no data exists on the contamination of maize grains, yet maize is a main staple food of the Swazi nation and its consumption is high. The purpose of the study was to carry out an investigation on the occurrence of aflatoxins on maize grown in various homesteads. Maize samples were collected from different areas in Hhohho, Manzini, Shiselweni and Lubombo regions. Extraction with methanol:water (85:15 v/v) was followed by detection with HPLC-PDA for the analysis of aflatoxins. Results showed that B_1 was the most pre-dominant toxin with the lowest concentration at 0.4 ppb and the highest at 25 ppb, both samples were from the Shiselweni region. A total of 90 maize samples were collected for analysis, it was determined that overall contamination with G_2 was 20% with 13% above 4 ppb- the European Union (EU) maximum recommended limit. B_1 contamination was at 31%, overall, with 18% above 2 ppb- the EU maximum recommended limit. Concentrations for G_1 and B_2 could not be determined due to poor resolution of peaks. These results show that people in Swaziland are being exposed to aflatoxins through feeding on contaminated maize. There is a growing urgency for the Swaziland government to come up with strategies of teaching the people about food safety.

Key words: aflatoxins, *Aspergillus*, maize, contamination, carcinogen, toxin, Swaziland, HPLC-PDA

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INTRODUCTION

Growing maize in Swaziland is more of a cultural practice than an agricultural one since maize is a staple food of the Swazi nation and therefore, its consumption is fairly high. Along busy roads and bus stations, there are always people selling roasted or boiled maize as a way of life and means of generating money for many families. It is estimated that rural households use approximately 86% of land on Swazi Nation Land (SNL) for growing maize [1].

Aflatoxins are known to contaminate many agricultural commodities around the world. Contamination is more pronounced in maize, cottonseed, tree-nuts and peanuts [2]. Aflatoxins are a group of naturally occurring toxins which are produced predominantly by an *Aspergillus* species and contaminate food and feedstuffs under favourable conditions; *Aspergillus* grows optimally at 25 ℃ [3]. The genus *Aspergillus* includes over 180 species; however not all *Aspergillus* species are harmful to human and/or animal health [4]. *Aspegillus flavus* and *Aspergillus parasiticus* are among the *Aspergillus species* that are reported to be harmful to human and animal health as they are responsible for the production of aflatoxins [5]. The major types of aflatoxins commonly found in food and feedstuffs are B_1, B_2, G_1, and G_2 while aflatoxins M_1 and M_2 are metabolites of aflatoxins B_1 and B_2, which may be found in milk and milk products [6]. *Aspergillus flavus* produces only the B aflatoxins while *Aspergillus parasiticus* produces both B and G aflatoxins [5, 7]. Aflatoxins are colourless to pale yellow crystals which dissolve in polar solvents like methanol and chloroform [2]. The B aflatoxins exhibit blue fluorescence under UV (ultra violet) light while the G aflatoxins exhibit a green-yellow fluorescence under UV light thus the B and G designations respectively [8].

Contamination of crops can occur in two principal stages; which are pre- and post-harvesting. The extent of contamination varies with geographical location, agricultural practices of the farmers and susceptibility of crops to fungal activity during pre- and post-harvesting [9]. Contamination in pre-harvest stage is mainly promoted by drought stress, damage of the crop, high insect activity, poor timing of harvest and heavy rains at, near, or during harvesting [10, 11, 12]. Post-harvest contamination is mainly influenced by levels of humidity, temperature and aeration during drying and storage. The condition of the stored grain and length of storage also influences post-harvest contamination [11]. Figure 1 shows maize contaminated with aflatoxin, sample was obtained from Nsangwini in the Hhohho region.

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Figure 1: Maize contaminated with aflatoxins, Picture taken from Nsangwini in the Hhohho Region on 13th April 2013

The major routes of human and animal exposure to aflatoxins are direct ingestion of aflatoxin contaminated food/feed and the inhalation of dust particles from contaminated foods during harvesting and/or processing [13]. Susceptibility to aflatoxins in humans and animals varies with age, general health, dose and duration of exposure [3, 14]. Aflatoxin B_1 is the most abundant, most toxic and the most potent natural carcinogen and is classified as a Group 1 human carcinogen [15]. Populations of developing countries are the most susceptible to aflatoxicosis illnesses; this is because food safety regulations concerning crops at pre- and post-harvest stages are not as strict as in developed countries [3]. Aflatoxin exposure can result in acute aflatoxicosis which occurs when high concentrations of aflatoxins are consumed. Symptoms of acute aflatoxicosis include vomiting, weight loss, abdominal pain, jaundice, liver damage and death [12, 16].

