Evaluation of Mycotoxin Contaminations across Beans Food Chain in Eswatini

Marie Diana Earnshaw, Cyril Dlamini, Bongiwe Porri Dlamini, and Yoseph Assefa

ABSTRACT

Mycotoxins are naturally occurring toxic secondary metabolites produced by filamentous fungi contaminating various food and feed crops. Exposure to mycotoxins has been associated with mycotoxicosis, which accounts for a high percentage of diseases in developing countries where methods of food handling and storage are inadequate. For the first time, this study determined the diversity, prevalence and co-occurrence of mycotoxins across the beans value chain in Eswatini. A total of 226 beans samples received between 2001 and 2021 by mycotoxin laboratory at Malkerns research were analysed and more than 59.3% of the samples were found contaminated with mycotoxins. Results confirmed that aflatoxins (AFs) and Zearalenone (ZEN) were the main contaminating mycotoxins in beans. These mycotoxins occurred singly and co-occurred as mixtures in the samples analysed. Considerable differences regarding the prevalence of these mycotoxins were observed between samples from different beans value chain actors and between sampling years. Implications of the results on the pre and postharvest handling of beans in Eswatini and the health hazard that mycotoxins pose to humans and animals were discussed. The importance of clear and enforceable regulations on levels of mycotoxin in beans and other dietary products is emphasised and the need for research on the deleterious effects of mycotoxins and their metabolites on animal and human health is underlined.

Keywords: Co-occurrence, Eswatini, Maize grain, Maize meal, Mycotoxin, Value chain.

I. INTRODUCTION

Bean (Pharsalus vulgaris) is the second most important legume to Swazis after groundnut. The crop plays an important role in nutrition and livelihood of small-scale farmers in the country. Beans, as a source of cheap protein and micronutrients (iron and zinc), have been earmarked as vital to improving and safeguarding rural incomes, food security, and nutrition (Nassary et al., 2020). However, beans are mostly grown by smallholder farmers, who are disproportionately affected by the adverse effects of climate change (Pais et al., 2020). Most smallholder farmers in the country are low consumers of agricultural technology such as certified seed and fertilizer, relying mostly on saved seeds. This made beans production in Eswatini relatively low compared to the neighbouring countries and food insecurity is a persistent challenge. The annual production of dry beans over the decade was very low (averaging 887.2 tons/year) that the country imported 3087 tons of beans yearly on an average from South Africa to satisfy the national demand for the crop (FAOStat, 2019). In the year 2021, imported beans accounted for 88% of the total dry bean supplied to customers in the country (NAMBoard, 2022).

Climate change, nutrient depleted soil, crop pests and diseases, and poor post-harvest handling practices are to be blamed for the very low dry bean production. Like other crops, beans are also prone to microbial infections during production, processing and storage stages (Rawat, 2015). Prevalence of toxigenic Aspergillus, Penicillium and Fusarium spp., on beans has been reported in beans growing and non-growing regions in Sub Saharan Africa (SSA) (Bankole et al. 2006). These fungal infections usually lead to the accumulation of mycotoxins (Mielniczuk and Skwaryło, 2020) that can result in significant sickness and mortality, placing a profound burden on human productivity and development. Acute mycotoxicosis have been reported in Africa (Matumba, et al., 2014; Kimanya et al., 2015). In rural Eswatini, most households have low income and lack access to a broad array of foods with the majority of calorific intake...
restricted to one or two major crops. Beans constitute the major part of the daily diet of these families. These rural farmers sale the highest quality crops leaving the poor quality ones for household consumption. When they buy from market, they tend to purchase, low priced near to expire beans that is likely to be contaminated by mycotoxins due to economic reasons. The daily consumption of poor quality beans that leads to a prolonged exposure to low amounts of various mycotoxins is a risk factor for human diseases including cancer and childhood stunting (Matumba et al., 2014).

