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Research Article

Prevalence of Mycotoxins in Locally Produced Maize [*Zea mays* (L.)] in Eswatini

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Abstract

Maize is Eswatini's most important crop, but it faces problems like insufficient production and low productivity due to climate change, poor management practices, and susceptibility to mycotoxin contamination. Mycotoxins are secondary metabolites produced by fungi that thrive in the warm, humid conditions common in Eswatini and contaminate maize from the field to storage. Three hundred and twenty (320) maize samples were collected from the drying structures and storage facilities of farmers contracted with the National Maize Corporation (NMC). Ninety (90) of these samples were randomly chosen and analyzed using Thin-layer Chromatography (TLC) to test for mycotoxins. The results showed that 42.8% of the samples contained aflatoxins (AFB1, AFB2, AFG1, and AFG2) and Zearalenone. The study found that farmers often lack proper storage facilities, which creates an environment that is conducive to fungal growth and mycotoxin contamination. This poses a health risk to maize consumers in Eswatini, and the study suggests that proper regulation guidelines are needed to reduce these associated risks.

Keywords: Aflatoxin, Eswatini, Farmers, Maize, Mycotoxin, NMC

Introduction

Food contamination by either biological, chemical, or physical contaminants are common cause of food poisoning, spoilage, and other losses [1]. Climate change, poor management practices from field to storage and susceptibility to mycotoxin contamination of the maize grain are among the major concerns in maize production [2]. Mycotoxins are secondary metabolites affecting the maize crop from the fields to storage produced by fungal pathogens such as *Aspergillus flavus* and *Fusarium verticillioides* which prefer warm and humid climatic conditions which are common in Eswatini [3]. Fungi of the genera *Fusarium* and *Aspergillus* are of economic importance in maize [4]. Over 300 mycotoxins have been identified [5].

Food that tested positive for mycotoxins is deemed not suitable for both human and animal consumption due to chronic effects they bring to health [6]. The susceptibility of maize to mycotoxin has notable health risks to consumers and a threat about sustainability to local producers. Maize is now more prone to mycotoxin toxicity in the world because of the negative impacts brought by climate change [7]. They further supported their claim in that this could be attributed to the fact that more insect pests, in the climate change era, survive in areas outside their area of origin which normally do not have the natural enemies for that crop pest. Maize borer and other stalk-boring maize pests increases the susceptibility of the maize to *Fusarium* spp. which are the major causes of stalk and ear rot resulting in increased levels of mycotoxin accumulation [8].

Eswatini is divided into four major agro-climatic zones which are namely the highveld, middleveld, Lowveld and the Plateau [9]. The middleveld is further divided into two zones which are the higher middleveld (wet middleveld) and lower middleveld (dry middleveld) while the lowveld consists of the eastern and western lowveld [10]. Most maize production is arguably done in the middleveld [11]. The highveld is the agro-ecological zone with the greatest potential for crop production [10]. The highveld receives the highest annual rainfall of 900mm while the upper middleveld and lubombo plateau receive 700mm annually per region and western lowveld receives 450mm annually [12]. Maize production in the highveld is affected by acidic soils because of excessive leaching [13]. The annual mean temperatures for the highveld and lowveld are 170C and 220C respectively [14].

Maize is the primary crop in the Kingdom of Eswatini, covering 80% of cultivated land and mainly grown for subsistence [15]. However, despite being the dominant crop in Eswatini's agricultural sector that plays a vital role in sustaining the livelihoods of its more than one million people, the nation persistently struggles to achieve self-sufficiency in maize, with suboptimal farming practices contributing significantly to this shortfall [10]. These risks might have detrimental effects on trade and economic growth in a country like Eswatini that is somehow dependant in agriculture [16]. Information on mycotoxin food contamination is limited in Eswatini although contamination may occur in the field or postharvest stages of the crop [17]. The purpose of the study was, therefore; to assess the prevalence of mycotoxins in locally produced maize in Eswatini.

Materials and Methods

Study Area

The study was conducted in the kingdom of Eswatini, targeting all four geographic regions of the country which were divided into six subdivisions of agro-ecological zones (highveld, upper middleveld, lower middleveld, western lowveld, eastern lowveld and Lubombo plateau) (Figure 1).