Aflatoxin exposure can also result in chronic aflatoxicosis, which manifests from being exposed to low concentrations over a long period of time. It is estimated that more than 5 billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods [17]. Studies suggest that there is a relationship between aflatoxin exposure and kwashiorkor (protein malnutrition) and aflatoxin intake in children [15, 18]. A study on children in Benin and Togo revealed that there was an association between stunting in children with chronic exposure to aflatoxins [19]. It has also been shown that there is a correlation between aflatoxin exposure and hepatocellular carcinoma (HCC), which is the most common form of liver cancer and is the third leading cause for cancer deaths with about 550,000-600,000 cases every year [17]. At least 300,000 of these deaths occur in China while the other 300,000 occur in countries in the Sub–Saharan Africa [20]. Preliminary evidence suggests an interaction

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between chronic aflatoxin exposure and immune-suppression which can result in susceptibility to infectious diseases such as malaria and HIV/AIDS [21].

Food safety issues do not only affect the general population of a country in terms of health but also the economy in terms of trade as well as tourism. The European Commission (EC) Regulation No. 1881/2006 is a legislation setting maximum levels for certain contaminants in foodstuffs [22]. The legislation has set maximum acceptable levels for the sum of aflatoxins (B_1+B_2+G_1+G_2) to be 4 ppb while the maximum acceptable level for aflatoxin B_1 is 2 ppb in maize and groundnuts intended for direct human consumption. The maximum recommended limits of aflatoxins according to this legislation are shown in Table 1. This presents a challenge in terms of trade for developing countries where contamination of crops with aflatoxins is still a problem. Trade rejection and discarded foods as a result of contamination with aflatoxins, as well as human healthcare costs and veterinary expenses due to aflatoxin exposure all lead to economic losses [21]. It is estimated that African economies lose about US$ 450 million each year in commerce due to lost trade as a result of aflatoxin contamination [23].

Swaziland has experienced droughts and erratic rainfalls which sometimes lead to flooding (excessive rainfalls) as a result of climate change; in December, 2013 the country experienced storms which caused damage to crops and infrastructure [24, 25]. Since droughts and flooding are also some conditions that favour aflatoxin contamination, maize would be vulnerable to contamination. Also, maize production in Swaziland is rain dependent, as a result, low yield would be harvested and this would increase food scarcity as well as poverty levels. Exposure to aflatoxin poisoning would also increase as poverty drives people to eat whatever is available no matter the quality.

The diet for many Swazi people is mostly limited to the staple food; maize. This implies that chances of aflatoxin exposure are high in Swaziland since there are no stringent rules in place regarding aflatoxins and also because the awareness of aflatoxins and their danger is lacking. The aim for this study was to carry out an investigation into the occurrence of aflatoxins B_1, B_2, G_1 and G_2 on maize the staple food of Swaziland collected from the four regions (Hhohho, Manzini, Shiselweni and Lubombo) of the country.

MATERIALS AND METHODS

Chemicals and Apparatus

A Genesis Nutri Plus blender (Verimark, South Africa), mechanical shaker, Spectroline E series UV lamp (Aldrich Steinheim, Germany), 20 x 20 aluminium sheets precoated with silica gel 60 F254 (0.2 mm thickness), separating funnels, Thermo Scientific Finnigan Surveyor Plus High Performance Liquid Chromatography system coupled with Photodiode array (HPLC-PDA) and Macherey Nagel C–18 column (250 x 4.6 mm, 5 µm particle size). Supelco aflatoxin mix standard (Sigma, Germany), containing concentrations of 1, 0.3, 1 and 0.3 µg/ml of aflatoxins B_1, B_2, G_1 and G_2, respectively dissolved in methanol. Millipore Millex-HV 0.45 µm, PVDF (Polyvinylidene fluoride)

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syringe filters, Mettler Toledo weighing balance, sodium chloride (analytical grade), methanol, acetonitrile, chloroform and n-hexane (all HPLC grade).

Sampling

Maize samples were collected from households in different areas in the four regions of Swaziland for a preliminary analysis. Samples were mainly collected from households with maize fields and metal silos for maize storage. Areas from which samples were collected were: Timphisini and Dvokolwako in the Hhohho Region, Ndinda, Luyengo and Ntondozi in the Manzini Region, Etjeni and Kontjingila in the Shiselweni Region and Macetjeni in the Lubombo Region as shown in Figure 2. A total of ninety maize samples were collected for further analysis from: Nsangwini, Nginamadvolo, Maguga, Mnyokane, and Nkhaba in the Hhohho Region, Luyengo, Mdonjane, Nhlulweni, Dvudvusini, and Ndwandwe in the Manzini Region, Mfishane and Endlovini in the Shiselweni Region, Mhlabubovu and Bhanganoma in the Lubombo Region as shown in Figure 2.