Mycotoxin contaminations occur along all pre and postharvest stages of the food system where crop may either be infested by mycotoxin producing fungi in the field or due to poor postharvest handling practices. Qualitative estimates of mycotoxins in the country are rare and quantification of mycotoxins in staple food has been overlooked due to lack of well-equipped laboratories, inadequate capacity in terms of expertise, inadequate funding, and dearth surveillance. Identification of mycotoxins in beans consumed in the country is, therefore, a necessary first step towards developing mycotoxin standards for major food crops and minimizes the health hazard related to mycotoxicosis. The study herein has compiled information on the laboratory analyses of mycotoxins in bean samples submitted to the Malkerns laboratory in Eswatini by different beans supply chain actors since 2001 and summarizes the mycotoxin groups and the trend in mycotoxin contaminations in beans supplied to consumers in the country. This should assist in guiding national authorities in the rational and specific measures for the management of beans quality to be supplied to consumers.

II. MATERIALS AND METHODS

Assessment of the bean supply chain in Eswatini and identification of the important value chain actors involved the collection of secondary data, documentation and expert consultations. The supply chain stages observed were primary production and harvesting, imports from South Africa, transportation of beans to the household, threshing and winnowing, drying of bean grains, storage and marketing by vendors, retailers and wholesalers. The contributions of local production and import to the beans supplied in the country were assessed. Then, the laboratory reports of all beans samples analysed at the Malkerns research mycotoxin laboratory between July 1, 2001 and December 31, 2021 were retrieved and the extent of Mycotoxin contamination in the submitted samples evaluated. Samples submitted by beans value chain actors were evaluated separately and the implications of the variation in Mycotoxin levels between actors were discussed. In accordance with currently accepted standards, samples collected in the reviewed period were analysed following the procedure described in Dlamini et al. (2022).

III. RESULT

A. An Overview of Eswatini Beans Supply Chain

After groundnut, beans are the second most important legume crop in Eswatini, and are produced in all regions but the crop does very well in the higher areas of the country (NAMBoard, 2021). In 2021, the total beans produced in the country was 659.6 tons (NMC, 2021), accounting for less than 18% of the total beans supplied to consumers in the country (Fig. 1). Beans import from South Africa in 2021 was estimated to be 4,832 tons (NAMBoard, 2021), contributing for more than 88% of the total national beans consumption (Fig. 1). The import from South Africa plays a major role in determining the price of beans in Eswatini and negatively affected the beans production in the country. Between 2005 and 2019, dry bean production in Eswatini stagnated between 815 to 775 tons. Production rose to 1100 tons in 2006 and 2010, to 1050 tons in 2009, to 1056 in 2011 and to 1000 in 2012 and 2013 (FAOSTAT, 2019). Units

The beans supply chain in Eswatini involves a number of stakeholders, as depicted in Fig.1. It involves farmers and vendors who are mainly dealing with locally produced beans and aggregators, donors, millers, Distributors wholesalers and retailers that trade both imported and locally produced beans. The supply chain also includes support system which consists of input suppliers, transporters, Agricultural Union and government offices. In 2021, the beans produced in the country accounted only for 17.7% of the volume consumed (Fig. 1).

At the field-level, large-scale growers and farmer cooperatives harvest produce and supply to the aggregators. Some large scale commercial growers and a significant number of the farmer cooperatives directly sale their produce to vendors, consumers and/or donor institutions. In contrast, smaller scale growers primarily sell produce directly at the farm gate, at open-air markets, and/or distribute to vendors. Produce from small-scale growers in most instances is not subjected to any form of postharvest treatment for maintaining quality or extending shelf-life. Aggregators, wholesalers, millers and distributors import beans from South Africa, transportation of beans to the household, threshing and winnowing, drying of bean grains, storage and marketing by vendors, retailers and wholesalers. The contributions of local production and import to the beans supplied in the country were assessed. Then, the laboratory reports of all beans samples analysed at the Malkerns research mycotoxin laboratory between July 1, 2001 and December 31, 2021 were retrieved and the extent of Mycotoxin contamination in the submitted samples evaluated. Samples submitted by beans value chain actors were evaluated separately and the implications of the variation in Mycotoxin levels between actors were discussed. In accordance with currently accepted standards, samples collected in the reviewed period were analysed following the procedure described in Dlamini et al. (2022).

Fig. 1. Summarized illustration of the Bean supply chain in Eswatini (adapted from FAO 2022 food loss assessment report).
Africa and supply to retailers, donors and consumers. These actors also buy beans from local farmers and supply to the urban and rural consumers. Donor institutes and Government offices assist farmers through training, research and provision of subsidies and aid.

