

Temporal Trends in Mycotoxin Contaminations in Maize Supplied to Consumers in Eswatini

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Abstract

Mycotoxin contamination of maize results in heavy economic loss and a potential risk for human being. The Eswatini population depends heavily on maize for nutritional needs. This commodity requires continuous monitoring and care from its site of production by smallholder maize producers and importers through to consumers along the marketing chains. Maize produced locally and imported from neighbouring countries is often contaminated with Mycotoxin, which, after ingestion, pose serious health hazard to the consumers. Mycotoxin can contribute to the causation of liver cancers, immune system disorders, and growth-related issues in children. Moreover, deaths in both humans and animals have also been reported after ingestion of Mycotoxin contaminated food. Studies have shown contamination of food and feed ingredients with Mycotoxin. This study places the maize value chain into context, summarizes results of laboratory analyses of maize grain samples for Mycotoxin in the years between 2001 and 2021 and presents the prevalence and diversity of Mycotoxin and discusses the present legislative regulation of maize quality implemented in Eswatini. There is a need to improve maize production and postharvest handling practices, which are the sources of Mycotoxin so prevalent in the maize marketed to the consumers in Eswatini.

Keywords

Co-occurrence, Eswatini, Maize grain, Maize meal, Mycotoxin

1. Introduction

Maize is a staple food in the Kingdom of Eswatini, and the most cultivated crop constituting 95% of the country's cereal production [1]. The crop is cultivated by most small-scale farmers in the communal Swazi Nation Land (SNL) for household food security purposes and livelihood support. Maize production remains low in Eswatini. The country is still unable to meet the local demand through local production that it has not yet reached self-sufficiency. Maize import to Eswatini increased from 50 thousand tonnes in 2001 to 180 thousand tonnes in 2020 growing at an average annual rate of 9.77% [2]. In, Maize is available many Eswatini marketplaces in sufficient quantities, but the quality of this commodity supplied to consumers may vary.

As a staple food for millions of people in the country, maize requires continuous monitoring and care from its site of production by smallholder maize producers and importers through to consumers along the marketing chains. Maize produced locally and imported from neighbouring countries is often contaminated with mycotoxins. Mycotoxins are toxic low molecular weight and very stable secondary metabolites, produced by several fungi that frequently contaminate maize along the entire production chain, especially under conducive pre- and post-harvest conditions [3]. Over 300 mycotoxins have been identified [4] and the most common mycotoxins are: aflatoxins (AF); ochratoxin A (OTA); citrinin; patulin; deoxynivalenol (DON), T-2 toxin (T2) and HT-2 toxin (HT2); fumonisins (FUM) and zearalenone

(ZEN) [5, 6]. Maize is often contaminated simultaneously with various mycotoxins [7]. Mycotoxin co-occurrence may arise from colonization by more than one fungal species which would produce different types of mycotoxins or colonization by one fungal species that produces more than one type of mycotoxin [7]. Exposure of animals and human beings to multiple mycotoxins may lead to additive, synergistic or antagonist toxic effects [8]. Published studies on the combined effects of multiple mycotoxins suggest possible greater toxic effects of the mixtures compared to individual mycotoxins [9].

The consumption of maize contaminated by mycotoxins may cause a number of severe toxic effects in both animals and humans. Due to the health risk associated with the intake of foodstuffs contaminated by mycotoxins, the European Union has established the maximum levels of certain mycotoxins allowed in maize and its derived products intended for human consumption [10]. Unfortunately, many countries have no regulation on the maximum residue limits for mycotoxins and Eswatini is not an exception. In a worldwide survey of mycotoxin regulation carried out by FAO in 2003, only 15 countries in Africa were reported to have regulations for mycotoxins [11]. The inadequate technical, human and economic resources may be reasons for the lack of regulation on maximum level of Mycotoxin in foodstuff and feeds in developing countries [12].

Although mycotoxin contamination is a major concern in Eswatini, to our knowledge, no study has been conducted on dietary mycotoxins in the country, mainly due to lack of advanced laboratory equipment, inadequate research funds, and limited surveillance systems and expertise. Food safety management in the country is limited to moisture testing at the harvest and occasional mycotoxin contamination and grading of samples from farmers and traders. Inspections of stores and maize in transit are very rare and not rigorous. Mechanism of detecting mycotoxin levels is inexistent and there is no obligatory regulation on the distribution and use of contaminated maize products. Information on the incidence of mycotoxin contamination in maize and its implications on consumers' health in the country is lacking. Hence, this study highlight, update and discuss the available information on the incidence of mycotoxins in maize supplied to Eswatini consumers. The study discusses the public health implications and the recommends strategies for control of mycotoxins in maize grain and maize meal supplied to consumers in the country.