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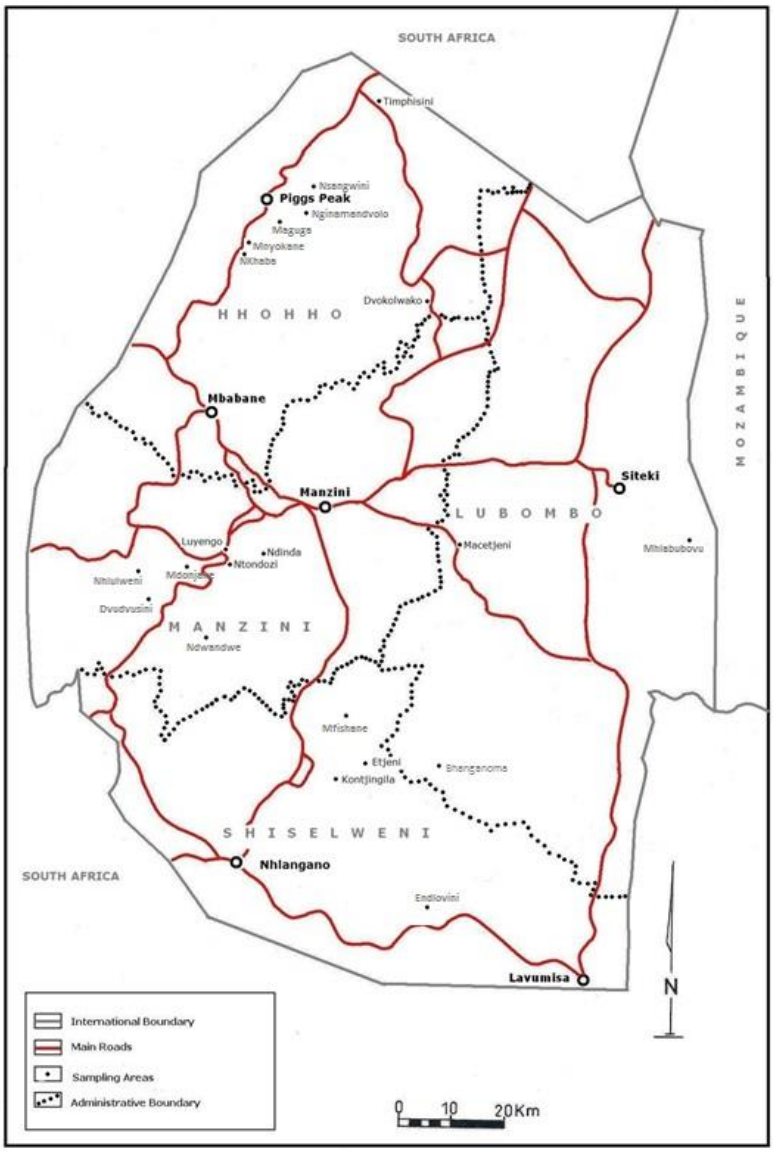

Figure 2: Map of Swaziland Showing Sample sites

Extraction of Aflatoxins

A 50 g ground sample was weighed into an Erlenmeyer flask and 200 ml methanol:deionized water (85:15 v/v) was added [26]. The mixture was shaken on a mechanical shaker for an hour. About 40 ml of the extract was partitioned with 40 ml of 10% sodium chloride (NaCl). This was followed by the addition of two 25 ml hexane portions and the phases were allowed to separate. The hexane layer was drained out and discarded while the aqueous layer was collected into another separating funnel. Two 25 ml portions of chloroform ($CHCl_3$) were added to the funnel and the phases were allowed to separate after shaking for a few seconds. The chloroform layer was evaporated to dryness using a Buchi multivapor capable of parallel evaporation of twelve samples at 60 ℃. The residue was then dissolved in 3 ml of dichloromethane (CH_2Cl_2) and the aflatoxin extract stored in a refrigerator until time for further analysis.

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Extracts of some samples (chosen at random) were spiked with 20 µl of aflatoxin standard.

Detection of Aflatoxins

Screening of samples for the presence of aflatoxins was carried out using thin layer chromatography (TLC) technique. Extracts were spotted along with standards on 10 x 10 cm plates and developed using chloroform:acetone (90:10 v/v) followed by toluene:ethyl acetate:formic acid (60:30:10 v/v/v) and viewed under UV light at 254 and 365 nm. Extract was filtered using a PVDF membrane filter. Detection using High Performance Liquid Chromatography (HPLC) was carried out at 365 nm with deionized water:methanol:acetonitrile (60:20:20 v/v/v) as mobile phase, injection volume of 20 µl and flow rate set at 1ml/min. The HPLC was used in identification and quantification of the aflatoxins present in the samples.

RESULTS

Preliminary analysis: TLC Analysis

TLC analysis was performed as a semi-qualitative step to identify whether aflatoxins were present before proceeding to HPLC analysis. On observing plates under UV lamp, spots corresponding to aflatoxins could be seen as shown in Figure 2.