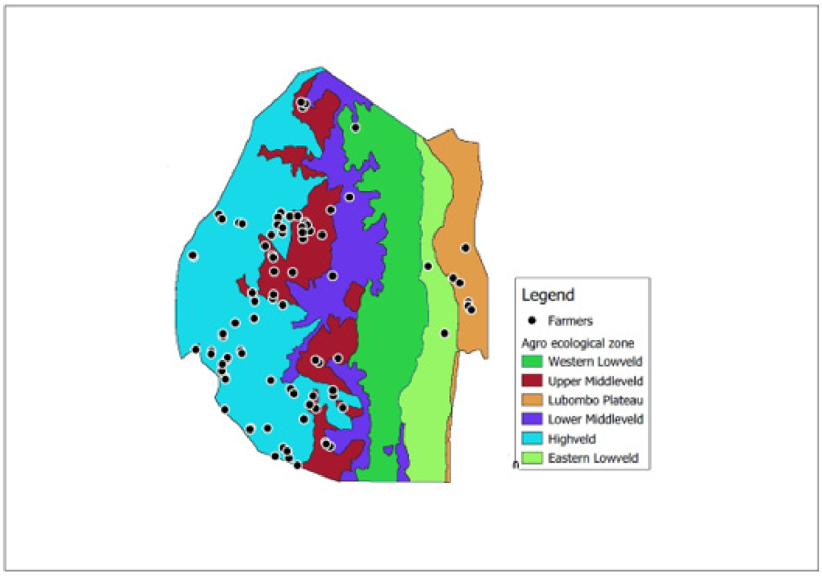

Figure 1: Locations Where Samples Were Collected from Local Farmers.

Tool for Data Collection and Procedure

Samples were collected from drying cribs and storage containers to test for the presence of mycotoxins. Physical observations on the condition of drying structures and storage structures were done during collection of samples. Samples were collected between August 2023 and May 2024.

Sampling procedure: The target population for this study was the active contracted maize farmers from the National Maize Corporation's database in 2023. A stratified random design was used to get samples according to six agroclimatic regions of Eswatini (western lowveld, eastern lowveld, upper middleveld, lower middleveld, highveld and the Lubombo plateau). A semi-structured questionnaire was administered from the survey population and results were recorded for analysis. A total of 320 samples were collected from 156 farmers from a population of 253 contracted farmers from NMC's 2023 farmer database which was calculated using Yamane's (1967) formula [18]. The 320 samples were then reduced to 90 samples which were randomly selected for TLC analysis (Table 1).

Table 1: Maize samples collected, and proportion of samples tested for mycotoxin contamination.

Agroclimatic Zone	Number of Samples Collected	Number of Samples Analyzed (TLC)
Lubombo Plateau	38	12
Highveld	120	23
Upper Middleveld	88	20
Lower Middleveld	28	14
Eastern Lowveld	24	11
Western Lowveld	22	10
Totals	320	90

Sample preparation and TLC analysis: Steps outlined for Thin-layer Chromatography (TLC) were followed [19]. 500 grams of each sample was ground into fine powder that can pass through a 0.85mm sieve by a Ramtoms blender. 50 grams of weighed sample were transferred into a blender. 250 ml of methanol was added into the sample which was then stirred for 2 minutes using a blender. 50 ml of hexane and sodium chloride were added as the polarization process continued. The mixture was shaken in a funnel and fumes released using a knob which was followed by extraction of the bottom layer of the sample. 125 ml of the extract was put into a separating funnel followed by the addition of 50ml of chloroform, 5 grams of cupric carbonate and 5 grams of sodium sulphate. Anti-bumping granules were added into a round bottom flask to the vial and filtered through a 12.5cm filter paper which was then heated until it disappeared on a heating mantle (Figure 2).

Figure 2: Filtration of Sample Solutions Through a Filter Paper.

2 ml of chloroform were added to the vial and then taken to a dark room for 5 minutes shaking on a shaker. It was then plotted on a TLC plate (Thin Layer Chromatography plate) to determine the presence of mycotoxins (B1, B2, G1, G2 and Zearalenone). The TLC plate was then inserted into a developing tank until it was fully soaked, and ultraviolet light was used to detect presence of mycotoxins. The presence of mycotoxin was found using visual comparisons with mycotoxin standards.

Data analysis: The data obtained was analyzed using descriptive statistics. Percentages and frequency counts were used in analyzing results.

Results

Type and Location of Storage Structures

About 53.3% of the farmers interviewed use metal tanks for maize storage whilst the least number of farmers (4.9%) use plastic tanks which are mostly (11.8%) used by farmers from the Lubombo plateau which are followed by farmers from western lowveld at

10%. About half of the respondents (50.1%) store their maize at an average distance ranging from 4 to 10 metres away from nesting structures and where animals are kept, with the rest storing their maize above 10 metres away (Table 2).

Table 2: Maize Storage Structures of Interviewed Farmers from the Six Agroecological Zones.