2. Materials and Methods

After approval by the Malkerns research station, we reviewed the laboratory reports of all maize grain samples analysed at the Malkerns research mycotoxin laboratory for Mycotoxin contamination between July 1, 2001 and December 31, 2021. Samples submitted by maize value chain actors for analysis and samples collected from the actors by inspection teams were treated similarly with no consideration to their prior history. In accordance with currently accepted standards, samples collected in the reviewed period were analysed following the procedure described in Alshannaq and Yu (2017). Only qualitative analysis described in the procedure was adopted for sample analysis in this study, quantitative analysis was not performed due to lack of equipment and expertise to quantify the results. The steps followed in the analysis of samples in this study are as described in the diagram below.

3. Results

A total of 892 maize samples diagnosed from 2001 to 2021 were included in this study, with 651 (73%) processed maize and 241 (27%) maize grain. Of the maize grain samples, 14 (5.8%) were diagnosed with Aflatoxin B₁ (AFB₁), 12 (5%) Aflatoxin B₂ (AFB₂), 9 (3.7%) Aflatoxin G₁ (AFG₁), 10 (4.1%) Aflatoxin G₂ (AFG₂), 7 (2.9%) Zearalenone (ZEN) and 22 (9.1%) with multiple mycotoxins (Table 1). Among the maize meal samples, 37 (5.7%) were diagnosed with Aflatoxin B₁ (AFB₁), 31 (4.8%) Aflatoxin B₂ (AFB₂), 24 (3.7%), Aflatoxin G₁ (AFG₁), 27 (4.1%) Aflatoxin G₂ (AFG₂), 17 (2.6%) Zearalenone (ZEN) and 16 (2.5%) with multiple mycotoxins. Mycotoxin contamination for maize products in the studied period was 14.5%, with Aflatoxins recorded in 118 (91.5%) of the positive maize products. In both product types (maize grain and meal), AFB₁ and AFB₂ were the most common mycotoxins diagnosed. Zearalenone was diagnosed as part of multiple mycotoxins only in 6 (4.7%) of the contaminated samples and as a single mycotoxin in 11 (8.5%) maize samples. Multiple Mycotoxin diagnoses were common in maize grain 22 (9.1%) than in maize meal samples 16 (2.5%) (Table 1). Of the 892 samples received by the laboratory, a total of 129 (14.5%) were diagnosed with mycotoxins. More than half of the Mycotoxin contaminated samples (n = 67) were found to be positive for Aflatoxins (Table 1).

The number of samples diagnosed with mycotoxins fluctuated from zero in the year 2017 to a maximum of 17 samples in the year 2010. There observed a strong positive correlation between the number of maize samples received, Mycotoxin contamination and co-occurrence of Mycotoxin in the years considered in this study. The highest incidence of mycotoxins in maize grain was recorded in the year 2011 with 7 (26.9%) maize grain samples received by the laboratory diagnosed positive to mycotoxins. Of these Mycotoxin contaminated samples three of them (42.9%) were diagnosed with multiple mycotoxins (Figure 2a). The highest incidence in mycotoxin contaminated maize meal samples was recorded in the year 2010 with 11 (45.8%) of the maize meal samples diagnosed positive to mycotoxins (Figure 2b).

Co-occurrence of mycotoxins in contaminated maize meal samples was recorded in 2018 where 66.7% of the Mycotoxin contaminated samples were diagnosed with multiple Mycotoxin (Figure 2b).