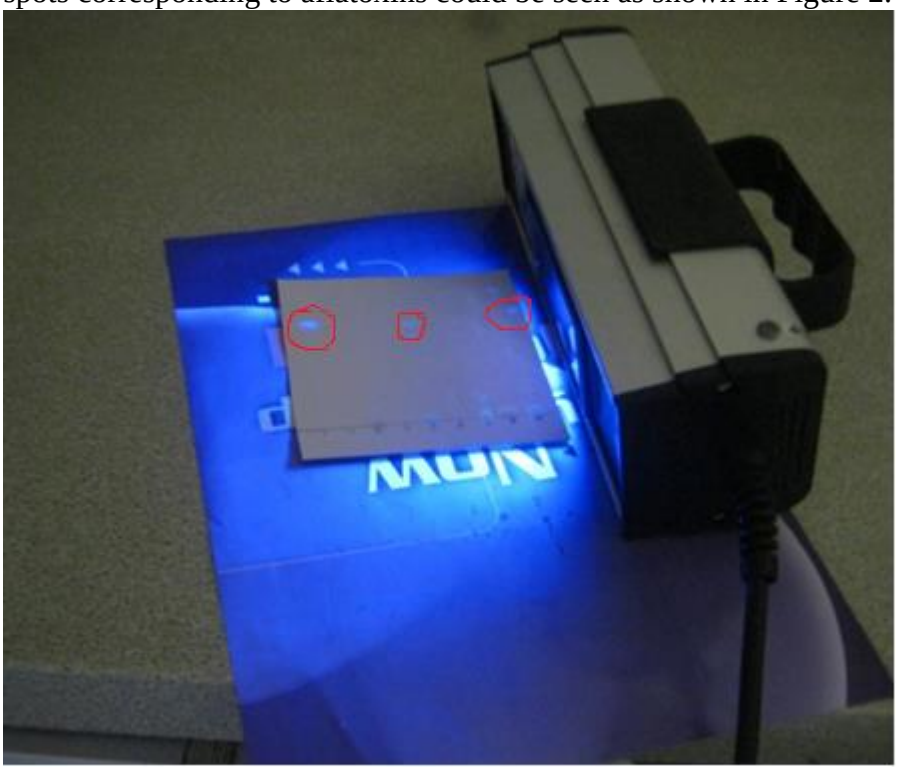

Figure 3: TLC plate examined under UV light

HPLC Analysis

Preliminary analysis of maize samples showed that samples from Luyengo B, Ntondozi B and Macetjeni A were contaminated with B_1, however, only Luyengo B sample was above 2 ppb. Nine maize grain samples indicated presence of G_2 with the highest

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concentration of 2.5 ppb and the lowest concentration of 0.1 ppb for samples from Ndinda B and Macetjeni B, respectively. Only two samples showed G_1 contamination with concentrations of 1.2 ppb and 2.1 ppb for samples from Ndinda D and Ntondozi B, respectively. There were seven samples which showed the presence of B_2 with the highest concentration being 7 ppb and the lowest concentration of 0.3 ppb for samples from Etjeni A and Ntondozi A, respectively. Semi-quantitative analysis of maize samples for total aflatoxin showed that only two samples; Etjeni A and Luyengo B with concentrations of 7.8 and 5.5 ppb respectively, were above the EU recommended limit for total aflatoxins which is 4 ppb.

Concentrations of aflatoxins and the semi-quantitative results for total aflatoxin contamination from the preliminary survey of maize are shown in Table 2. Only three samples showed presence of aflatoxin B_1 in maize as shown in Figure 4 against the EU maximum acceptable limit which is set at 2 ppb.