	Lubombo Plateau	Highveld	Upper Middleveld	Lower Middleveld	Eastern Lowveld	Western Lowveld	Average
No. of Storage Structures Sampled	17	60	44	14	11	10	26
Types of Storage Structures							
Bins	5,90	1,70	4,50	35,70	36,40	30,00	19,00
Sacks	5,90	0,00	9,10	35,70	45,40	40,00	22,70
Metal Tanks	76,40	95,00	81,80	28,60	18,10	20,00	53,30
Plastic Tanks	11,80	3,30	4,50	0,00	0,00	10,00	4,90
Distance of storage from nesting structure							
4 - 10 metres	70,60	8,30	43,20	64,30	54,50	60,00	50,10
Above 10 metres	29,40	91,70	56,80	35,70	45,50	40,00	49,90

Detection of Mycotoxins on Samples

The most prevalent single mycotoxin which was present in the samples was aflatoxin G1 (AFG1) which was at 11.7 % while 14.1 % samples had more than one mycotoxin detected (Figure 3). Above 57% of the samples tested had no presence of the mycotoxins that were tested with the Lubombo plateau having the least percentage (41.7%) of negative samples (Figure 3).

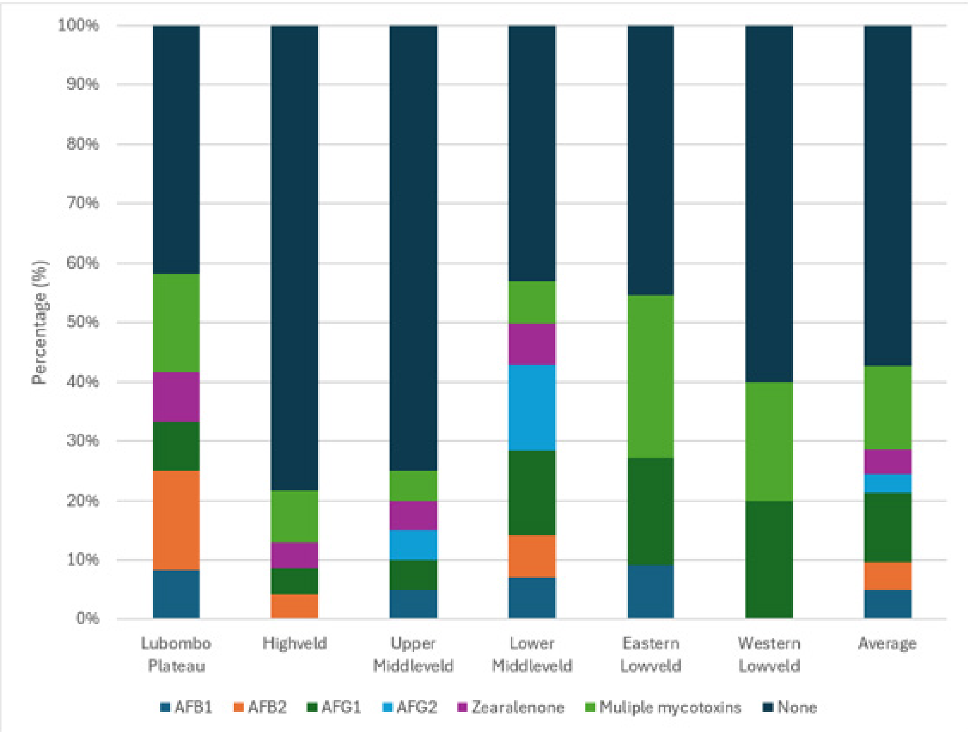

Figure 3: Mycotoxins detected from Maize Samples Collected in the study.

Prevalence of Mycotoxins from Samples Collected from Drying and Storage Structures

The presence of mycotoxins was mostly prevalent on storage sacks at an average of 31.31%, with the lower middleveld and western lowveld having the highest at 50,28% and 50% respectively on this type of structure (Table 3). Most positive mycotoxins in drying structures were detected on samples from floor mats with an average of 18.02% but the Lubombo plateau had an alarming 43.08% on samples taken from roof tops (Table 3). Maize cribs had the lowest (average of 2.37%) when compared to other drying structures while metal tanks had the least positive samples (average of 1.67%).

Frequency of Mycotoxins at Drying and Storage Structures

Most positive samples (average 64.29%) were detected from samples collected from storage structures when compared to drying structures (Table 4). The most predominant mycotoxin at drying was Zearalenone with a frequency of 60% while multiple mycotoxins had a frequency of 90% at storage when compared to its presence in drying structures. The most common single mycotoxin at storage was AFB2 with a frequency of 80% from samples collected from storage structures (Table 4).

Table 3: Prevalence of mycotoxins in maize from different drying and storage structures.

	Lubombo Plateau	Highveld	Upper Middleveld	Lower Middleveld	Eastern Lowveld	Western Lowveld	Average
Type of Drying or Storage Structure							
Roof tops	43,08	0,00	0,00	0,00	16,67	0,00	9,96
Mats	14,23	19,81	20,00	12,43	16,67	25,00	18,02
Maize Crib	14,23	0	0,00	0,00	0,00	0,00	2,37
Field Drying	0,00	19,81	20,00	12,43	16,67	0,00	11,49
Bins	14,23	39,62	10,00	24,86	16,67	25,00	21,72
Sacks	14,23	0,00	40,00	50,28	33,32	50,00	31,31
Metal Tanks	0,00	0,00	10,00	0,00	0,00	0,00	1,67
Plastic Tanks	0,00	20,76	0,00	0,00	0,00	0,00	3,46

Table 4: Mycotoxins prevalence in maize from drying and storage structures.