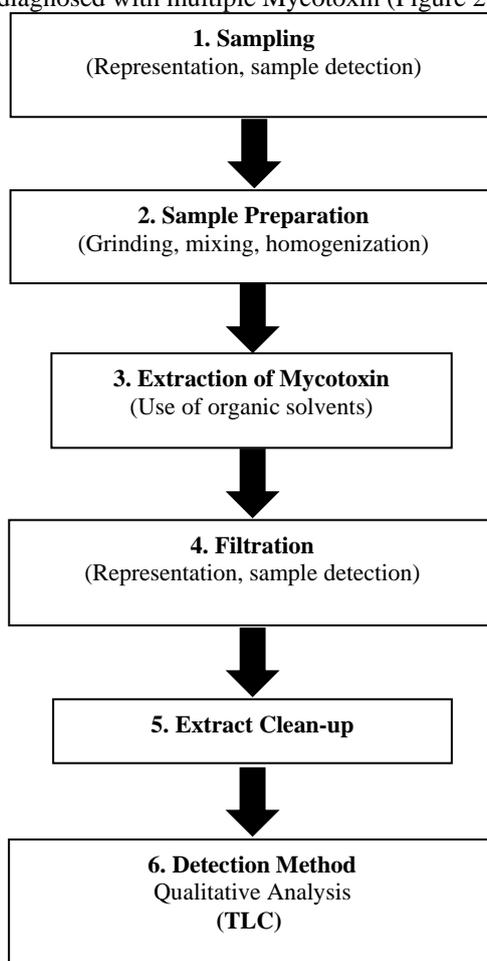


Figure 1. Flow diagram of steps involved in mycotoxins analysis in maize grain and meal samples.

Table 1. The incidence of mycotoxin in maize samples diagnosed from 2001 to 2021 by mycotoxin group and product type

Product type	Number (%) of samples with mycotoxin						
	No samples analysed	Aflatoxin B ₁ (AFB ₁)	Aflatoxin B ₂ (AFB ₂)	Aflatoxin G ₁ (AFG ₁)	Aflatoxin G ₂ (AFG ₂)	Zearalenone	with multiple mycotoxins
Maize Grain	241 (27)	14 (5.8)	12 (5)	9 (3.7)	10 (4.1)	7 (2.9)	22 (9.1)
Maize meal	651 (73)	37 (5.7)	31 (4.8)	24(3.7)	27(4.1)	17(2.6)	16 (2.5)
Total	892 (100)	51 (5.7)	43 (4.8)	33 (3.7)	37 (4.1)	24 (2.7)	38 (4.3)

In total 18 different mycotoxin combinations were diagnosed in maize grain and meal samples analysed between 2001 and 2021. Nine of these mycotoxin combinations were unique to maize grain samples and six mycotoxin combinations were unique to maize meal. Three mycotoxin combinations (AFB₁, AFB₂; AFB₁, AFB₂, AFG₁ and AFB₁, AFG₁, AFG₂) occurred in both maize grain and maize meal samples (Table 2). The co-occurrence of different multiple mycotoxins varied. AFB₁, AFB₂ was the most frequently documented mycotoxin co-occurrence, with 12 (31.6%) records in total. This multiple mycotoxin was followed in number of available detections by AFB₁, AFB₂, AFG₁, and AFB₁, AFG₁, AFG₂ which were diagnosed 3 (7.9%) times. The remaining nine Mycotoxin combinations (Table 2) were unique to one

of the product (maize meal or maize grain). The presence of these was very low with records ranging from 1 (2.6%) to two 2 (5.3%) (Table 2).

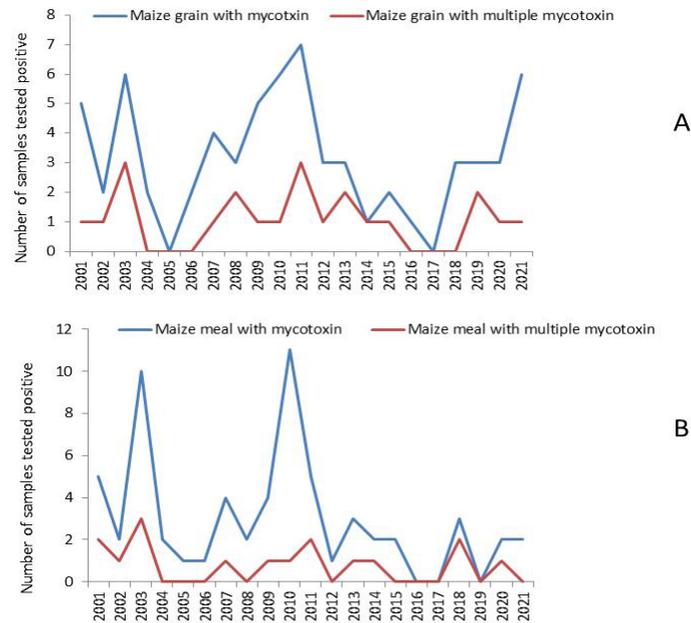


Figure 2. Trends in mycotoxin contamination of maize grain and maize meal supplied to consumers in Eswatini from 2001 to 2021.