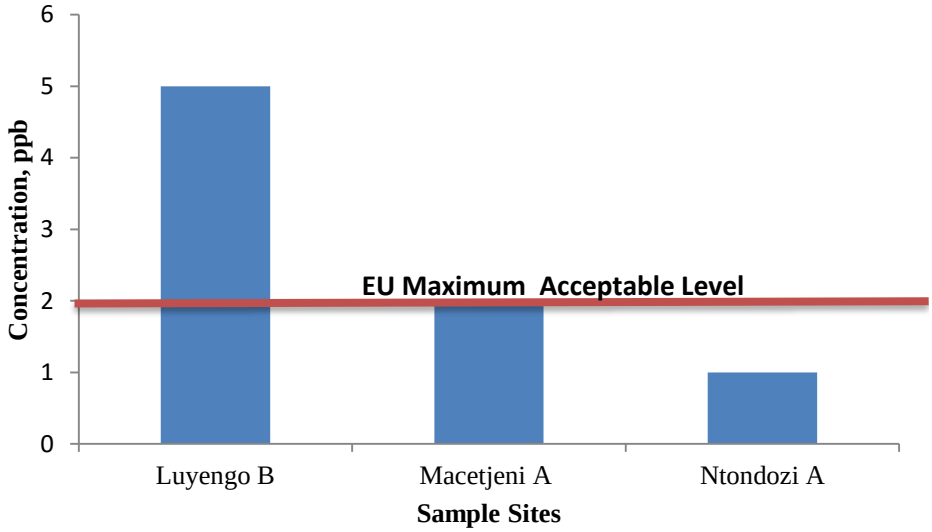

Figure 4: Concentrations of aflatoxin B_1 in maize samples

Occurrence of Aflatoxins in Maize by Regions in Swaziland

Figures 5, 6, 7 and 8 show the concentrations of aflatoxins B_1 and G_2 in maize from the Manzini, Shiselweni, Lubombo and Hhohho regions respectively. Investigations on the occurrence of aflatoxin in maize grown in Swaziland revealed that there was contamination with aflatoxins. Table 3 gives the concentrations of aflatoxins that were determined. The highest concentration determined for G_2 was 47 ppb in a sample from the Manzini region, while the highest B_1 concentration determined was 25 ppb in sample from the Shiselweni region. Of the ninety samples that were analysed 31% were contaminated with aflatoxin B_1, with 18% above 2 ppb, while contamination with aflatoxin G_2 was 20% with 13% above 4 ppb. Table 4 shows the occurrence of B_1 and G_2 above 2 ppb and 4 ppb respectively for the four regions of Swaziland.

Maize samples from the Shiselweni region were found to be the most contaminated, as out of the 18% (samples contaminated with B_1 above 2 ppb), 8% were from the Shiselweni region as shown in Table 5. Hhohho and Shiselweni regions were the most

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contaminated with G_2 both with 4% contamination above 4ppb while the least contaminated was the Lubombo region with 1%. Aflatoxin B_1 was the more dominant toxin which eluted consistently in the retention time expected if present in a sample. This is the most dangerous of the aflatoxins due to its carcinogenic effects on human and animal health.