	Drying or Field	Storage
Type of Mycotoxin		
AFB1	30,00	70,00
AFB2	20,00	80,00
AFG1	27,00	73,00
AFG2	33,00	67,00
Zearalenone	60,00	40,00
Multiple Mycotoxins	10,00	90,00
None	70,0	30,00
Average	35,71	64,29

Discussion

The results of our study indicate that a majority of the farmers (53.3%) in Eswatini rely on metal tanks for maize storage. This is a positive finding, as metal tanks are generally more effective at protecting grain from moisture, pests, and rodents compared to more traditional methods [20]. The use of metal tanks is particularly high in the Highveld (95.0%), Upper Middleveld (81.8%), and Lubombo Plateau (76.4%) zones. This is likely due to the higher rainfall and humidity in these regions, which necessitates better storage to prevent spoilage.

In contrast, the use of sacks and bins is more prevalent in the Eastern and Western Lowveld, as well as the Lower Middleveld. Sacks, in particular, are less protective and more susceptible to moisture absorption and pest infestation, which can directly contribute to mycotoxin development. Studies reported that insect density and grain damage significantly increase in grain stored in plastic tanks for more than six months [21]. The data shows that 22.7% of farmers use sacks on average, with the Eastern Lowveld having the highest usage at 45.4%. The highest number of respondents who use bins for storage were found in the eastern lowveld, lower middleveld and western lowveld at 36.4%, 35.7% and 30% respectively. Storage in bins and sacks were reported to promote insect infestation and increased grain damage because it becomes difficult to control [22].

Almost half of farmers interviewed (50.1%) had storage structures found in unclean surroundings which were less than 10 metres away from nesting structures like poultry houses, cattle kraals and other structures for keeping domestic animals. This proximity increases the risk of contamination from rodents, birds, and insects, which can introduce fungal spores and toxins into the stored maize. Previous studies discovered that maize storage structures found above ten metres away from these structures had lower levels of mycotoxin contamination compared to closer structures as close structures [23]. The high percentage of farmers storing maize in close proximity to potential contaminant sources is recorded in this study a serious concern for food safety.

The data on mycotoxin detection reveals a significant problem. While 57% of samples tested negative for the specific mycotoxins, a notable percentage of samples were contaminated. The high prevalence of mycotoxins could be results of poor crop management practices, improper harvesting and post-harvest handling [24]. The prevalence of aflatoxin G1 (AFG1) as the most common single mycotoxin (11.7%) and the high percentage of samples with multiple mycotoxins (14.1%) are particularly alarming. The co-occurrence of multiple mycotoxins can have synergistic and additive toxic effects, posing a greater health risk than a single mycotoxin [25].

The results also highlight regional variations in contamination. The warmest parts of the country; the eastern lowveld, western lowveld, Lubombo plateau and lower middleveld had the highest of the most prevalent mycotoxin (Aflatoxin G1) with 18.2%, 20%, 8.3% and 14.3% prevalence of Aflatoxin G1 respectively. The coolest parts of Eswatini being the highveld and upper middleveld had the lowest mycotoxin prevalence with 78.3% and 75% negative samples respectively. These findings agree with previous study that concluded that hot and dry weather conditions favor mycotoxin development [26]. A combination of differences in environmental factors, specific farming practices and/or storage practices between the regions might have contributed to variation in contaminations [27].

Mycotoxin prevalence is significantly higher in storage structures than in drying structures. On average, 64.29% of positive samples were from storage, compared to 35.71% from drying. This indicates that while contamination begins during the drying process, it is during storage that the mycotoxins proliferate and reach high levels. The data shows a dramatic increase in multiple mycotoxins (from 10% in drying to 90% in storage) and AFB2 (from 20% to 80%) during the storage period, confirming this trend. The frequency of Zearalenone, however, was higher during drying, which is in agreement with results of studies that suggested this mycotoxin to be more associated with pre-harvest or early post-harvest contamination [28].

Conclusion

The prevalence of mycotoxins on the analyzed maize samples indicates that Eswatini maize consumers are in high risk of mycotoxin health associated outcomes. The prevalence of diseases and illnesses associated with hunger and lack of access to proper healthcare in the country may worsen the current conditions if more consumers are exposed to consumption of contaminated maize. Proper postharvest handling, relevant infrastructure, skills and knowledge are key factors in mitigating the results of lack of mycotoxin regulations in Eswatini.

Acknowledgement

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Conflict of Interest

No conflict of interest.

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