B. Beans Samples Analysed

Summary of the beans samples submitted to the Mycotoxin laboratory between 2001 and 2021 are indicated in Table 1. The overall results confirm that aflatoxins (AFs) and Zearalenone (ZEN) are the main contaminating mycotoxins in beans. These mycotoxins occurred singly and co-occurred as mixtures in samples analysed. Considerable differences regarding the prevalence of these mycotoxins were observed between samples from different actors and between sampling years. From a total of 226 beans samples analysed for Mycotoxin contamination between 2001 and 2021, only 92 (40.7%) samples were diagnosed to be negative to mycotoxins. More than fifty nine (59.3%) of the samples examined were contaminated with mycotoxins with the most frequently diagnosed mycotoxin contaminations being Aflatoxin B1 (AFB1), 51 (22.6%) Aflatoxin B2 (AFB2), 48 (21.2%), Zearalenone (ZEN) 42(18.6%) and multiple mycotoxins 50(22.1%) (Table 1). Other mycotoxins recorded in the analyses were Aflatoxin G1 (AFG1), 32 (14.6%) and Aflatoxin G2 (AFG2), 11 (4.9%).

Aflatoxin B1, Zearalenone and multiple mycotoxins are the most often detected mycotoxins with values of positive samples frequently above 50%. The highest incidence of Aflatoxin B1 and Zearalenone contaminated beans sample was diagnosed in 2018 in 13 samples from a donor organisation where all (100%) were diagnosed to be contaminated by AFB1 and ZEN as multiple mycotoxins.

Comparison of beans samples for trends in occurrence and co-occurrence of mycotoxins in the years included in this study shows high prevalence and temporal variations. In the first seven years (2001-2007) Aflatoxin G1, Aflatoxin B1 and Zearalenone were the predominant mycotoxins recorded. From the beans samples submitted to the laboratory between the years 2001 and 2007, 8.2% were contaminated with Aflatoxin G1, 11.3% Aflatoxin B1 and 9.1% with Zearalenone (Fig. 2). In these years, multiple mycotoxins were not recorded. The prevalence of Mycotoxin contamination has shown a marked increase in the years between 2008 and 2014. There was also a change in the importance of mycotoxins. In these years, 37.7% of the beans samples analysed were diagnosed with Aflatoxin B1. The relative importance of zearalenone dropped to 5% and the importance of Aflatoxin B2 increased from 4.9% in the previous seven years to 14.4% in the years 2008 to 2014.

More than 17% of the beans samples analysed in these years exhibited the co-occurrence of mycotoxins (Fig. 2). AFB1 + ZEN were the most commonly diagnosed Mycotoxin mixtures in the analyzed beans samples. In the last seven years (2015-2021) the incidence of Aflatoxin B2 in the beans samples showed a marked increase reaching 49.5% from 14.4% in the years 2008 to 2014. In these years, none of the beans samples analyzed were diagnosed with AFG2 and the incidence of AFB1 and AFG1 was lower than the levels in the previous seven years. The co-occurrence of Mycotoxin doubled from what it was in the previous seven years to reach 11.1% (Fig. 2).

It is important to consider the sources of beans samples and the trend of contamination. Thus, the numbers of beans samples submitted by beans food value chain actors and the trends in Mycotoxin contaminations have been compared for the samples analysed in the twenty years (2001-2021) included in the study (Fig. 3A). Fig. 3A shows that majority of the beans samples submitted to the laboratory for analysed originate from donor institutes. Beans samples from donors constituted for 82.1% of the samples analysed in the years between 2001 and 2007. The proportion of beans samples sourced from donors increased in the years between 2008 and 2014 to 87.3% and showed a slight reduction in the last seven years (2015-2021) to 60.8% (Fig. 3A). Millers and distributor contributed to 15.5%, 9.5% and 19% of the beans samples diagnosed for Mycotoxin in the first (2001-2007), second (2008-2014) and third seven years (2015-2021) respectively. Samples from government institutes were submitted for analysis only in the years between 2008 and 2021. Similarly, there was no beans sample received by the laboratory from aggregators in the years between 2008 and 2014. The number of beans samples from aggregators was very few in the first and the last seven years included in the study (Fig. 3A). There were no samples submitted to the laboratory from venders, farmers, wholesalers and retailers.