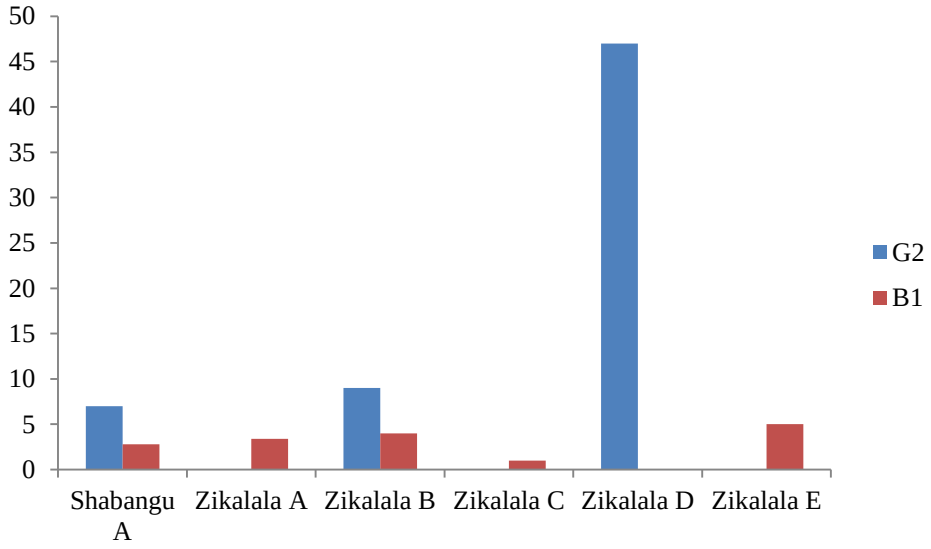

Figure 5: Concentrations of aflatoxins B_1 and G_2 in Manzini Region

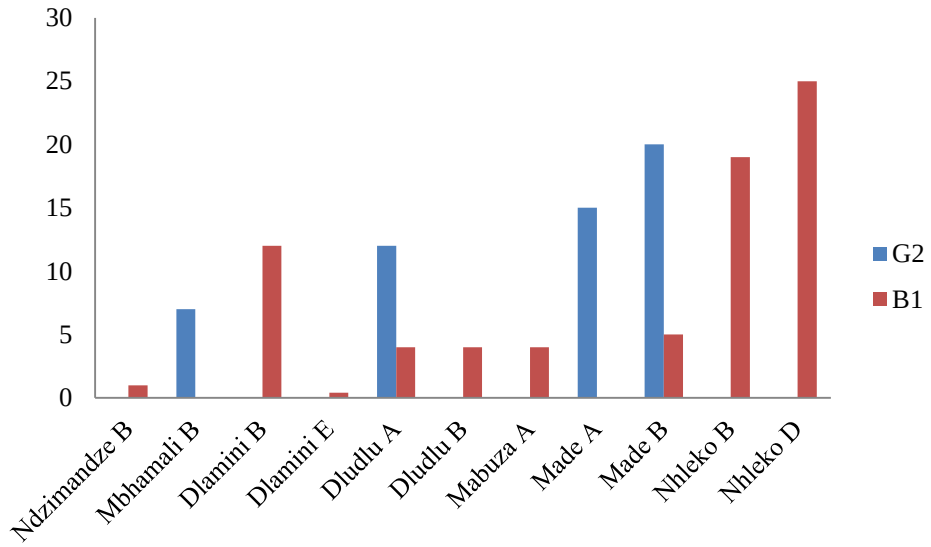

Figure 6: Concentrations of B_1 and G_2 in the Shiselweni Region

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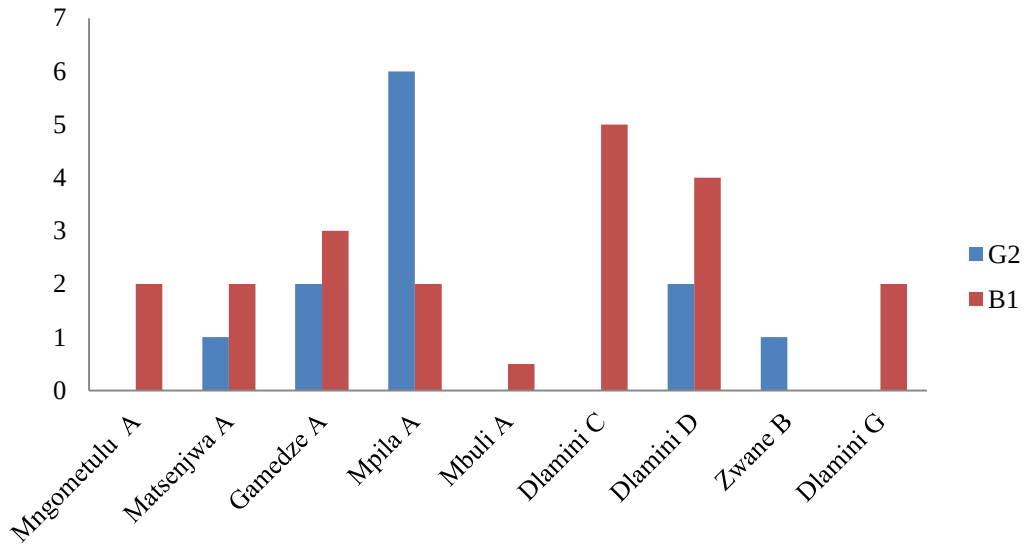

Figure 7: Concentrations of B_1 and G_2 in the Lubombo Region

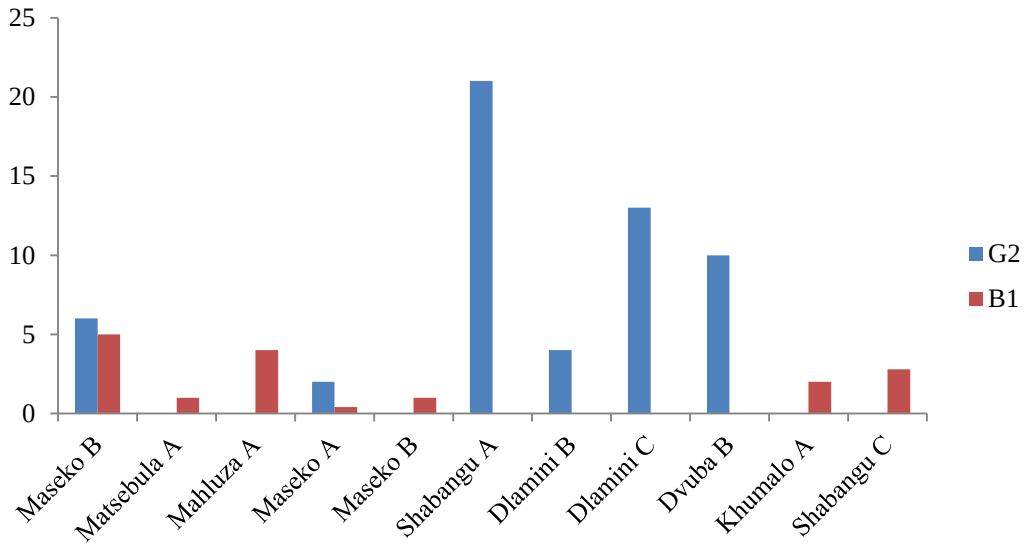

Figure 8: Concentrations of B_1 and G_2 in maize Hhohho Region

DISCUSSION

Maize production in Swaziland is mainly rainfall dependent, therefore, lack of rainfall, high temperatures and storms have greatly compromised maize harvest in Swaziland [25]. Such conditions also favor contamination of maize with aflatoxin which would compromise the health of the population feeding on this maize. Aflatoxin contamination in maize samples was analysed for this study, it was discovered that some maize samples were contaminated with aflatoxin B_1 and G_2. Overall contamination was 31% for aflatoxin B_1, the aflatoxin content exceeded the EU maximum recommended level set at 2 ppb for 18% of the sample with the highest

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determined concentration being 25 ppb. Aflatoxin B1 was found to be the most predominant toxin which is line with the findings obtained from analyzing foodstuffs constituting the diet of the Swazi population [27]. Positive results were also determined for aflatoxin G_2 in 20% of the samples, of the positive samples 13 % were above 4 ppb (EU Maximum recommended level for sum total of the aflatoxins).