Table 2. Mycotoxin co-occurrence in maize supplied to consumers in Eswatini

Product type	Mycotoxins combinations recorded	Co-occurrence	
		No	Percentage of the multiple mycotoxins with this combination
Maize Grain	AFB ₁ , AFB ₂	8	36.4
	AFB ₁ ,ZEN	2	9.1
	AFB ₂ , AFG ₂	2	9.1
	AFG ₁ , AFG ₂	1	4.5
	AFG ₂ , ZEN	1	4.5
	AFB ₁ , AFB ₂ ,AFG ₁	1	4.5
	AFB ₂ ,AFG ₂ ,ZEN	2	9.1
	AFB ₂ ,AFG ₁ , ZEN	1	4.5
	AFB ₁ , AFG ₁ , AFG ₂	1	4.5
	AFB ₁ , AFG ₂ , ZEN	1	4.5
	AFB ₁ , AFB ₂ , AFG ₁ , ZEN	1	4.5
	AFB ₁ , AFB ₂ , AFG ₁ , AFG ₂ , ZEN	1	4.5
Maize Meal	AFB ₁ , AFB ₂	4	25
	AFB ₁ ,AFG ₁	2	12.5
	AFB ₁ ,AFG ₂	2	12.5
	AFG ₁ , ZEN	1	6.3
	AFB ₁ , AFB ₂ ,AFG ₁	2	12.5
	AFB ₁ , AFB ₂ ,ZEN	1	6.3
	AFB ₁ , AFB ₂ ,AFG ₂	1	6.3
	AFB ₁ , AFG ₁ , AFG ₂	2	12.5
AFB ₁ , AFG ₁ , ZEN	1	6.3	

4. Discussion

The number of maize samples diagnosed by Mycotoxin laboratory at Malkerns (892) from 2001 to 2021 was very few as compared to the volume of maize consumed in the country. The sources of the samples were also skewed to the aggregators and wholesalers who are expected to handle relatively higher quality maize product. Samples received from small scale farms were very few and maize sold by local traders (venders) was not represented. Considering the few sample size and the sources of these samples, the Mycotoxin contamination level 129 (14.5%) reported in this study is likely to be an underestimate of the prevalence of mycotoxins in maize products supplied to Eswatini consumers. Because maize is a staple and also the main cash crop for small scale farmers and venders, the highest quality crop is often supplied to the market, leaving the poor quality one for home consumption predisposing the population to consumption of mycotoxin contaminated foods [13, 14]. Studies of fungal infection and mycotoxins in maize stores of small scale farmers from different agroecological zones of Benin showed aflatoxin contamination in 25% of the maize stores. In a similar study conducted in the Sudan Savanna, 56% of the maize stores were contaminated with aflatoxins after six months of storage [15]. In Southern Africa, maize and maize products are reported to be frequently contaminated with unacceptable levels of mycotoxins [16]. High levels of fumonisin contamination in maize have been reported in several studies in Malawi, South Africa and Zimbabwe [17, 18, 19]. A recent report from Murashiki et al. (2017) showed 100% FB₁ contamination in 388 samples from rural households in Shamva and Makoni districts of Zimbabwe. Results of these studies reveal that investigations into mycotoxin occurrence are very important for the assessment of consumer exposure to these contaminants via maize products as the major constituents of the human diet in the region. Research in this field is also of the essence for compiling legislation governing mycotoxin presence in maize and maize products, since no maximal or guidance values have insofar been established in the country for any dietary product. Strategies to manage mycotoxin contamination in foods need to be designed and implemented. Different awareness programmes have to be carried to inform people of the danger of consuming and commercialising mouldy products.