---


<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Average</th>
<th>Maximum</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin G1 (AFG1)</td>
<td>1.5</td>
<td>4</td>
<td>14.2</td>
</tr>
<tr>
<td>Aflatoxin G2 (AFG2)</td>
<td>2.4</td>
<td>2</td>
<td>4.9</td>
</tr>
<tr>
<td>Aflatoxin B1 (AFB1)</td>
<td>0.5</td>
<td>13</td>
<td>22.6</td>
</tr>
<tr>
<td>Aflatoxin B2 (AFB2)</td>
<td>2</td>
<td>5</td>
<td>21.2</td>
</tr>
<tr>
<td>Zearalenone</td>
<td>2.4</td>
<td>13</td>
<td>18.6</td>
</tr>
<tr>
<td>Multiple mycotoxins</td>
<td>2</td>
<td>13</td>
<td>21.1</td>
</tr>
</tbody>
</table>

DOI: http://dx.doi.org/10.24018/ejbio.2022.3.3.369
Mycotoxin contamination occurred extensively in all the samples submitted by beans value chain actors. However, beans samples from aggregators and government institutes were the highest in mycotoxin contamination with Mycotoxin prevalence of as high as 100%. In the beans samples from Donors and distributors, the prevalence of Mycotoxin contamination were relatively lower with levels ranging from 48% to 83% (Fig. 3B). The overall Mycotoxin prevalence in the beans samples analysed ranged from 50% in the first seven years (2001-2007) to 71% in the last seven years (2015-2021).

IV. DISCUSSION

This work demonstrates the significant occurrence and co-occurrence of five important mycotoxins namely Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2 and Zearalenone in Eswatini beans. This finding is important because beans are staple foods in Eswatini. The four Aflatoxins recovered from the beans samples in this study are the major naturally produced aflatoxins which are both acutely and chronically toxic to animals and human beings (Negash, 2018). Exposures to these aflatoxins can lead to aflatoxicosis, which accounts for a high percentage of diseases in developing countries (Kimanya et al., 2021). Acute aflatoxicosis can cause death to humans and animals while chronic high levels can lead to a gradual deterioration of health through liver damage and immunosuppression (Negash, 2018). Aflatoxins B1 and G1 have been categorized as Group-1 carcinogens (Nazhand et al., 2020). Commonly, AFB1 is the most carcinogenic, mutagenic, and teratogenic compound of the aflatoxins recovered in this study (Alpsoy, 2010). Aflatoxin B1 obstructs DNA, RNA and protein synthesis, causing immuno-suppressive, hormonal and teratogenic effects (Aasa, 2021). Exposures to aflatoxins have been found to cause hormonal imbalance in children, leading stunting/growth faltering in children in different parts of Africa (Aasa, 2021; Matchado, 2019). Evidence also suggests that there may be an interaction between chronic mycotoxin exposure and malnutrition, immunosuppression, impaired growth and diseases such as malaria and HIV/AIDS (Matumba et al., 2014; Kimanya et al., 2015).

Zearalenone is an oestrogenic toxin, primarily produced by Fusarium graminearum and Fusarium culmorum (Caglayan et al., 2020). These species of Fusarium are generally found on plants primarily grown in temperate regions (Rogowska et al., 2019) but the mycotoxin they produce was frequently reported from grains produced in tropical and subtropical regions of Africa (Doko et al., 1996; Bankole et al., 2010). The effect of zearalenone in animals is a well-defined syndrome. Exposure to this contaminant is accompanied by reduced levels of progesterone and serum testosterone in the bloodstream resulting in infertility and reduced incidences of pregnancy in animals like cows, pigs and rats (Yang et al., 2007; Rai et al., 2019). Zearalenone toxicity is recurrently associated with reproductive syndromes in farm animals and intermittently with hyperactive oestrogenic disorders in human beings. It has been categorized as a Group 3 carcinogen by the International Agency for Research on Cancer (IARC) due to its unclassifiable carcinogenicity to humans with inadequate evidence (Rai et al., 2019). Zearalenone has been implicated in several incidents of precocious pubertal changes in children (Kuiper-Goodman et al., 1987).