Swaziland can be defined into four geographical regions; Highveld, Middleveld, Lowveld and Lubombo region. These geographical regions vary according to altitude with the Highveld having average altitude between 1050 and 1400 m, Middleveld between 400 and 1000 m, Lowveld between 150 and 400m and Lubombo between 400 m and 777 m [28]. The Shiselweni region consists of both the Highveld and Lowveld regions [29]. In this study samples from Shiselweni were found to be the most contaminated samples overall, with aflatoxin B_1 concentrations ranging between 0.4 ppb and 25 ppb. Previous research on food items concluded that there was increasing contamination with decreasing altitude [27]. These findings explain the wide range between the lowest and highest contamination levels within Shiselweni.

Another effect of climate change is change in rainfall patterns as has been the case in Swaziland [30]. This has created confusion in terms of planting and harvesting seasons resulting in two scenarios are possible as a consequence. The first one is that people would wait for the first rains which in most cases are now delayed and therefore, due to food shortages people would find themselves harvesting maize too early. The danger in this would be that the maize would not have matured fully and the moisture levels in the grains would be high enough to increase chances of contamination with aflatoxins. The second possible scenario would be for people to plant during the first rains and hope for a good harvest, however should a dry spell with scorching temperatures be experienced in the country as it happened in 2013 [25], growing maize cobs would then be vulnerable to aflatoxin contamination as a result of drought stress and insect activity.

CONCLUSION

Concentrations of aflatoxins were determined for the samples from the four regions of Swaziland. It is of great concern that 18% of the samples analysed gave concentrations of aflatoxin B_1 exceeding the maximum recommended limit of 2 ppb set by the EU. Concentrations of G_1 were also determined and it was found that 12% of the samples had concentrations above 4 ppb which is the maximum recommended limit for a sum total of all the aflatoxins. This implies that Swazis are being exposed to aflatoxins.

RECOMMENDATION

It is recommended that a follow up study be done in which , clean-up methods must be used in order to improve on the resolution of the peaks, for example the use of immune affinity column and QuEchERS kits. Also, more samples should be collected for analysis since aflatoxins are heterogeneous. In addition, the effect of climatic conditions on contamination should be investigated. Further, people should be made aware of the high concentrations (above EU maximum recommended limit) of aflatoxins in some areas through the Ministry of Agriculture and the Ministry of Health.

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Table 1: EU maximum acceptable limits of aflatoxins in some food commodities

Food Commodity	**Intended use**	**Maximum levels (µg/kg),**	
		B_1	**B_1, B_2, $G_1$and G_2**
(a) Groundnuts	To be subjected to sorting , or other physical treatment before human consumption or use as an ingredient in foodstuffs	8.0	15.0
(b) Groundnuts	Direct human consumption or for use as an ingredient in foodstuffs	2.0	4.0
(c) Maize	To be subject to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs	5.0	10
(d) All cereals and all products derived from them	Direct human consumption	2.0	4.0
(e) Processed cereal based foods	Baby foods for infants and young children	0.10	___

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Table 2: Preliminary analysis of aflatoxins in maize in ppb

Site	G_2	G_1	B_2	B_1	Total aflatoxin
Etjeni A	0.8	ND	7	0	7.8
Kontjingila A	2	ND	ND	0	2
Kontjingila B	0	ND	ND	0	ND
Luyengo A	1	ND	ND	0	1
Luyengo B	0.5	ND	ND	5	5.5
Macetjeni A	0	ND	ND	2	2
Macetjeni B	0.1	ND	ND	0	0.1
Ndinda A	0.8	ND	ND	0	0.8
Ndinda B	2.5	ND	ND	0	2.5
Ndinda C	ND	ND	ND	0	ND
Ndinda D	0	1.2	0.6	0	1.8
Ntondozi A	0	ND	0.3	0	0.3
Ntondozi B	0	2.1	0.4	1	3.5
Ntondozi C	0	ND	0.5	0	0.5
TimphisiniA	0	ND	ND	0	ND
Timphisini B	1	ND	2	0	3
Timphisini C	0.3	ND	0.7	0	1

ND: not determined

G_2, G_1, B_2, B_1 = aflatoxins analysed

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Table 3: Occurrence of aflatoxins by regions in Swaziland in ppb

(ND: Not Determined)