Five Mycotoxin types (Aflatoxins B1, B2, G1, G2 and Zearalenone) and mixture of these were isolated from samples in this study. All the mycotoxins isolated in this study are among the most important and highly toxic mycotoxins (Margherita et al., 2012) and reported to be the most common mycotoxins in maize [8]. Aflatoxins (B1, B2, G1 and G2) are considered to be the group of mycotoxins of greatest concern from a global perspective [8]. They are primarily produced by *Aspergillus flavus*, *A. parasiticus* and in rare cases, by *A. nomius* [6]. Aflatoxins may contribute to growth stunting during early childhood and together with other mycotoxins, are commonly suspected to play a role in development of edema in malnourished people as well as a causal or aggravating factor in the pathogenesis of kwashiorkor, a frequent condition in African children [20, 21]. Aflatoxin B₁ (AFB₁) has been extensively linked to human primary liver cancer in which it acts synergistically with hepatitis B virus (HBV) infection and was classified by the International Agency for Research on Cancer (IARC) as a human carcinogen (group 1 carcinogen) [22, 243, 24]. It is worth to note that this Mycotoxin was the most commonly diagnosed toxin in the maize grain and maize meal samples in this study. Studies carried out in Eswatini and Mozambique helped establish the link between aflatoxins, liver cancer and HBV infection [25]. In South Africa, cases of kwashiorkor, marasmus, and underweight that were reported correlated with findings of impaired liver function. Katerere et al. [30] reviewed available studies and data for a link between chronic aflatoxicosis and infant malnutrition in Southern Africa and concluded that there is mounting evidence implicating aflatoxin contamination as an important factor in infant under-nutrition, increased morbidity and mortality due to negative impact on immune function and micronutrient absorption. Immune modulation effects of aflatoxins may intensify health impacts of major diseases troubling Africa such as malaria, kwashiorkor and HIV/AIDS [20, 26]. Zearalenone is commonly produced by various *Fusarium* species such as *F. culmorum*, *F. graminearum* and *F. sporotrichioides*. It is most often found in maize, yet it can also be observed in other grain crops such as wheat, barley, sorghum, mealeet, and rice [27, 28]. Zearalenone has relatively low toxicity but is a naturally occurring endocrine-disrupting chemical and has been associated with clinical manifestations of hyper-oestrogenism in humans, including gynecomastia with testicular atrophy in rural males in Southern Africa [29].

These mycotoxins co-occurred in some of the maize grain and maize meal samples diagnosed. A total of eighteen different combinations of the AFs and ZEN were diagnosed in this study. Multi-mycotoxin contamination of predominantly consumed food commodities can exert serious health problems in consumer populations. The toxins may be carcinogenic, mutagenic, teratogenic, estrogenic, neurotoxic, hepatotoxic, nephrotoxic and cytotoxic or may induce immunosuppression in humans [20, 27, 28]. Acute mycotoxicoses have been reported in Africa and prolonged exposure to low amounts of various mycotoxins is a risk factor for human diseases including cancer and childhood stunting [20, 21]. However, the toxicity level of mycotoxins combinations cannot always be predicted based upon their individual toxicities. Multi-exposure may lead to additive, synergistic or antagonistic toxic effects [29]. The data on combined toxic effects of mycotoxins are limited, thus the health risk from this multi-exposure is not well-known.

The fluctuation in the prevalence of mycotoxins in maize grain and maize meal in this study may be a result of the variation in climatic conditions. In a survey of pre-harvest maize ear rot diseases in Zambia, Mukanga et al. [32] re-

ported a relationship between disease incidence and climatic data such as rainfall, relative humidity and temperature. Mohale et al. [33] reported seasonal variation in mycotoxin levels of maize samples from Lesotho. Rheeder et al. [33] reported that drought conditions in 2003 led to a substantial increase in fumonisin levels in two areas in South Africa, dry sub-humid Centane, compared to humid sub-tropical Mbizana. These reports indicate the importance of pre and postharvest management of maize and maize products in minimizing customers' exposure to mycotoxins. The warm and humid climate conducive for toxigenic fungi growth has made foods to be at high risk of mycotoxin contamination in Eswatini. The widespread use of traditional storage practises by Eswatini farmers contribute to a considerably to Mycotoxin contamination and warrants investigation into finding appropriate storage technologies for different agro ecological zones. The maize quality control systems in country are weak with analyses mainly executed on few samples collected from aggregators, the quality of locally produced maize, imports, maize product in retail markets, local traders and maize based foods and drinks need to receive regulatory attention. Since the harmful effects of mycotoxins are not known to the population, a mass awareness campaign backed by enforceable government regulations has the potential to minimize the risk to human health very quickly. It is imperative to have clear and enforceable regulations showing the standards for mycotoxin content in maize and other dietary products. Further research is required to identify the deleterious effects of the ever-growing family of mycotoxins and their metabolites on animal health and their impact on human health.

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