Results of the current study revealed the temporal increase in the prevalence of multiple mycotoxin contamination in beans supplied to customers in the country. The observed co-occurrence may have resulted from colonization of beans by more than one fungal species which produced different types of mycotoxins or due to colonization by one fungal species that produced more than one type of mycotoxin. Published studies on the combined effects of multiple mycotoxins suggest possible greater toxic effects of the mixtures compared to individual mycotoxins (Gruber-Dorninger et al., 2019). The confirmed occurrence and co-occurrence of mycotoxins in beans is unavoidable and poses a unique challenge to food safety. However, it is possible and important to regularly monitor the exposure of animals and human being to mycotoxins through food and feed. The severity of the toxic effects of mycotoxins on animals and human health is reported to be dependent on the type(s) of mycotoxin; the amount and duration of exposure and the age, health and sex of the exposed individual (Darwish, 2019; Bennett and Klich, 2003). Regulating the amount of mycotoxins in food and feed is, therefore, essential to minimize the risks of mycotoxicosis in human beings and other animals.

Over 100 countries have developed specific limits for mycotoxins in foodstuffs (FAO, 2004; Wu and Mitchell, 2016; Eskola et al., 2018). In Africa, only few countries such
as Ivory Coast, Egypt, Kenya, Malawi, Nigeria, South Africa and Senegal have permitted level of mycotoxins in food and feed ingredients (Assefa and Geremew, 2018). Unfortunately, Eswatini is one of the countries without a regulation on the level of mycotoxins in food and feeds. It is imperative to determine the presence, levels and co-occurrence of various mycotoxins in food and feeds in the country. The information on the prevalence and diversity of mycotoxin provided herein is an indication of the risk of mycotoxicosis in Eswatini consumers who depend on beans as staple food. The information can also be used to justify the need for implementation of mycotoxin management strategies and enforceable regulations on food safety in the country.

Prevalence, diversity and co-occurrence of mycotoxins reported in this study are based on samples submitted by donor organizations, Aggregators, government offices, millers and distributors. Beans handled by farmers, venders, wholesalers and retailers are not represented. Donor organizations, aggregators, millers and distributors are mainly handling imported beans which are usually the highest quality crops with less mycotoxin contaminations than locally produced beans. Moreover, these actors are well aware of mycotoxins and follow proper post-harvest handling practices. In contrary, rural beans farmers and venders have reduced awareness on the health risk associated to consumption of mycotoxin and the pre and post-harvest handling practices to minimize mycotoxin contaminations. These factors are reported to be causes for the wide spread occurrences of mycotoxins at frequently high levels in grains handled by smallholder farmers (Kristine and Florian, 2018). Devastatingly, crops which are stored improperly by venders and smallholder farmers under favourable temperature and prompting humidity for a long time facilitate mould growth and can be subject to mycotoxicosis. Ahmad and Jae-Hyuk, (2017). Including beans sample from venders and small scale farmers is essential to get a clear picture on the prevalence and diversity of mycotoxins in beans in the country. Including supermarket rejects which are often sold at lower prices as animal feed and beans grains shelved in retail shops after their expiry dates may indicate the levels of exposure of consumers and animals to mycotoxosis.

Data on human exposure to single and multiple mycotoxins in Eswatini is lacking, but the few available studies indicated that Eswatini population is chronically exposed to major mycotoxins (Martin et al., 1971). The present study provided new data on mycotoxin risk in Eswatini, revealing for the first time the diversity, prevalence and co-occurrence of different mycotoxins in beans consumed in the country. The data presented herein can serve as a basis for future studies on the health risk associated with mycotoxin, as a teaching material in mass awareness campaigns and as an as a basis in casting enforceable government regulations to minimize the mycotoxicosis risk to human health in Eswatini.

REFERENCES
Matchado, A. J. (2019). Dietary Factors Associated With Child Linear Growth In Rural Malawi, Doctoral dissertation, University of California, Davis, USA.

DOI: http://dx.doi.org/10.24018/ejbio.2022.3.3.369