Region	Area	Site	Aflatoxin G_2	Aflatoxin G_1	Aflatoxin B_2	Aflatoxin B_1
M	Ndwandwe	Malambe A	O	ND	ND	0
A	Mdonjani	Sibandze A	0	ND	ND	0
N	Luyengo	Shabangu A	7	ND	ND	2.8± 0.2
Z		Zikalala A	0	ND	ND	3.39 ±0.03
I		Zikalala B	9	ND	ND	4 ±1
N		Zikalala C	0	ND	ND	1
I		Zikalala D	47	ND	ND	0
		Zikalala E	0	ND	ND	5
		Zikalala F	0	ND	ND	0
		Fakudze A	0	ND	ND	0
	Enhlulweni	Jele A	0	ND	ND	0
		Jele B	0	ND	ND	0
		Dlamini A	0	ND	ND	0
		Dlamini B	0	ND	ND	0
		Dlamini C	0	ND	ND	0
	Dvudvusini	Maseko A	0	ND	ND	0
		Maseko B	0	ND	ND	0
S	Ndlovini	Ndzimandze	0	ND	ND	0
H		A	0	ND	ND	1
I		Ndzimandze	0	ND	ND	0
S		B	7	ND	ND	0
E		Mbhamali A				
L		Mbhamali B				
W	Mfishane	Dlamini A	0	ND	ND	0
E		Dlamini B	0	ND	ND	12
N		Dlamini C	0	ND	ND	0
I		Dlamini D	0	ND	ND	0
		Dlamini E	0	ND	ND	0.4
		Dlamini F	0	ND	ND	0
		Dludlu A	12	ND	ND	4
		Dludlu B	0	ND	ND	4
		Mabuza A	0	ND	ND	4
		Mabuza B	0	ND	ND	0
		Made A	15	ND	ND	0
		Made B	20	ND	ND	5
		Nhleko A	0	ND	ND	0
		Nhleko B	0	ND	ND	19
		Nhleko C	0	ND	ND	0
		Nhleko D		ND	ND	25

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REGION	AREA	Site	Aflatoxin G_2	Aflatoxin G_1	Aflatoxin B_2	Aflatoxin B_1
L	Mhlabubovu	Mngometulu A	0	ND	ND	2
U		Matsenjwa A	1	ND	ND	2
B		Matsenjwa B	0	ND	ND	0
O		Maziya A	0	ND	ND	0
M		Ngwenya A	0	ND	ND	0
B		Ngwenya B	0	ND	ND	0
O		Gamedze A	2±1	ND	ND	3.1±0.4
		Ndzimandze A	0	ND	ND	0
		Mpila A	6	ND	ND	2.
		Mpila B	0	ND	ND	0
		Mbuli A	0	ND	ND	0.5
		Mbuli B	0	ND	ND	0
		Myeni A	0	ND	ND	0
		Mbhamali A	0	ND	ND	0
		Tsabedze A	0	ND	ND	0
		Dlamini A	0	ND	ND	0
		Dlamini B	0	ND	ND	0
		Dlamini C	0	ND	ND	5
		Dlamini D	2	ND	ND	4
	Bhanganoma	Dludlu A	0	ND	ND	0
		Penga A	0	ND	ND	0
		Shabangu A	0	ND	ND	0
		Simelane A	0	ND	ND	0
		Zwane A	0	ND	ND	0
		Zwane B	1	ND	ND	0
		Dlamini E	0	ND	ND	0
		Dlamini F	0	ND	ND	0
		Dlamini G	0	ND	ND	2
H	Nginamadvolo	Maseko A	0	ND	ND	0
H		Maseko B	6	ND	ND	5
O		Maseko C	0	ND	ND	0
H		Matsebula A	0	ND	ND	1
H		Matsebula B	0	ND	ND	0
O		Dlamini A	0	ND	ND	0
	Mnyokane	Mahluza A	0	ND	ND	4
		Mahluza B	0	ND	ND	0
		Mahluza C	0	ND	ND	0
		Mahluza D	0	ND	ND	0
		Maseko A	2	ND	ND	0.4
		Maseko B	0	ND	ND	1
	Nkhaba	Dvuba A	0	ND	ND	0
		Mhlanga A	0	ND	ND	0
		Mhlanga B	0	ND	ND	0
		Mthande A	0	ND	ND	0
		Shabangu A	21	ND	ND	0
		Shabangu B	0	ND	ND	0
	Nsangwini	Dlamini B	4	ND	ND	0
		Dlamini C	13	ND	ND	0
		Dvuba B	10	ND	ND	0
		Khumalo A	0	ND		2
	Maguga	Dlamini D	0	ND	ND	0
		Malindzisa A	0	ND	ND	0
		Shabangu C	0	ND	ND	2.8±0.2

Table 4: Occurrence of B_1 and G_2 above 2 ppb and 4 ppb in Swaziland

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Region	B_1	G_2
Shiselweni	8%	4%
Manzini	4%	3%
Lubombo	3%	1%
Hhohho	3%	4%

B_1, G_2: Aflatoxins

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